Edrolo

VCE BIOLOGY

Units 3 & 4

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Edrolo

At Edrolo, we're transforming the way the students learn and teachers teach.

Our mission is simple: to improve education.

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Our offices sit upon the lands of Australia's first peoples - the Wurundjeri people of the Kulin Nations. As we work together, we acknowledge this and pay our respects to Elders both past and present, and emerging leaders. Aboriginal and Torres Strait Islander peoples are advised that this book may contain names or images of people who are now deceased.

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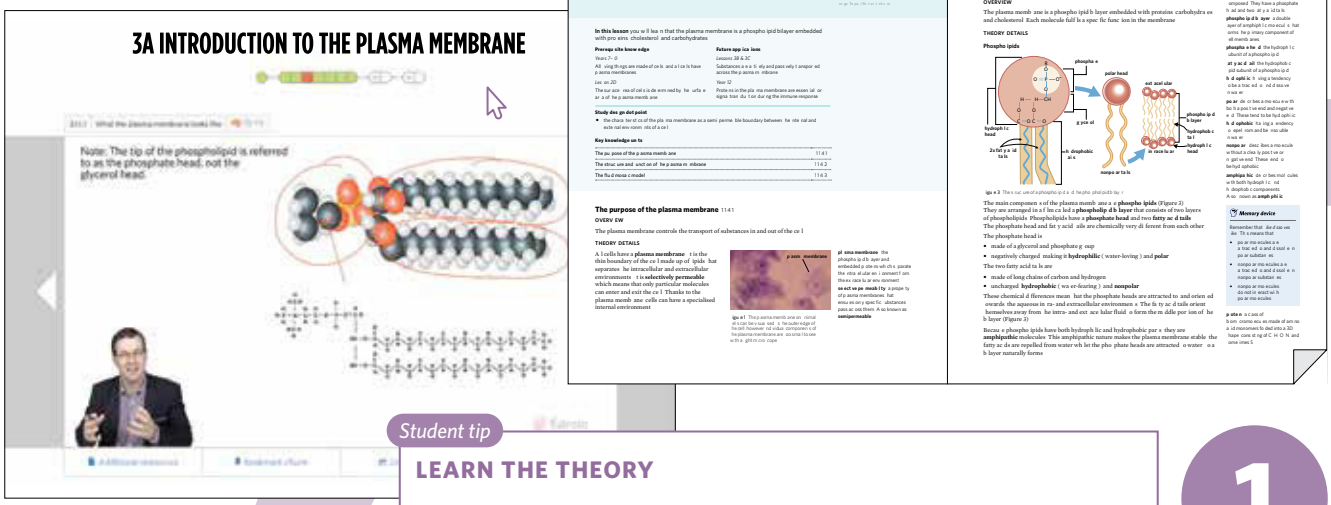
CONTENTS

USING THIS RESOURCE TO TEACH AND LEARN



Student tip

LEARN THE THEORY

Every dot point in your study design is covered in our video lessons and textbook theory – perfect to use for pre-learning, during class, and as revision.

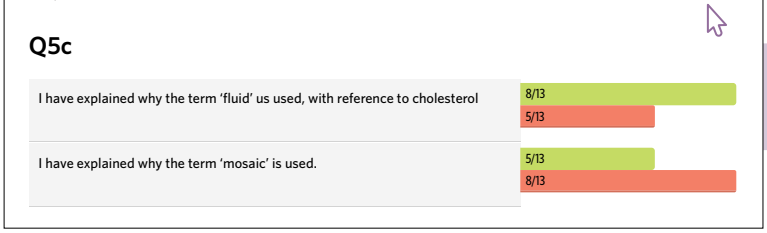
1

Teacher tip

EVALUATE STRENGTHS AND AREAS FOR IMPROVEMENT

Teachers see class-level data and individual student responses – use this to provide feedback, differentiate student learning, plan future lessons, and inform the revision program of your students.

4



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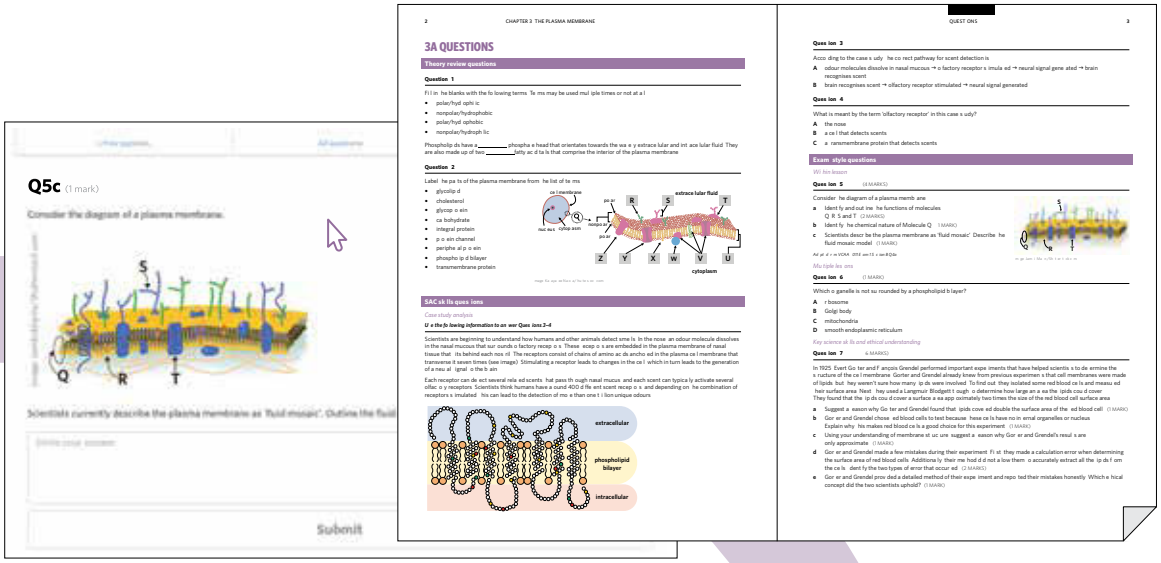


2

Student tip

CHECK FOR UNDERSTANDING

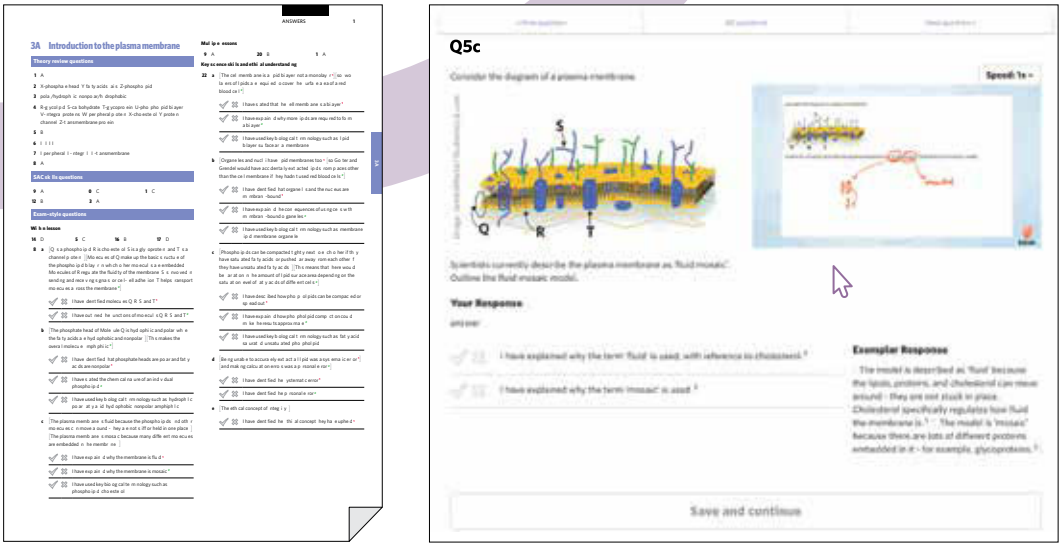
Each lesson has theory review questions, SAC skill questions, and exam-style questions so you can apply your knowledge in different ways to consolidate your learning. You'll also find tests/exams within each area of study.

3

Student tip

SELF-ASSESS AND GET FEEDBACK

At the back of your textbook you'll find exemplar responses and checklists for every SAC and exam-style question. In your Edrolo account, you'll find video solutions as well as the interactive checklists and exemplar responses. Use these answers to target your revision and get the greatest impact from your study time. This enables you to focus on the parts of the theory you struggled with, and ask your teacher for support if you get totally stuck!



FEATURES OF THIS BOOK

Edrolo's VCE Biology Units 3 & 4 textbook has the following features.

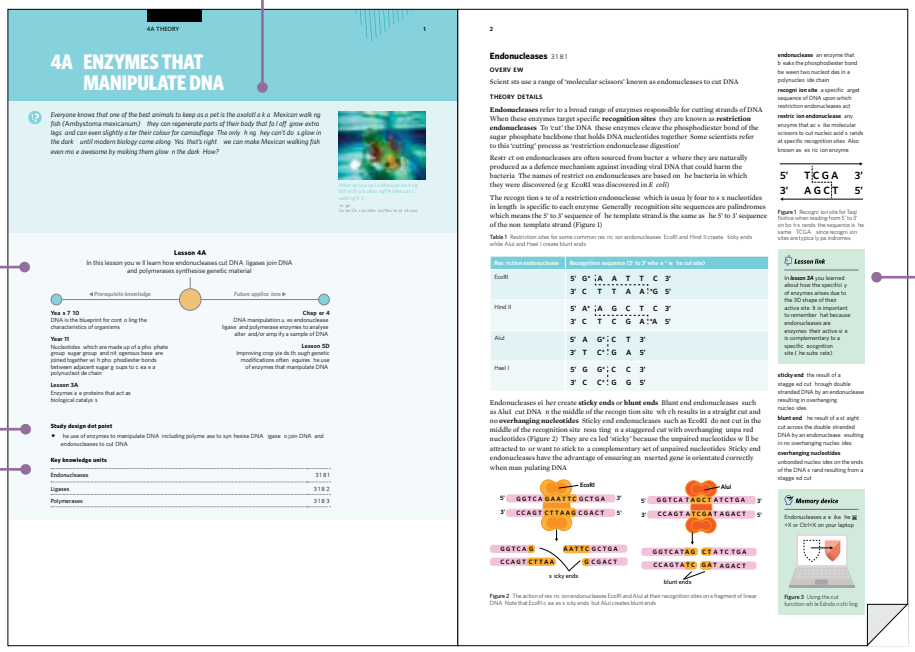
Hooks pose a real-life problem that you should be able to solve by the end of the lesson.

Explore boxes include memory devices, lesson links, case studies, and theory in action boxes which help broaden your understanding of the theory.

Learning timelines outline what you have already learned in previous lessons or from Years 7–10, what you will learn in this lesson, and how this knowledge will be used in future lessons or in Year 12.

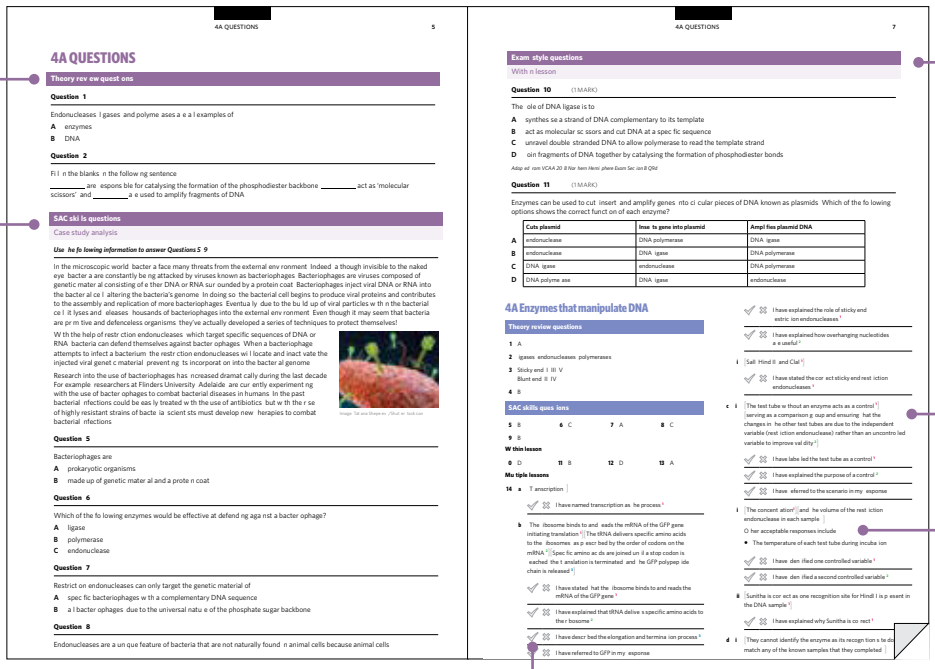
Study design dot points from the VCAA curriculum provide explicit links between our lessons and the syllabus.

Key knowledge units break down the theory into smaller chunks and can be used to help navigate the corresponding theory lesson videos online.



Theory review questions test if students can remember the basic theory and overcome common misconceptions. They are stepping stones between the content and exam-style questions.

Exam-style questions reflect the style of your end-of-year exam in Year 12. These include questions from both within the lesson and from multiple lessons, plus questions that test key science skills and ethical understanding in the context of the theory that you just learned.



Exemplar responses are provided for every exam-style question to show you what a full mark response could look like.

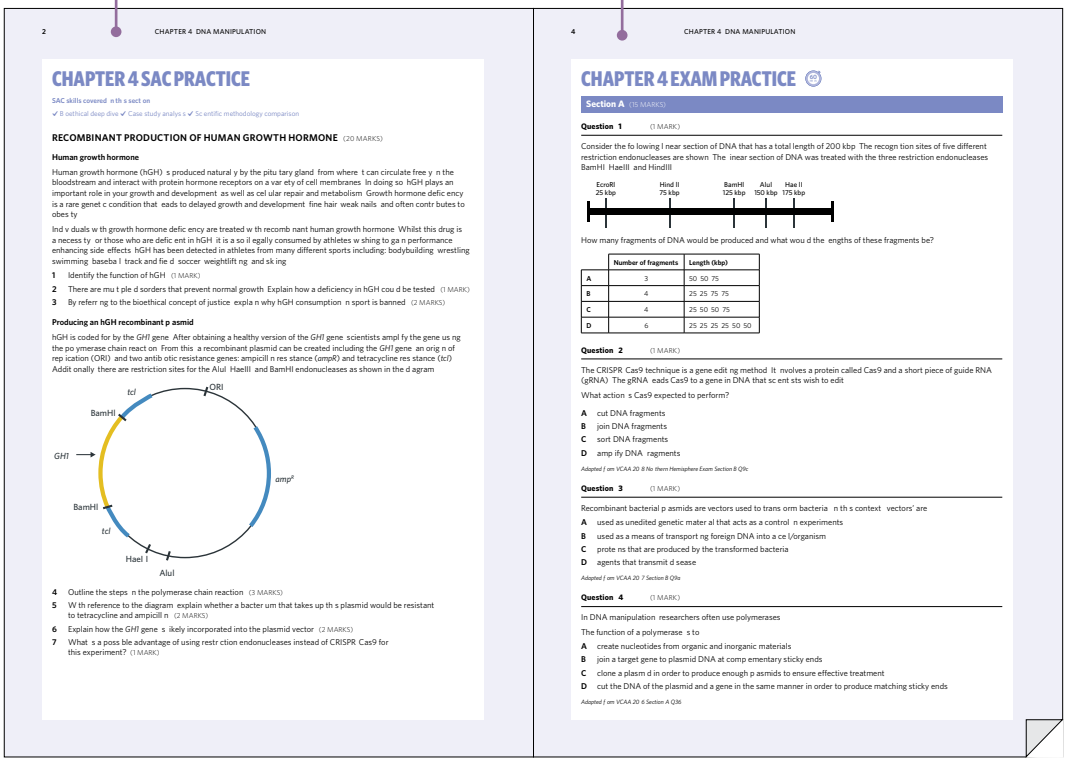
Other acceptable responses are included when there are multiple answers that could achieve full marks.

SAC skills questions build your skillset to tackle Year 12 SACs. You'll get to hone your ability to analyse case studies, evaluate bioethical issues, interpret data, and compare scientific methodologies.

Checklists break answers down into the smallest components required to get full marks. Checklists also show you how to articulate your response coherently, by including key terms or comparative language.

Practice SACs are activities that put your case study analysis, data analysis, scientific methodology comparison, and/or bioethical analysis skills to the test. It is important to develop these skills in Units 1 & 2, as these are the core SAC assessments in Units 3 & 4 from 2022 onwards.

The **practice exam** is a 20-mark set of questions that, if sat in 30 minutes, replicates the experience of a VCAA exam. Each chapter has a carefully selected ratio of multiple-choice and short-answer questions to reflect how the information is assessed on VCAA exams.



2 CHAPTER 4 DNA MANIPULATION

CHAPTER 4 SAC PRACTICE

SAC skills covered n th s sect on

✓ B oethical deep dive ✓ Case study analys s ✓ Sc entific methodology comparison

RECOMBINANT PRODUCTION OF HUMAN GROWTH HORMONE (20 MARKS)

Human growth hormone

Human growth hormone (hGH) s produced natural y by the pitu tary gland from where t can circulate free y n the bloodstream and interact with protein hormone receptors on a var ety of cell membranes In doing so hGH plays an important role in your growth and development as well as cel ular repair and metabolism Growth hormone defic ency is a rare genet c condition that eads to delayed growth and development fine hair weak nails and often contr butes to obes ty

Ind v duals w th growth hormone defic ency are treated w th recomb nant human growth hormone Whilst this drug is a necess ty or those who are defic ent in hGH it is a so il egally consumed by athletes w shing to ga n performance enhancing side effects hGH has been detected in athletes from many different sports including: bodybuilding wrestling swimming baseba l track and fie d soccer weightlift ng and sk ing

1 Identify the function of hGH (1 MARK)
2 There are mu t ple d sorders that prevent normal growth Explain how a deficiency in hGH cou d be tested (1 MARK)
3 By referr ng to the bioethical concept of justice expla n why hGH consumption n sport is banned (2 MARKS)

Producing an hGH recombinant p asmid

hGH is coded for by the *GH1* gene After obtaining a healthy version of the *GH1* gene scientists ampl fy the gene us ng the po ymerase chain react on From this a recombinant plasmid can be created including the *GH1* gene an orig n of rep ication (ORI) and two antib otic resistance genes: ampicill n res stance (*amp*R) and tetracycline res stance (*tc*R) Addit onally there are restriction sites for the AluI HaeIII and BamHI endonucleases as shown in the d agram

4 Outline the steps n the polymerase chain reaction (3 MARKS)
5 W th reference to the diagram explain whether a bacter um that takes up th s plasmid would be resistant to tetracycline and ampicill n (2 MARKS)
6 Explain how the *GH1* gene s ikely incorporated into the plasmid vector (2 MARKS)
7 What s a poss ble advantage of using restr ction endonucleases instead of CRISPR Cas9 for this experiment? (1 MARK)

4 CHAPTER 4 DNA MANIPULATION

CHAPTER 4 EXAM PRACTICE

Section A (15 MARKS)

Question 1 (1 MARK)

Consider the fo lowing l near section of DNA that has a total length of 200 kbp The recogn tion sites of five different restriction endonucleases are shown The inear section of DNA was treated with the three restriction endonucleases BamHI HaeIII and HindIII

How many fragments of DNA would be produced and what wou d the engths of these fragments be?

	Number of fragments	Length (kbp)
A	3	50 50 75
B	4	25 25 75 75
C	4	25 50 50 75
D	6	25 25 25 25 50 50

Question 2 (1 MARK)

The CRISPR Cas9 technique is a gene edit ng method It nvolves a protein called Cas9 and a short piece of guide RNA (gRNA) The gRNA eads Cas9 to a gene in DNA that sc ent sts wish to edit

What action s Cas9 expected to perform?

A cut DNA fragments
B join DNA fragments
C sort DNA fragments
D amp ify DNA ragments

Adapted f om VCAA 20 8 No thern Hemisphere Exam Section B Q9c

Question 3 (1 MARK)

Recombinant bacterial p asmids are vectors used to trans orm bacteria n th s context vectors' are

A used as unedited genetic mater al that acts as a control n experiments
B used as a means of transport ng foreign DNA into a ce l/organism
C prote ns that are produced by the transformed bacteria
D agents that transmit d sease

Adapted f om VCAA 20 7 Section B Q9a

Question 4 (1 MARK)

In DNA manipulation researchers often use polymerases

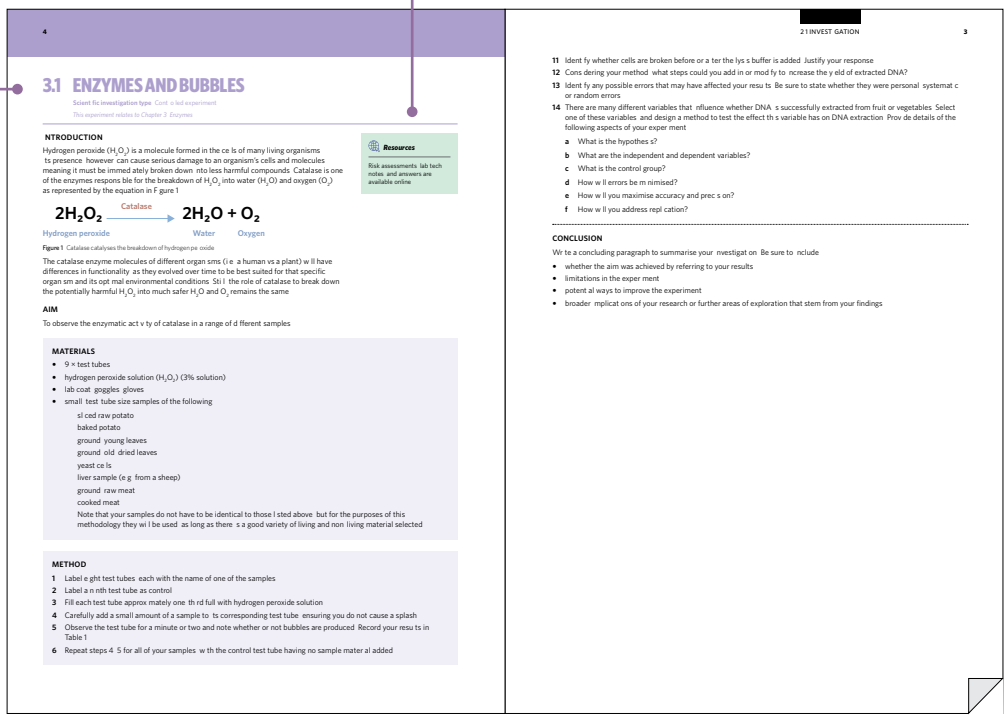
The function of a polymerase s to

A create nucleotides from organic and inorganic materials
B join a target gene to plasmid DNA at comp ementary sticky ends
C clone a plasm d in order to produce enough p asmids to ensure effective treatment
D cut the DNA of the plasmid and a gene in the same manner in order to produce matching sticky ends

Adapted f om VCAA 20 6 Section A Q36

Risk assessments, **lab tech notes**, and **answers** are available online

Scientific investigations can be found at the back of the book which follow one of the scientific investigation types in the VCAA Biology study design (2022-2026)



4

3.1 ENZYMES AND BUBBLES

Scient fic investigation type Cont o led experiment

This experiment relates to Chapter 3 Enzymes

NTRODUCTION

Hydrogen peroxide (H_2O_2) is a molecule formed in the ce ls of many living organisms ts presence however can cause serious damage to an organism's cells and molecules meaning it must be immed ately broken down nto less harmful compounds Catalase is one of the enzymes respons ble for the breakdown of H_2O_2 into water (H_2O) and oxygen (O_2) as represented by the equation in F gure 1

Resources

Risk assessments lab tech notes and answers are available online

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Hydrogen peroxide — Water — Oxygen

Figure 1 Catalase catalyses the breakdown of hydrogen pe oxide

The catalase enzyme molecules of different organ sms (i e a human vs a plant) w ll have differences in functionality as they evolved over time to be best suited for that specific organ sm and its opt mal environmental conditions Sti l the role of catalase to break down the potentially harmful H_2O_2 into much safer H_2O and O_2 remains the same

AIM

To observe the enzymatic act v ty of catalase in a range of d fferent samples

MATERIALS

- 9 × test tubes
- hydrogen peroxide solution (H_2O_2) (3% solution)
- lab coat goggles gloves
- small test tube size samples of the following
  - sl ced raw potato
  - baked potato
  - ground young leaves
  - ground old dried leaves
  - yeast ce ls
  - liver sample (e g from a sheep)
  - ground raw meat
  - cooked meat

  Note that your samples do not have to be identical to those l sted above but for the purposes of this methodology they wi l be used as long as there s a good variety of living and non living material selected

METHOD

1 Label e ght test tubes each with the name of one of the samples
2 Label a n nth test tube as control
3 Fill each test tube approx mately one th rd full with hydrogen peroxide solution
4 Carefully add a small amount of a sample to ts corresponding test tube ensuring you do not cause a splash
5 Observe the test tube for a minute or two and note whether or not bubbles are produced Record your resu ts in Table 1
6 Repeat steps 4 5 for all of your samples w th the control test tube having no sample mater al added

2 I NVEST GATION 3

11 Ident fy whether cells are broken before or a ter the lys s buffer is added Justify your response
12 Cons dering your method what steps could you add in or mod fy to ncrease the y eld of extracted DNA?
13 Ident fy any possible errors that may have affected your resu ts Be sure to state whether they were personal systemat c or random errors
14 There are many different variables that nfluence whether DNA s successfully extracted from fruit or vegetables Select one of these variables and design a method to test the effect th s variable has on DNA extraction Prov de details of the following aspects of your exper ment
   a What is the hypothes s?
   b What are the independent and dependent variables?
   c What is the control group?
   d How w ll errors be m nimised?
   e How w ll you maximise accuracy and prec s on?
   f How w ll you address repl cation?

CONCLUSION

Wr te a concluding paragraph to summarise your nvestigat on Be sure to nclude

- whether the aim was achieved by referring to your results
- limitations in the exper ment
- potent al ways to improve the experiment
- broader mplicat ons of your research or further areas of exploration that stem from your findings

CHAPTER

1

General skills

The key science skills and ethical understandings are a core component of the study of VCE Biology and apply across Units 1 to 4 in all areas of study. In designing teaching and learning programs for each unit and in assessing student learning for each outcome, teachers should ensure that students are given the opportunity to develop, use, and demonstrate these skills in a variety of contexts, including when undertaking their own investigations and when evaluating the research of others. As the complexity of key knowledge increases from Unit 1 to 4, and as opportunities are provided to undertake scientific investigations, students should aim to demonstrate the key science skills at a progressively higher level.

1A KEY SCIENCE SKILLS

If you were at school in Australia in Year 7 or 8, at some stage you would have found yourself in a line of students waiting to receive a vaccination for the human papillomavirus (HPV). How did you feel about this? Happy that you got to miss a bit of class? Or did you have to blink back tears of anxiety?

HPV can cause genital warts and a number of cervical and anal cancers. The HPV vaccine is taken in two doses six months apart and became free for school students in 2007 as part of the National HPV Vaccination Program. Before this program was introduced, four out of every five people contracted HPV at some stage in their life. Now, as a result of the vaccine:

- *cases of genital warts have decreased by 90% in people under 21*
- *90% of cervical cancers and 96% of anal cancers will be prevented*
- *Australia is set to be the first country in the world to eliminate cervical cancer.*

So how did the scientists make this powerful vaccine? Did they mix random concoctions of chemicals together with the hope they might destroy the virus? Did they hold up beakers of coloured water to the light and peer seriously at them like some mad scientist? Or were they following an age-old, systematic process of discovery?

This won't hurt a bit...
Image: Embrace of Beauty/Shutterstock.com

Lesson 1A

In this lesson you will learn the key science skills (KSSs) required to plan, conduct, analyse, and present the results of scientific investigations.

◀ *Prerequisite knowledge* *Future applications* ▶

Years 7–10
You've followed the scientific method, written practical reports, and identified variables before. Now, you need to build on those foundations to broaden and deepen your science skills.

Year 11
In Year 11, you were first introduced to these key science skills and applied this knowledge to concepts such as the plasma membrane, homeostasis, and body systems.

Lesson 1B
None of the skills in this lesson can be used if your investigation is unethical – in lesson 1B, you'll learn how to identify and analyse bioethical issues.

Year 12
The skills taught in this lesson are applicable across the VCE Biology course, and will be assessed in many of your SACs and exams.

Study design dot points

- develop aims and questions, formulate hypotheses, and make predictions
- plan and conduct investigations
- comply with safety and ethical guidelines
- generate, collate, and record data
- analyse and evaluate data and investigation methods
- construct evidence-based arguments and draw conclusions
- analyse, evaluate, and communicate scientific ideas

Key knowledge units

What are key science skills?	0.0.0.10
Designing and planning investigations	0.0.0.11
Conducting investigations	0.0.0.12
Analysing and presenting results	0.0.0.13

What are key science skills? 0.0.0.10

OVERVIEW

Key science skills are the capabilities students demonstrate when designing, conducting, analysing, and presenting scientific investigations.

THEORY DETAILS

What are key science skills and why are they important?

Key science skills (KSSs) are a set of capabilities that VCE Biology students are expected to learn over Units 1–4 (listed on pages 7 and 8 of your study design). You can use KSSs in all realms of life, however, and not just in the Biology exam. This is because, at its most fundamental level, science is about discovering the truth in the world around us – which is clearly something we should all be doing!

key science skills (KSSs) the set of capabilities that VCE Biology students must learn to design, conduct, analyse, and report valid experiments

You demonstrate KSSs when you ask questions like:

- do I believe this? Why?
- what evidence supports this conclusion?
- is this evidence trustworthy?
- is it weak or strong evidence?
- is there evidence that undermines this conclusion, or supports another position?

When your friend gossips to you about their neighbour, or your grandad complains that 'kids these days are spoilt', or you read a tweet from your favourite celebrity – you can ask these questions to decide for yourself what to think.

You will use KSSs more rigorously and methodically in your VCE science subjects. In these classes, you will learn to distinguish between weak (e.g. an **opinion**) and strong (e.g. data from a well-designed **controlled experiment**) evidence. You will also collect, analyse, and draw conclusions by:

opinion the personal belief or viewpoint of an individual which typically has not been verified as fact

controlled experiment an investigation into the effect of an independent variable on a dependent variable, while keeping all other factors constant

- designing your own investigation/s, or
- examining someone else's investigation/s.

Less formal evidence, such as anecdotes and expert opinions, can be helpful to consider when drawing conclusions. However, data gathered from investigations that are guided by KSSs is broadly considered more 'trustworthy' and **reliable**. This is because KSSs help you to reduce **bias**, minimise the effects of **errors**, and ensure results are not due to chance.

reliable describes an experiment, tool, or measurement that produces similar results when repeated and reproduced

bias an inclination to favour a particular position or outcome

error differences between observed values and the true value

The rest of this lesson will walk you through the KSSs you can use to design and examine scientific investigations. Whilst there are many different types of scientific investigations, we'll mostly focus on controlled experiments, which allow scientists to manipulate specific variables and control their studies to a high degree. You can be asked to demonstrate KSSs in SACs and on exams, so we have included KSS questions at the end of every lesson in this book.

Example

ACING VCE BIOLOGY

Let's say you want to use your newly developed KSSs to answer an age old question – how do you ace VCE Biology? How does one even use KSSs to answer this? Some scientific investigations you could undertake to answer this question include:

- surveying top-performing VCE Biology students from the previous year, collecting data on study habits and lifestyle
- analysing the research of other scientists and coming to your own conclusions based on the strengths and weaknesses of their investigations
- setting up an experiment where one group of students tries one study technique, and another does not try it, then comparing the marks they get on a test.

Theory in context

FRAMEWORKS FOR KNOWING WHAT IS 'TRUE'

Using KSSs to arrive at knowledge is often tied to ideas around the 'scientific method', which has characterised how many cultures around the world have approached natural science since the 17th century. However, the stringent adherence to KSSs is only one particular means of determining what is 'true'.

Other ways of seeking the truth can provide a more holistic approach to knowledge. For example, Indigenous Australians have a much longer history of developing knowledge which is often focused on the interconnections between individuals, habitats, and ecosystems. An example of this holistic, or 'big picture' knowledge of Indigenous Australians is fire management. According to Koori Country Firesticks (2017), Aboriginal fire management removes ground vegetation using cool burns that move slowly over small areas, taking place up to several times a year. This:

- reduces the fuel load
- protects the canopy of trees (so fruits and seeds are preserved; insects, birds, and climbing mammals have a place to hide; and shade is maintained after the fire)
- doesn't burn hollow logs (maintaining habitat)
- moves slowly so animals can escape
- manages weeds
- results in quicker return of native plants to the area.

Furthermore, the practice allows easier access to **Country**, cleans up important pathways, maintains cultural responsibility, and is part of ceremonies. In contrast, European 'hazard reduction burns' tend to involve hotter and less frequent fires that have the single goal of reducing fuel load.

Many people are calling for the integration of KSSs and Indigenous knowledge. These people point out that Indigenous ways of knowing share many characteristics with KSSs. Both place importance on observation, questioning, **hypothesis** testing, experimentation, and application. Indigenous ways of knowing have scientific rigour through thousands of years of repetition, but can also change if new evidence arises. These methods of scientific inquiry enabled Australia's first peoples to thrive on this continent for many tens of thousands of years, in good health and in a sustainable way.

Want to learn more about the intersection between Indigenous knowledge and KSSs? Here are some places to start:

Watch – this 10 minute video by the ABC about cool burns youtube.com/watch?v=RM72NtXxyLs&feature=youtu.be

Listen – to this podcast about Indigenous knowledge and science audioboom.com/posts/5380644-why-western-science-urgently-needs-aboriginal-holistic-knowledge-to-tackle-21st-century-issues

Read – this article about flaws in research into hazard reduction burns theconversation.com/the-burn-legacy-why-the-science-on-hazard-reduction-is-contested-132083

Country an area that is traditionally owned and looked after by an Aboriginal language group or community, or by certain people within that group. The term may indicate more than simply a geographical area – it is also a concept that can encompass the spiritual meaning and feelings of deep connection and attachment associated with that area

hypothesis a testable statement that describes how experimenters expect the dependent variable to change as the independent variable changes

Designing and planning investigations 0.0.0.11

OVERVIEW

Designing a scientific investigation involves: constructing a research question and aim; identifying your independent, dependent, and controlled variables; formulating a hypothesis; selecting a methodology; designing a repeatable, reproducible, and valid method; following ethical and safety guidelines.

THEORY DETAILS

Constructing a research question and aim

Most scientists start investigations by noticing something unusual, or a pattern, in the world around them. They might notice that a particular plant has useful properties, students perform poorly on tests when hungry, or that birds fly around the MCG lights at 11 pm. Then, scientists need to narrow the scope of their inquiry down to one question that they wish to answer. Table 1 outlines the requirements for a **research question**.

research question a testable, achievable, and specific question that an investigation sets out to answer

Table 1 Elements of a research question

Research question must be	Explanation	Bad example	Good example
Testable	You must be able to measure the factors you are interested in.	'How do sea monkeys grow?	'What is the effect of salinity on the life cycle of sea monkeys?'
Achievable	The scientist must have the funding, ethical approval, and resources available to answer the question.	'What happens to test scores if we prevent all school students from eating on the day of a test?'	'What is the average test score for students at this particular school if they have fasted for 0, 4, 8, or 12 hours?'
Specific	Only particular individuals will be sampled at particular times and locations.	'Is bird behaviour affected by light pollution?'	'Is silver gull (*Chroicocephalus novaehollandiae*) nighttime behaviour affected by light pollution in Melbourne from June to September?'

Sometimes you need to do a bit more background research to settle on a final research question. You may even go through a few draft questions as you refine it to become more testable, achievable, and specific. From the research question, it is usually pretty easy to develop an **aim**. The aim is the objective of the investigation and typically starts with the word 'To'. For the research questions above, the aims would be:

- to determine if the salinity of water affects the duration of life cycle stages in developing sea monkeys
- to determine if fasting before tests affects student performance
- to determine if silver gull (*Chroicocephalus novaehollandiae*) nighttime behaviour is different in light-polluted Melbourne compared to non-light-polluted areas.

Note that, where required, we include scientific names for species in research questions and aims.

aim the objective of an investigation or experiment

Image: fishmonger/Shutterstock.com

Figure 1 Indigenous knowledge has made significant contributions to science, including the identification of potential new materials from native plants like spinifex grasses.

Example

HOW POWERFUL IS A POWER NAP?

If you're studying VCE Biology, you're probably quite keen to investigate if there is something you can do to improve your results on assessments. You might have lots of friends who swear by the 'cram' method before exams, where they try to fit as much information in their head in the minutes, hours, and days prior. Your mum, meanwhile, always tells you that 'if you have a problem, sleep on it' and that this will help you understand and solve it. Is there anything to either of these two learning strategies? Can either of them improve your memory and performance on tests?

Considering this, we devised a first version of a research question to investigate:

'Is cramming or napping a better study method?'

We realised pretty quickly that this research question has some problems:

- It's not testable – how do you measure if something is a 'better' study method?
- It's not specific – who is participating? What does cramming look like? How long do participants nap for?

From here, we worked on a second draft of a research question:

'Do Year 11 Biology students at this school remember more if they cram or nap for one hour after a class?'

This question was much more testable, given that a test can be administered to measure how much our research participants actually remember. It was also much more specific, given that the people being studied are identified as Year 11 Biology students from a certain school. We've also made the research question more achievable by making the duration of the experiment one hour and using easily accessible participants (rather than, for example, all VCE students in Victoria).

Using this information, devise an aim for the investigation:

Aim: _____

_____

Suggested answer
Aim: To determine if cramming or napping improves the memory of Year 11 Biology students at this school.

Identify independent, dependent, and controlled variables

Notice that creating a testable, achievable, and specific research question means that investigations tend to end up measuring the effect of one variable on another variable. The variable that is being affected is the **dependent variable (DV)**, while the variable that is being manipulated is the **independent variable (IV)**. We can identify the IVs and DVs in the research questions we looked at previously:

- DV – duration of life cycle stages; IV – water salinity
- DV – test score; IV – time spent fasting prior to test
- DV – seagull nighttime behaviour; IV – light pollution or no light pollution.

A **controlled variable** (also known as a constant variable) is a factor that remains the same throughout the experiment in an effort to reduce the chance of this factor influencing the DV. To identify variables you need to control in your investigation, consider other factors that might cause your DV to change. For example, when testing the effect of activity level (IV) on occurrence of heart disease (DV), you would want to make sure that each participant was of a similar age. If this factor wasn't constant, it would be an **uncontrolled variable** that could potentially affect the results, making the experiment inaccurate and invalid (Figure 2).

dependent variable (DV) the factor/s measured in the experiment that are changed when the IV is manipulated

independent variable (IV) the factor/s that is/are manipulated in an experiment

controlled variable a factor that is kept constant throughout the experiment. Also known as a **constant variable**

uncontrolled variable a factor that is not kept constant or accounted for throughout the experiment. Also known as an **extraneous variable**

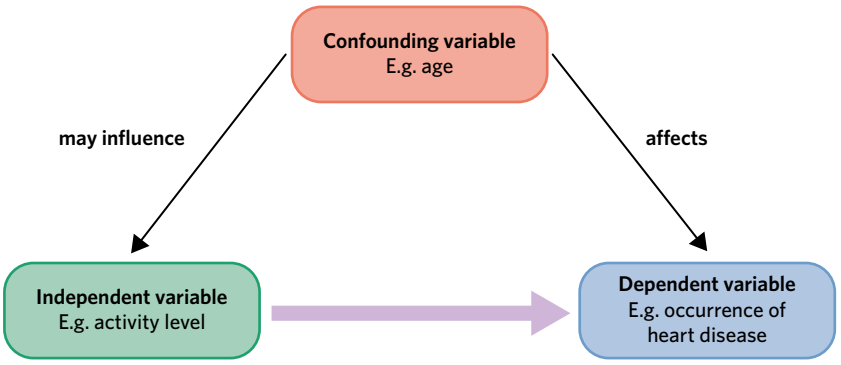


Figure 2 In this experiment, scientists are interested in determining if activity levels directly impact a person's likelihood of developing heart disease. Age is another variable in this experiment since it could influence a person's activity level and a person's likelihood of developing heart disease (older people are more likely to develop heart disease). If it is not controlled for (by only including people of a similar age in the experiment) it will serve as an uncontrolled variable, making it difficult to determine if exercise alone has an impact on heart disease.

Memory device

Remember the IV-DV-TV! Old TVs had antennae on top of them. When you moved the antennae, it affected what you saw on the screen. In this way, the antenna is the thing you manipulate (the IV) and the image is the thing you watch/measure (the DV).

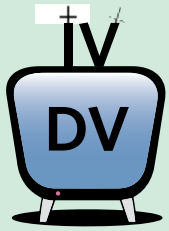


Figure 3 The IV-DV-TV

Example

HOW POWERFUL IS A POWER NAP?

Given the research question 'Do Year 11 Biology students at this school remember more if they cram or nap for one hour after a class?', use the template 'If [change in IV], then [change in DV]' to generate a hypothesis.

Note that sometimes you are also required to include an explanation that explains why you've made the prediction. In this case, your hypothesis template could be 'If [change in IV], then [change in DV] because [existing evidence]'.

_____

_____

Suggested answer
There are two IVs – napping and cramming – so we could write two hypotheses: 1) 'if students nap after learning, then they will get increased test scores'; or 2) 'if students cram after learning, then they will get increased test scores.' Or, we could write a single hypothesis that includes both IVs: 'if students nap or cram after class, then they will get increased test scores.' You could also hypothesise that the interventions do not increase test scores.

Formulate a hypothesis

From your aim, question, and variables, you can then build a hypothesis. A hypothesis is more than 'what you expect to happen' during your experiment. It should:

- be a testable statement
- describe how you think your IV will affect your DV, including the direction of change (increase/decrease etc).

Your hypothesis will either be supported or refuted by your results. A simple hypothesis format is 'If [change in IV], then [change in DV].'

Select a scientific investigation methodology

Now that you've got your research question, it's time to figure out how to actually get to the answer by conducting a scientific investigation!

Scientific investigations can be undertaken in a variety of ways depending on your research question and aim. We call these broad frameworks for inquiry the scientific investigation **methodologies**, and they help guide how you will design your specific **methods** (the actual steps in your experiment). For instance, if you want to learn what species of bacteria live on human skin, it might make sense to use a classification and identification methodology. But, if you want to understand cause and effect, you'd want to perform a controlled experiment (where you test the effect of an IV on a DV, while controlling all other variables). Controlled experiments are often difficult to set up properly, however they can provide very reliable results, and most of the KSSs you will learn in this lesson relate directly to controlled experiments. The methodologies you can use to answer research questions are outlined in Table 2.

methodology the strategy or overarching framework followed in a scientific investigation

method the steps followed in a scientific investigation

Table 2 The nine scientific methodologies prescribed by the VCAA

Methodology	Description	Example
Case study	An investigation of an event or problem that involves a real or hypothetical situation. Case studies can take many forms including historical analysis, role-play of an imagined situation, or designing a solution to a real-world problem.	Researching a bioethical dilemma such as the de-extinction of woolly mammoths, then preparing a debate or essay presenting your analysis and conclusions
Classification and identification	Classification is the arrangement of individuals or objects into logical, manageable sets. We use identification to recognise where new individuals or objects belong in these sets.	Creating a classification tree or phylogeny showing how Australian marsupials are related
Controlled experiment	An investigation into the impact of an IV on a DV, controlling for all other variables.	Testing if introducing a new gene into tomatoes protects the plants from pests
Correlational study	Observing and recording events that have not been manipulated or controlled to understand associations that exist between variables. Typically still measures the effect of an IV (or multiple IVs) on a DV, but the IV is not manipulated by the experimenter and some conditions may be less controlled than in a laboratory experiment.	Recording how environmental conditions such as day length and temperature affect timing of leaf fall in different deciduous plant species
Fieldwork	A correlational study or controlled experiment set up outside a controlled environment (e.g. the classroom), usually in a selected ecosystem. Typically still measures the effect of an IV on a DV, however, conditions may be less controlled than in a laboratory experiment.	Measuring the distribution of sea snails across the intertidal region
Literature review	The collation and analysis of other people's scientific findings or viewpoints concerning a particular topic. Consideration of the reliability of sources and methods is important in literature reviews. They are used to provide background information on a topic of interest and/or identify potential areas of research.	A report summarising past research about Indigenous Australian agriculture and aquaculture
Modelling	The construction of a model or representation that approximates an object or event. This could be a drawing, a 3D structure, an equation, a moving structure, etc., and can be used to describe systems or make predictions.	A flow chart showing the biochemical reactions that take place during photosynthesis
Product, process, or system development	Design of an object, process, or system to meet a human need.	Designing a pot that delivers different water levels to indoor plants depending on the plants' needs
Simulation	The process of using a model to observe and predict what may happen in a real or theoretical system.	Using masking tape to make a large-scale map of the body's osmoregulatory system in your classroom, then have students act out what happens to different hormone levels in different conditions

Example

HOW POWERFUL IS A POWER NAP?

There are a couple of methodologies we could use to figure out if cramming or napping improves memory:

- a case study of exemplary students, where we survey and record what those students did over Years 11 and 12
- a literature review of studies that investigate what helps students perform well in high school.

However, these investigations may provide weaker evidence than a controlled experiment:

- case studies only look at a very small group of people, and the information we get from it might not be accurate (former students might, for example, overreport the amount of napping they did)
- a literature review may not include studies specific to the region or subject we're interested in, so the results may not be relevant.

Given that we are interested in a cause-effect relationship and have identified a DV, an IV, and several variables to keep constant, we can design a controlled experiment that gives us reliable and meaningful results.

Design a repeatable, reproducible, and valid investigation

For controlled experiments, there are some broad rules around what needs to be included in your experimental design. These rules also help ensure that your experiment is:

- **repeatable** – you can repeat your experiment and get the same results over and over again
- **reproducible** – other scientists could follow your method and get the same results over and over again
- **valid** – your experiment actually measures what it claims to be measuring.

If your experiment is not repeatable, reproducible, or valid, then the results are typically not going to be useful, reliable, or meaningful. To ensure you can trust your results, you need to design a strong method. Here are some tips for ensuring your methods are repeatable, reproducible, and valid:

repeatable an experiment/measurement in which scientists, using the methods they designed, can obtain the same result multiple times

reproducible an experiment/measurement in which a group of scientists, using methods designed by others, can obtain the same results as another group's experiment

valid a measurement or experiment that actually tests what it claims to be testing

Identify your experimental group/s and control group/s

The **experimental group** has individuals exposed to your IV treatment or intervention. There may be different levels of your experimental group. For instance, if you are testing the effect of a new pesticide on crop yield, your experimental groups could be three groups of crops exposed to either low, medium, or high levels of pesticide.

experimental group a group of individuals/samples in which the independent variable is manipulated. Also known as the **treatment group**

Control groups are used as a comparison with experimental groups and every controlled experiment should include at least one control group. Control groups can be samples that are not exposed to any level of the IV, which means we do not expect it to produce any results. These are known as negative controls. Alternatively, controls can be groups where you would expect to see a result. Scientists apply a treatment to this group which induces a well-understood effect on the DV which can be compared against the effects of other IVs. These are known as positive controls.

control group a group of individuals/samples that are not exposed to the independent variable. Also known as an **experimental control**, **control treatment**, or **the control**

Negative controls are the most common and should be present in all controlled experiments. If they do produce results, we know that something other than the IV (an uncontrolled variable) may be causing the change in the DV and our method is flawed. In our pesticide and crop yield experiment, a negative control group would be a field not exposed to the pesticide at all, while a positive control group would be a field exposed to an already-existing pesticide that is known to be effective at protecting crops from pests.

Examiners' tip

Be careful not to mix up control groups and control variables. Controlled variables are factors that must be kept constant during your experiment whereas a control group is a sample that is not exposed to the independent variable.

Theory in context

MIND OVER MATTER

Placebo groups are often used as a type of control group, especially when testing medicines. Placebos are medicines/procedures that seem identical to the treatment medicine/procedure, but have no active ingredients and do not result in therapeutic benefit. This means that the participants do not know if they are part of the treatment group or the placebo group. So, if a treatment involves giving participants a pill, the placebo group would be given a pill that looks like the drug, but has no active ingredients (e.g. a sugar pill). A standard negative control, meanwhile, would just be a group of participants who receive no pill (so they know they aren't receiving treatment).

In such studies, we often note an improvement in patients treated with the placebo. This improvement is known as the 'placebo effect' and is due to the psychological beliefs of the person (i.e. if you believe you are going to get better, you will probably get better). Scientists have even figured out how to boost the placebo effect to make a medicine more effective. For example, they've learned that antidepressant pills that are yellow are more effective than the same pill of a different colour.

placebo a substance that has no active ingredients or side effects

Table 3 Examples of research questions alongside potential experimental and control groups

Research question	Experimental group	Control groups
Does the drug we have developed kill bacteria?	Bacteria in a Petri dish exposed to the drug	Bacteria in a Petri dish not exposed to the drug
Are humans injected with our newly developed vaccine protected from the influenza virus?	Humans injected with the vaccine	Humans injected with a vaccine that is already widely used to provide immunity against influenza
Does gene X make banana plants produce more fruit?	Banana plants with gene X	Banana plants without gene X

Example

HOW POWERFUL IS A POWER NAP?

What would the experimental group/s and control group/s be for our experiment on cramming and napping?

Well, we obviously need to get one group to nap after learning, and one group to cram after learning:

- Experimental group #1 – cramming
- Experimental group #2 – napping

We also need a control group, where the treatments of cramming and napping are not applied, but everything else remains constant. Perhaps the best control group would involve participants 'taking a break' at the same time as the experimental groups experience the cramming or napping intervention. It would be important that the control participants are awake and do not revise during this time.

- Control group – awake break

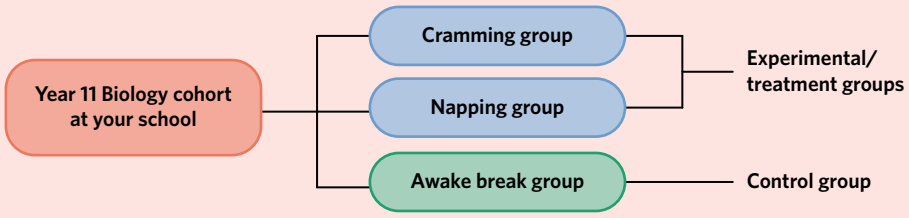


Figure 4 Diagram showing how the Year 11 Biology cohort is divided into experimental and control groups

Is the 'awake break' group an example of a negative or positive control group? What will the results from this group tell us?

_____

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Suggested answer
This is a negative control group, unexposed to any treatment so that we can compare the results of this group to the experimental groups. Having a negative control group will tell us if applying the intervention alters student memory of the lesson.

When you are thinking about your experimental and control groups, you also need to think practically about how each will be treated. This means asking questions like:

- what tools will I use to take measurements of each group?
- how often will I take measurements of each group?
- how long will the experiment run for?

Example

HOW POWERFUL IS A POWER NAP?

Let's make sure we know the details of how we're going to treat each of the groups over the course of the experiment.

- What tools will I use to take measurements?

We need to test if students have remembered what they learned in the class prior to the intervention (or awake break). This can be measured using a 30-minute test on the material covered.

- How often will I take measurements?

Given that memory can be both short-term and long-term, it would be prudent to test students immediately after the intervention, but also one week later. Therefore, we'll give them two tests – Test 1, immediately post-intervention, and Test 2, one week later. The tests will cover the same content but have different questions. As we need enough questions for 2 × 30-minute tests, the pre-intervention lesson should be quite long – perhaps 2 hours.

- How long will the experiment run for?

In total, the experiment will run for one week. Learning and Test 1 will take place on day 1, and Test 2 will take place on day 8.

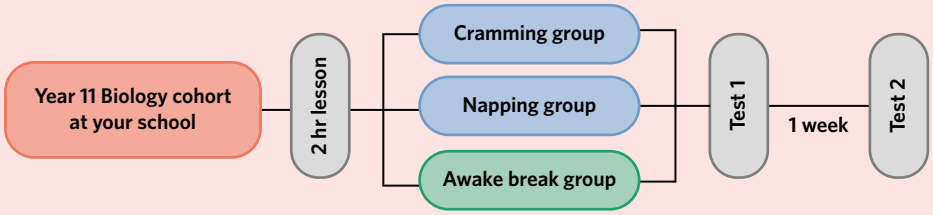


Figure 5 The design of the napping and cramming experiment

Replicate your experimental and control groups

Replication involves having multiple experimental and control groups. Using our crop and pesticide example, instead of having four different fields exposed to either no pesticide, low, medium, or high levels of pesticide, a replicated experiment would ensure there were two or more fields exposed to each treatment.

replication the process of running your test/experiment multiple times

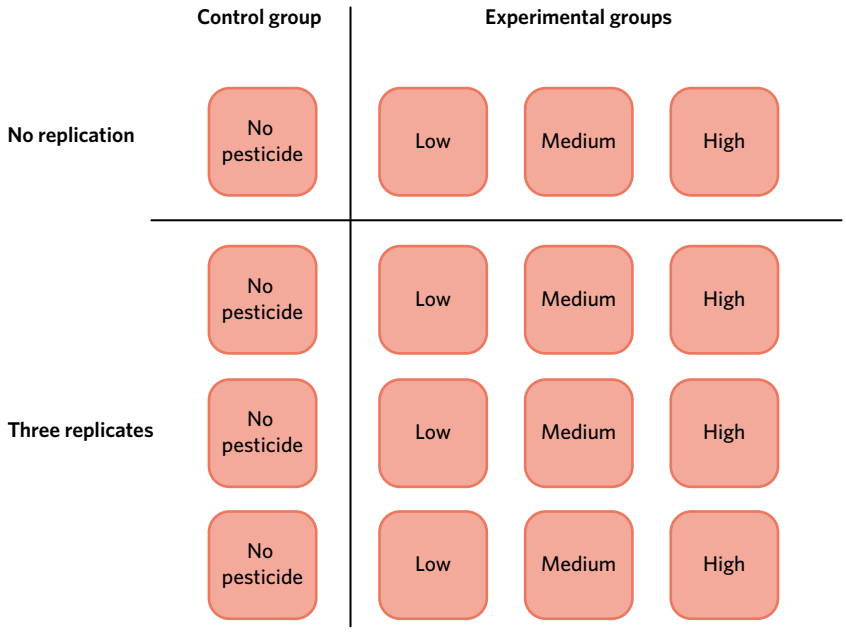


Figure 6 An example of replicated and unreplicated experimental designs testing the effect of pesticides on crop yield

Increasing replication is good scientific practice because:

- You can find out if your results are **precise**
  - Precise results indicate that your method is valid and reliable, and that you may be able to assume the same results would be found in a larger sample
  - If you get a wide spread of values across **replicates**, then results are imprecise
  - If replicates get similar results, your results are precise.
- You can take the average of your results
  - This reduces the impact of **outliers** and **random error**
  - This might make your results more **accurate**, as it may bring your final values closer to the **true value**.

Sometimes there is not enough funding, time, or resources to replicate an experiment many times. Nevertheless, you must design treatment groups with at least two replicates if you want to be able to trust your results. Depending on the field of Biology, it may be standard practice to replicate treatments hundreds or even thousands of times.

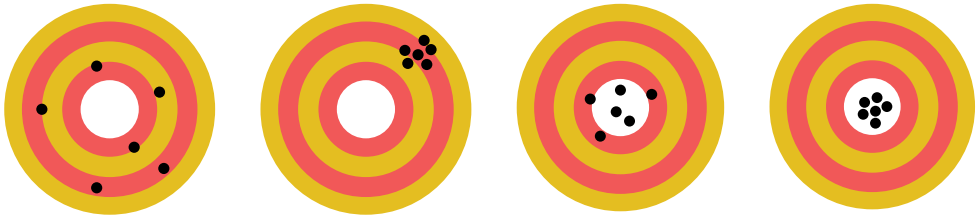


Figure 7 Accurate results are close to the true value, whereas precise results have very little spread around the mean value.

precise two or more measurements that closely align with each other

replicates multiple measurements that are exposed to the same level of the IV, are very close in value, and are close to the 'true' value of the quantity being measured

outlier a reading that varies drastically from other results

random error variation in results caused by uncontrollable conditions between replicates, resulting in a less precise spread of readings. Can be reduced using more replicates or refining the measurement process

accurate how close a measurement is to the true value

true value the value that would be obtained by a perfect measurement without the influence of errors

Examiners' tip

It is important to note, however, that calculating the average of your results after replicating the experiment only brings your final values closer to the true value if the range of your data (maximum value-minimum value) isn't too large. In other words, if your data has a large range and you calculate the average, your final results will actually be further away from the true value.

Example

HOW POWERFUL IS A POWER NAP?

To replicate our sleep study, we need to make sure that there is more than one person in each of the experimental and control groups. Ideally, we also have equal numbers of people in each group. So, if we have 90 Year 11 VCE Biology students participating in the study, there would be 30 students in experimental group #1, 30 students in experimental group #2, and 30 students in the control group. This means that the experiment has 30 replicates.

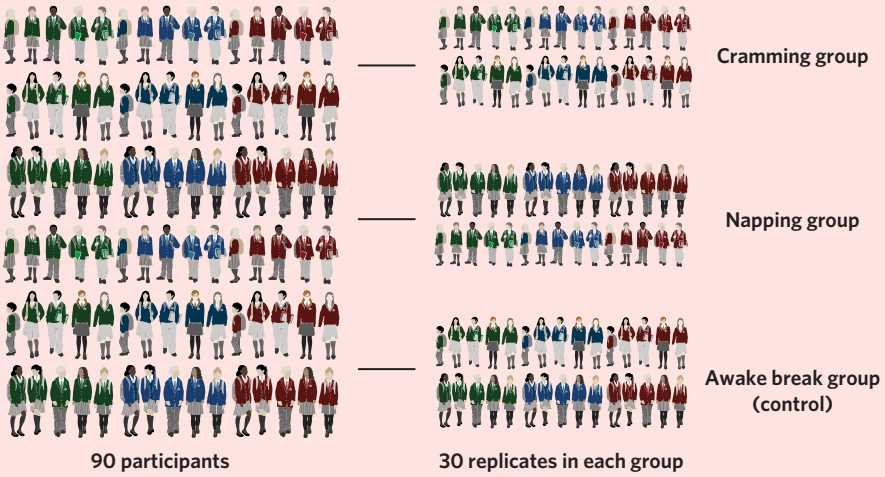


Image: Katrine Glazkova/Shutterstock.com

Figure 8 Diagram showing that there are 30 replicates in each group.

Decide how to sample your groups

It is hard to take measurements of every single individual in a **population**, so scientists tend to collect data on only a small subset of that population called a **sample**. However, because sampling only looks at a subset of a population, scientists need to be careful that their samples are:

- **representative** – accurately reflects the characteristics of the entire population
- **unbiased** – unaffected by prejudice or an inclination towards finding a specific result.

It is a good idea to get as large a sample size as possible, as this will increase the likelihood that you have collected representative and unbiased data. A larger sample size also means that you will have a better understanding of the precision of your data and can take averages to reach a final value that should be more accurate than if you only took a smaller sample. To help ensure samples are representative and unbiased, scientists can use sampling techniques like those outlined in Table 4.

population a set of similar objects or individuals that are studied in a scientific investigation

sample a subset of the larger population being studied

representative a sample that accurately reflects the characteristics of the larger population

unbiased a sample or measurement that is unaffected by a scientist's expectations

Table 4 Different sampling techniques

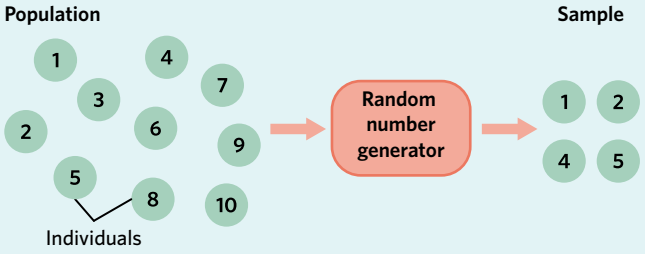
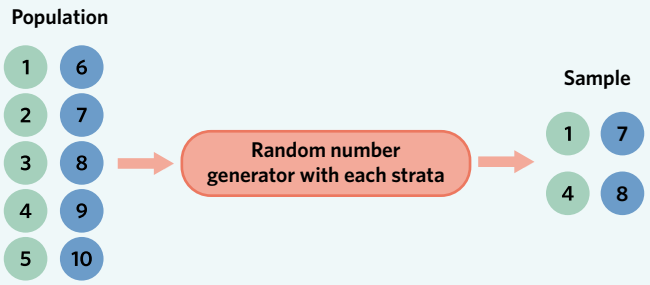
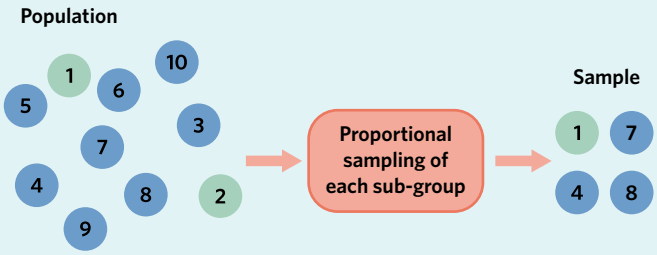
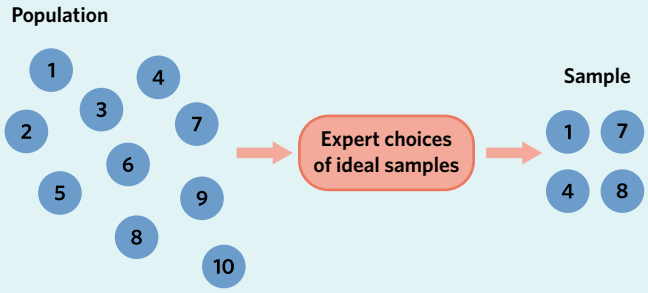
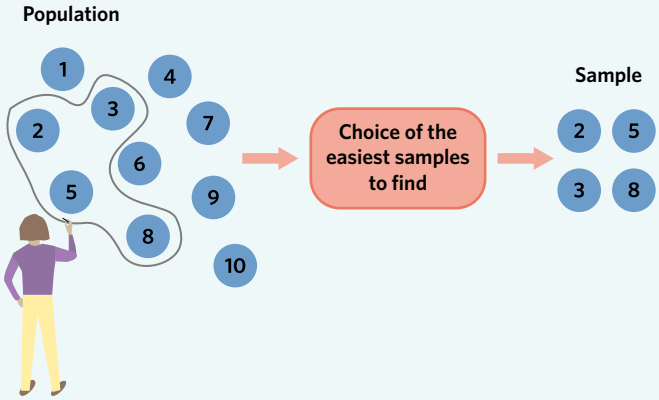
Sampling technique	Definition
Random sampling	Random sampling ensures each member of the population is equally likely to be included. 
Systematic sampling	Systematic sampling involves taking samples at regular intervals along an environmental gradient (such as depth, soil type, rainfall, altitude, or temperature). 
Stratified sampling	When a population has clearly defined zones or characteristics, and you wish to sample proportionately from each zone, you may wish to use stratified sampling.  *cont'd*

Table 4 Continued

Sampling technique	Definition
Judgement sampling	Also known as selective sampling, the researcher chooses which individuals (or asks an expert's advice) to sample according to their needs. Judgement sampling can be biased and lead to unrepresentative data, so should only be used when necessary. 
Convenience sampling	This type of sample is taken from a group of individuals who are easy to reach. Convenience sampling can lead to biased and unrepresentative samples that make results unreliable, so should be avoided where possible. 

Example

HOW POWERFUL IS A POWER NAP?

Sampling technique

In this experiment, the 90 Year 11 VCE Biology students (we can say n = 90 to explain that the sample size is 90) at your school are a sample of all Year 11 VCE Biology students that exist. We chose these students using convenience sampling – they are the students who we know and are easily available to participate.

To strengthen the experiment, we can ensure that the sample of 90 students are randomly allocated into the experimental and control groups. For example, we could assign each student a number from 1–90, then use a random number generator to determine which intervention they receive: the first 30 cram (n = 30), the next 30 nap, and so on. This is important as it minimises the risk of all 'high-achieving' students being accidentally placed into the same group, which would make that treatment appear really successful.

Of course, there is a strong possibility that students at our sample school are not representative of VCE Biology students in general. For example, your school may be more linguistically diverse than the average Victorian school. You'll need to decide how this affects your results in your discussion.

cont'd

Example

HOW POWERFUL IS A POWER NAP? –CONTINUED

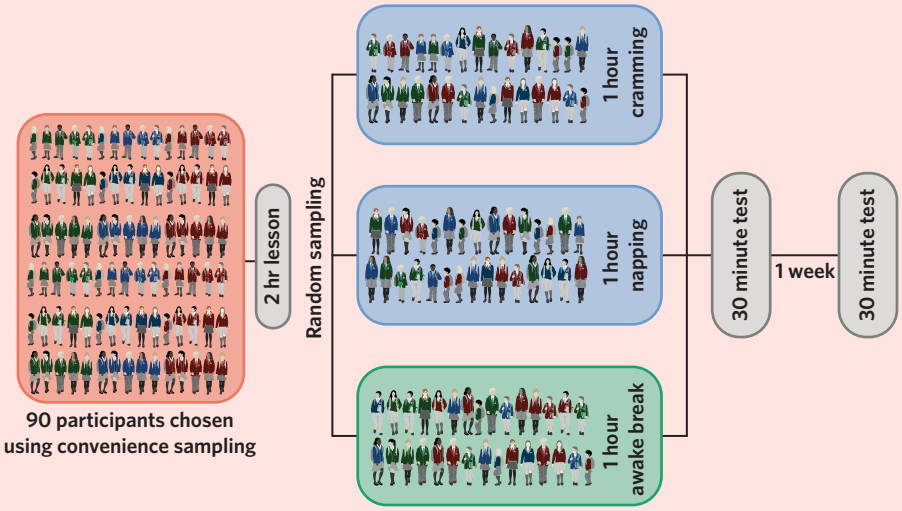


Image: Katrine Glazkova/Shutterstock.com

Figure 9 Experimental design to measure if cramming or napping improves student memory

Can you think of any other reasons why your school may not be representative of VCE Biology students?

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Suggested answer
The postcode a school is in, access to resources, the diversity of its culture, the influence of teachers and parents, and many other factors may mean that the sample school is far from 'average'.

Minimise the potential for error throughout the method

There are three main types of errors that you should plan to avoid during your experiment, outlined in Table 5. When you are designing your method, you should make sure you choose appropriate equipment to use for measurement, calibrate equipment where needed, and build in a sufficient number of replicates to minimise error. It is also important to identify parts of the method where errors may occur (e.g. during delicate or complex processes), then either find ways to reduce the risk of error or practice the process prior to conducting the experiment.

Table 5 The error types you need to know for VCE Biology

Error type	Description	How to avoid
Personal	Mistakes or miscalculations made by the experimenter. Counting incorrectly, rounding to the wrong decimal place, or labelling samples incorrectly are all examples of personal errors.	Repeat the experiment again. For measurements relying on human accuracy (e.g. counting plant numbers), you can get two or three people to make the same measurement.
Systematic	Errors which cause results to differ from the true value by a consistent amount each time, typically due to faulty equipment or calibration. They affect the accuracy of the experiment, and cannot be minimised through replication.	Re-calibrate your instruments, or use more reliable equipment.
Random	Errors which are caused by unpredictable variations in the measurement process and result in a spread of readings. For example, when a quantity is estimated by reading between the lines on a measuring cylinder – is it 5.6 mL or 5.7 mL? Perhaps we'll just say 5.65 mL. Random errors reduce precision.	Replicate the experiment, increase the sample size, refine the measurement process, or use more precise measuring equipment.

Theory in context

QUANTIFYING UNCERTAINTY

Some instruments are more precise than others. For instance, the screen height of an iPhone X could be 14.9 cm (ruler), 14.86 cm (vernier calipers), or 14.859 cm (micrometre screw gauge). Clearly, there is more **uncertainty** associated with the ruler measurement than with the micrometre screw gauge measurement. You may wish to quantify the uncertainty associated with measuring instruments in your methods. Digital devices like scales typically state the uncertainty on a sticker somewhere. For analogue instruments like rulers and measuring cylinders, uncertainty is a bit trickier.

If you have to set up the instrument before measuring (e.g. with a ruler, you need to put it in place before measuring), then the uncertainty is the smallest measurement. On the ruler shown in Figure 10, the uncertainty is ± 1 mm. However, when you don't need to set the instrument up before measuring (e.g. a measuring cylinder, or a thermometer), then the uncertainty is half of the smallest measurement. In the measuring cylinder shown in Figure 11 the smallest measurement is 1 mL, so the uncertainty is ± 0.5 mL. Note that the uncertainty assigned to standard digital stopwatches is ± 0.1 of a second due to human reaction time.

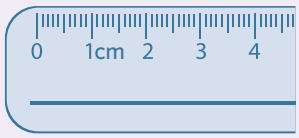


Figure 10 A section of a ruler that has an uncertainty of ± 1 mm

Image: oFFsoRRy/Shutterstock.com

Figure 11 A measuring cylinder that has an uncertainty of ± 0.5 mL

personal error mistakes or miscalculations due to human fault. Can be eliminated by performing the experiment again correctly

systematic error errors which cause results to differ by a consistent amount each time, typically due to faulty equipment or calibration, resulting in a less accurate result. Can be reduced by calibrating and maintaining instruments

uncertainty a quantification of the error associated with a measurement, often represented by the symbol '±' after a reading

Write your method out clearly

Once you know your treatment groups, replication number, sampling method, and have identified any methodological stages which may introduce error, you should write the steps of your experiment out clearly. Remember that anyone should be able to follow your method exactly – other scientists won't be able to reproduce your results if they can't follow your method.

Follow ethical and safety guidelines

Before starting your experiment, you need to ensure that your method is **ethical**. Ethical conduct is valued so highly in modern day science that, at universities and research facilities, experimental procedures must be presented to an ethics board before being permitted to proceed.

To check if your experiment is ethically sound before starting, you should ask yourself the following questions:

- Is my method designed to avoid harming living things or ecosystems as much as possible?
- Has this research considered the beliefs, perceptions, customs, and cultural heritage of those involved in, or affected by, the experiment?
- Are all participants aware of the risks associated with this research and have they provided their consent?
- If I make a great discovery, will there be equal access to, and fair distribution of, any benefits that have arisen from this research?

Memory device

You can think of the characteristics of a good controlled experiment as a checklist (RICHES):

- Replication
- Independent variable/ dependent variable
- Control group
- Hypothesis
- Errors are minimised
- Sample is large and randomly collected.

ethics a field of knowledge that helps individuals exercise moral judgment and determine what is right and wrong

- Will I acknowledge all sources of funding and help for this research?
- Will I be transparent about any errors with the data or methods?
- Is the identity of participants protected?

If you answered 'No' to any of these questions, your experiment may not be ethical and you may need to revise your method. You will learn more about ethical decision making in lesson 1B.

Example

HOW POWERFUL IS A POWER NAP?

There may be some ethical issues with our experiment on napping and cramming. For example:

- Have participants provided fully informed consent to be a part of the experiment?
- As the test is being undertaken on minors, do we need to get consent from their parents as well?
- Are there language, gender, or cultural differences that may influence student experience of the experiment?

Can you think of any other ethical questions to consider for this investigation? List them below.

_____

_____

It's important to have reasonable answers to these questions, and others, before starting the experiment.

Suggested answer

- If we use a test to measure student memory, will the score on their test affect their school results?
- Is there a safe place for students to nap?
- Will we share the results with the participants?
- Will participating in the experiment cause undue stress or anxiety for students?
- Do any of the students have a pre-existing sleep condition which means that they should avoid napping during the day?
- Can students leave the study if they wish?

Comply with safety guidelines

It is likely that, during Year 11 and 12, your teacher will ask you to take ownership of your own safety during an experiment by doing a risk assessment. This involves writing down all potential risks in an experiment, keeping in mind any contextual factors that may affect the safety of the experiment, and identifying ways to minimise these risks (Table 6).

Table 6 Examples of potential risks, contextual factors, and risk minimisation strategies during scientific investigations

Aspect of risk assessment	Examples
Possible risks	• Sharp objects • Flammable material • Hazardous chemicals • Open flames • Culturing of microorganisms
Contextual factors	• The experience of staff and students with the procedure • The behaviour of the class • Allergies of students and staff • Facilities available
Strategies to minimise risk	• Wearing personal protective equipment like gloves, lab coats, and enclosed footwear • Using fume hoods and other safety equipment where needed • Tying back long hair • Following instructions from the teacher and lab technicians • Washing hands after lab work • Keeping lab benches and equipment **sterile** • Conducting experiments in isolation • Conducting experiments in negative pressure rooms

sterile surgically clean and free from contamination by microorganisms. Also known as **aseptic**

You can undertake a risk assessment online (e.g. riskassess.com.au) or use a printed template provided by your school. The online risk assessments are helpful because they typically outline standard handling procedures for all equipment and safety data sheets for chemicals.

Example

HOW POWERFUL IS A POWER NAP?

Identify one possible risk that would need to be in this experiment's risk assessment.

_____

_____

Suggested answer
Acceptable responses include: stress from having to cram, stress from having to sit tests, stress from using up valuable VCE study time, and stress from potential disruptions to the sleep cycle.

Conducting investigations 0.0.0.12

OVERVIEW

During your investigation, you should focus on collecting unbiased, accurate, and precise raw data. In addition, you need to work cooperatively with classmates, teachers, and lab technicians to achieve the most reliable results.

THEORY DETAILS

Specific, answerable research question? Check. Appropriate research methodology? Check. Now it's time to start getting some data to actually answer your question!

Generate and collate data

If you're collecting your own data, we say you're collecting **primary data**. As you write results down in your logbook, the data is considered **raw**. Once you start graphing it or presenting it in tables, we describe that data as **transformed**. If you're getting results from someone else (e.g. a previous class, online data banks, or scientific papers) we say you're collecting **secondary data**.

primary data results collected from experiments, interviews, or surveys undertaken by the researcher

raw data results that have not been processed, manipulated, or formatted for use

transformed data results that have been converted from their raw format into a more visually comprehensible format that is easier to analyse

secondary data results from sources other than the researcher's own investigations

When collecting primary data, it's important that you note down any observations from the experiment. In particular, write down any potential errors that may have occurred while conducting the experiment. Some examples of observations to collect during the experiment include:

- potential moments of contamination
- any personal errors, including spills or breakages
- general observations such as scents or colour changes
- any inconsistent treatment of experiment and control groups
- potential uncontrolled variables that may be affecting results.

Example

HOW POWERFUL IS A POWER NAP?

Here is an example of the raw data we collected for our cramming and napping memory experiment. Notice that we have:

- tried to keep it neat and organised by using a computer rather than handwriting
- used clear headings so that anyone can interpret the data
- included a column for observations so that we can track any potential uncontrolled factors.

Student #	Group	Test 1 score	Test 2 score	Observations
1	Cram	90	88	
2	Nap	80	82	Loud noise may have disturbed nap
3	Break	42	33	
4	Break	54	52	May have dozed off during break
5	Break	70	53	
6	Cram	82	84	Only read notes, did not write anything down or highlight

Figure 12 An example of what raw data might look like

Lesson link

By writing down any potential errors that occur during the experiment and then discussing them in your report, you are communicating your results with integrity – a key ethical concept discussed in ***lesson 1B***. In doing so, you are basically saying 'my results say X, which is significant because Y, but make sure you're aware that Z happened during the experiment and that could make the results a bit dodgy'. This gives readers full autonomy to draw their own conclusions with all the required knowledge at their fingertips.

Analysing and presenting results 0.0.0.13

OVERVIEW

Once you've finished conducting your experiment, you need to interpret and present your results. This typically involves transforming your data into a graph or table, determining any potential sources of error, drawing conclusions from your data, and then communicating your findings to a specific audience.

THEORY DETAILS

After your investigation, you need to start thinking about what your results mean and how to communicate them. There are four steps to follow in order to do this (Figure 13), which we'll go through in more detail below.

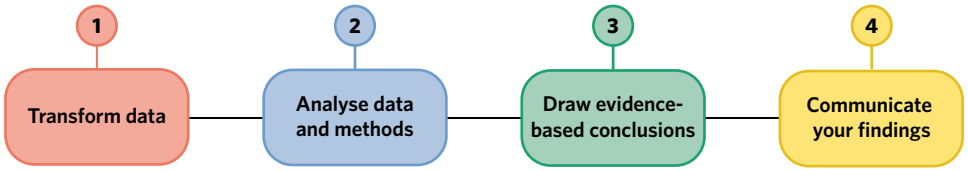


Figure 13 Steps to presenting and communicating results

1. Transform your data

A crucial part of being a scientist is communicating your results clearly and honestly. In practical reports and posters, raw data is not usually presented because it can be hard to read, repetitive, irrelevant, or messy (and, frankly, sometimes a bit boring!). Instead, data is manipulated so that the main result, pattern, or trend is obvious. Tables are not always the best way to show trends, so results sections will typically include graphs and charts.

The type of graph you choose depends on the type of data that you have collected. Table 7 outlines the different types of data you may collect, and how you can represent that type of data.

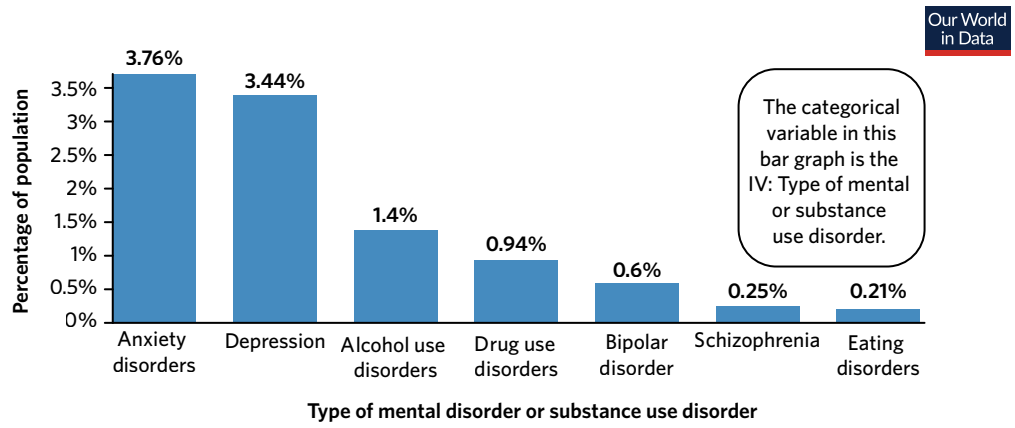
Table 7 Types of data you may collect about variables and how they are best graphed

Type of variable		Explanation	Typically graphed using
Numerical	Continuous	Data that can take any value between a set of real numbers. In other words, continuous data can include decimals and fractions e.g. height (178.87 cm), age (16 years 2 months 4 days...), mass (65.87 kg)	Line graph or scatter plot
	Discrete	Data that can be counted and takes a particular value. Discrete data cannot take a fraction of that value e.g. count of individuals (1, 2, 3)	Bar graph
Categorical	Ordinal	Data that can be logically ordered e.g. size (small, medium, large), fin health score (1 = no fin damage, 2 = some fin damage, 3 = most of fin surface damaged), attitudes (agree, neutral, disagree)	Bar graph or pie chart
	Nominal	Data that cannot be organised in a logical sequence, e.g. gender (male, female, nonbinary, other), nationality (Australian, Chinese, South African, Egyptian), hair colour (brown, black, blonde, red)	

numerical variable a factor that is measured as a number such as height, count of population, and age

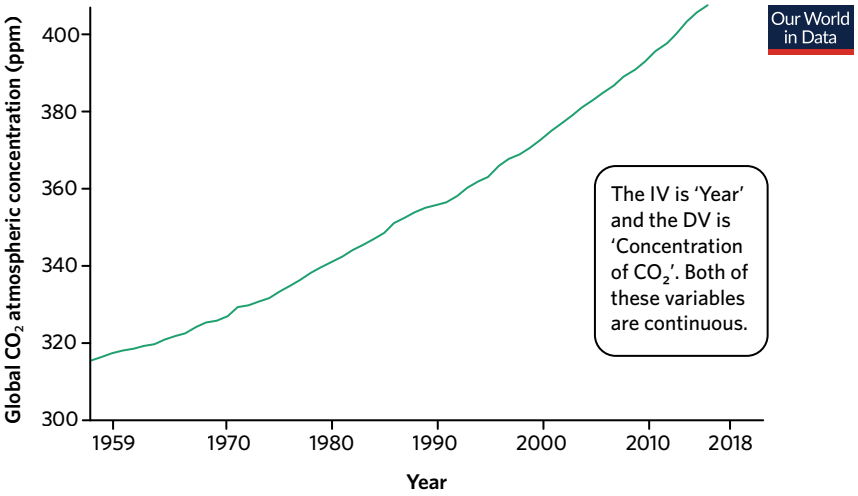
categorical variable a factor that is qualitative, typically describing a characteristic such as gender, birth order (1st, 2nd, 3rd), or nationality

Bar graphs (Figure 14) are typically used to display categorical and discrete data, whereas line graphs (Figure 15) and scatter plots (Figure 16) display continuous data. Scatter plots are particularly useful if you wish to visualise the relationship between two continuous variables (e.g. amount of rainfall and number of species in an ecosystem). If one variable is categorical but the other is continuous numerical, bar graphs usually work well. Note that, typically, the IV is presented on the x-axis and the DV is presented on the y-axis.



Source: Global Burden of Disease Collaborative Network (2017) adapted by Ritchie and Roser (2019)

Figure 14 Bar graph showing the prevalence of mental disorders and substance use disorders in 2017



Source: Keeling (1974) and the National Oceanic and Atmospheric Administration (2018) adapted by Ritchie and Roser (2019)

Figure 15 Line graph showing the change in global carbon dioxide atmospheric concentration over the past 60 years

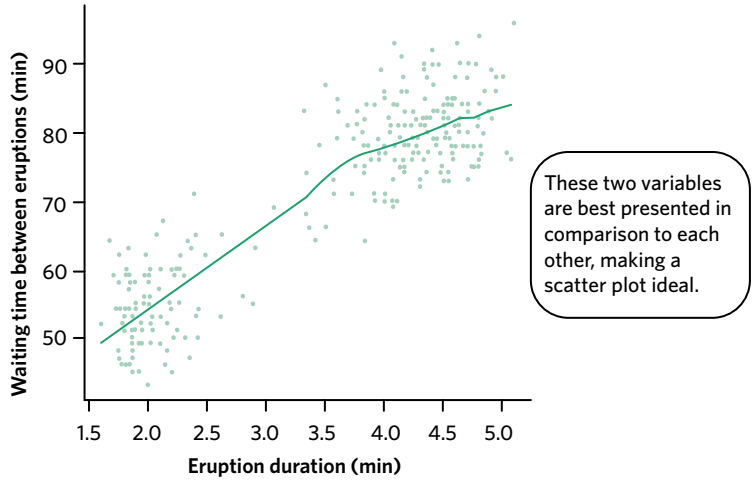


Figure 16 Scatter plot showing that the longer the wait time between eruptions of the geyser Old Faithful, the longer the duration of the next eruption.

During experiments, you may record continuous data enabling you to create a scatter plot. For example, you may record the oxygen concentration in a sealed jar with a plant inside every five minutes. Because both variables are continuous you can make the scatter plot a line graph by drawing a **trendline** also known as a line of best fit. The trendline will help readers determine if the relationship between the two variables is positive, negative, or non-existent. A line of best fit may pass through all the points, some of the points, or none of the points (Figure 17). A good rule of thumb when drawing a line of best fit is to ensure the number of points above and below the line are equal.

trendline a line that shows the main pattern followed by a set of points on a graph. Also known as a **line of best fit**

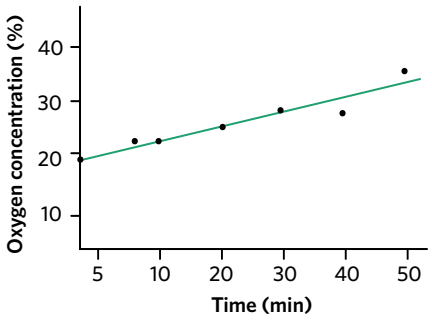


Figure 17 The line of best fit showing the general trend between two variables on a scatter plot

Once you've drawn up your graph on paper or on the computer, you need to format it to maximise clarity and to ensure it fits scientific conventions. Some guidelines for formatting are:

- ensure the graphics are clear and easy to read
- the scale should be appropriate for the data, and labelled clearly
- ensure the graphs do not have coloured backgrounds or grid lines, unless required to present results clearly
- axis labels should be formatted in sentence case (Not in Title Case and NOT ALL CAPS). Only the first letter of the first word should be capitalised, as well as any proper nouns
- any calculations should be presented in a clear, non-repetitive manner (e.g. by using one sample calculation)
- each graph should have a figure number and caption underneath
- each table should have a table number and title above
- tables should have units written in the column or row headings only, and not in the cells within the table
- the results section also includes text. The text should summarise the key findings for each graph in 1–2 sentences, including if the result supports the hypothesis.

Example

HOW POWERFUL IS A POWER NAP?

In our experiment, the DV was memory, as measured by a score on a test. The IV was study technique – cramming or napping. Classify the DV and IV as categorical or numerical, and continuous, discrete, ordinal, or nominal.

Suggested answer
Score on test = numerical, discrete (because you either get the mark or don't get the mark)
Study technique = categorical, nominal

Example

HOW POWERFUL IS A POWER NAP?

There are so many ways to present the data on test scores. Here, we'll walk through how you could transform the data you collect on study technique and memory.

If you turn our raw data point (Figure 12) into a graph, you'll see what score each student received in each week (Figure 18).

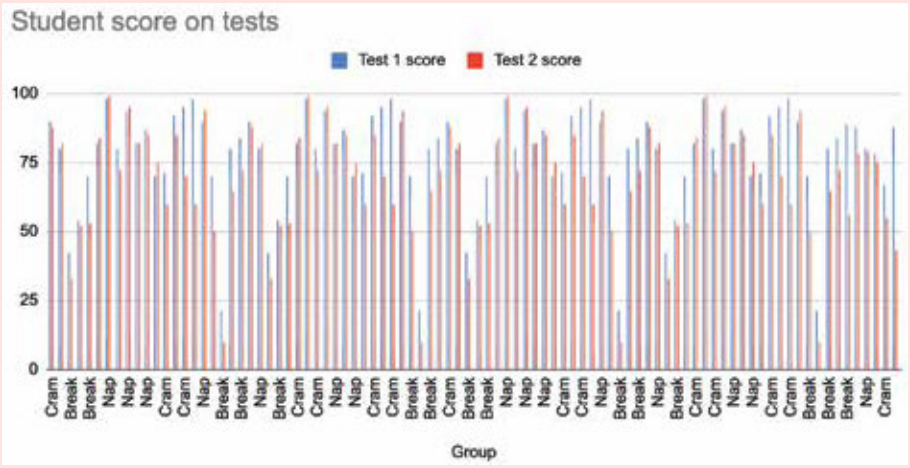


Figure 18 Raw data represented on a bar graph

Figure 18 is easier to read than a table, but is still a little confusing: you can see that the students performed differently, but you can't see if there is a difference between groups that used different study techniques. In essence, the graph is not telling a story yet. Ultimately, we want our graph to clearly answer our research question 'Do Year 11 Biology students at this school remember more if they cram or nap for one hour after a class?'. To do this, we need to figure out the mean score each group achieved on the tests. You can calculate this by summing up all the test scores within a group, then dividing that number by 30 (the number of participants).

Group	Test 1 mean score	Test 2 mean score
Break	62.03	49.13
Nap	85.40	86.73
Cram	86.23	72.47

Figure 19 The mean test scores for the students who breaked, napped, and crammed after learning.

We can then use the means to create a graph that shows our results clearly. Look at the graphs in Figure 20. Which do you think is most appropriate for representing our data, (a) or (b)? Why?

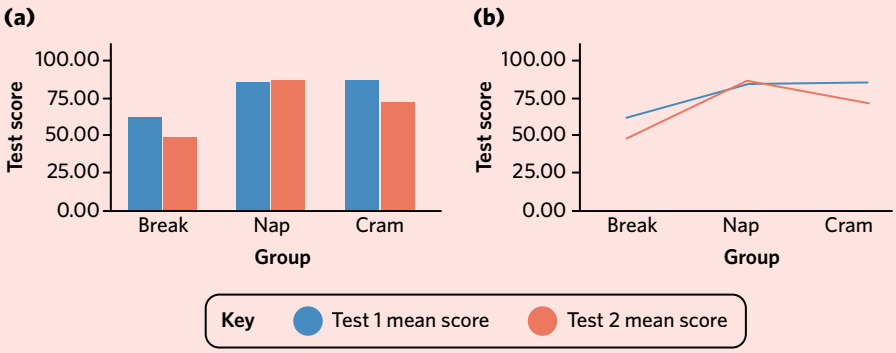


Figure 20 **(a)** A bar graph showing the average score on tests 1 and 2 using different study techniques and **(b)** a line graph showing the average score on tests 1 and 2 using different study techniques

Suggested answer

Graph A represents the data best because neither the IV nor the DV is continuous. In particular, using a line graph for (b) implies that there are values between 'Break', 'Nap', and 'Cram' (e.g. half break-half nap), which is not the nature of these nominal variables.

2. Analyse your data and method

Once you visualise your results clearly, you can:

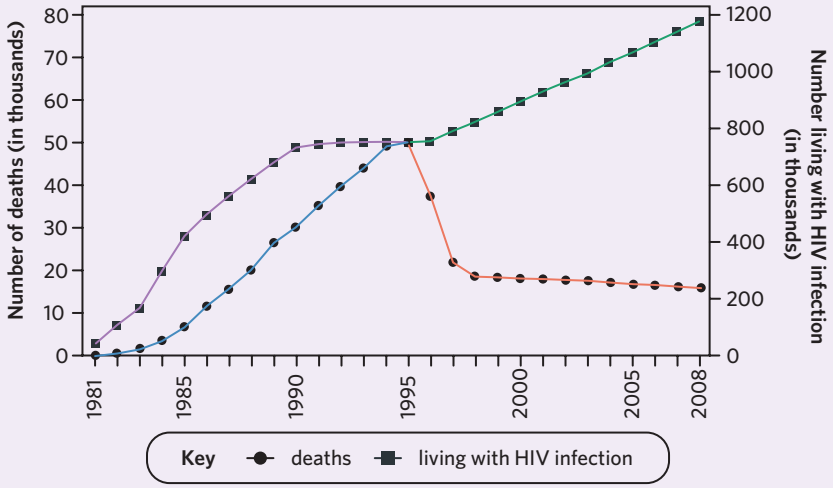
- determine if your hypothesis is supported or rejected
- reflect on your data and method to decide if your experiment is valid and reliable.

It is usually pretty easy to tell if the data support your initial hypothesis – you simply check if the data follow the pattern you expect, or if it does not. Often, you may find that the hypothesis is partially supported by your results. This is always really interesting, as your next step is to think of reasons why the pattern was not consistent. It may be due to an error in your method, or due to an unknown uncontrolled variable.

Theory in context

During exams, you may be asked to describe data before you explain it. A good plan of attack to describe the data is to divide the graph into different sections.

For example, in this graph from the 2018 exam, it would be difficult to describe everything that is happening all at once. But we have superimposed different colours over particular sections of the data which makes it easier to interpret the line graph in sections.



Source: adapted from NIDA (2020)

Figure 21 An exam question that requires students to interpret a line graph

Here is an example of how you could describe this data:

The number of deaths from HIV rose steadily from 1981, reaching a peak in 1995 at 50 000. This was followed by a sharp decline in deaths from 1995–1997, until plateauing around 20 000 for the next ten years. Meanwhile, the number of people living with HIV rose from close to zero to 800 000 between 1981 and 1990. This number stayed at approximately 800 000 for five years before increasing linearly to 1 200 000 by 2008.

Note that the description includes numbers from the x and y-axis to contextualise the overall pattern.

Once you know what your results mean for your hypothesis, you can then dig deeper into the data and evaluate your method. Some questions you may wish to consider include:

- Method
  - Did anything happen during the experiment that might mean you can't trust a data point, or multiple data points?
  - Identify any personal, systematic, or random errors that may affect the accuracy and precision of your results.
- Data
  - Precision – are the results within replicate treatments similar or different? If there is a wide spread of results this could mean your instruments or processes were not valid and did not measure what you wanted to measure.
  - Accuracy – if you know what the true value should be, are the values you recorded similar or different? If they are different, this could mean an uncontrolled variable was affecting your results, your instruments were faulty, or that you were not collecting data carefully enough.
  - Outliers – are there any data points that stand out or do not follow the pattern? If so, did something happen when you collected that sample that could explain the anomaly? There may be a good reason to exclude outliers from your results, but make sure you report in your discussion that you did this and why.

The answers to these questions, and any others that may be relevant, should be brought up in the discussion section of a report, article, or poster.

3. Draw evidence-based conclusions

One of the beautiful and frustrating things about science is that things that are 'true' one day can be disproven the next. Scientists draw the most reasonable conclusions based on the evidence available at the time. If evidence to the contrary arises, what is 'true' can also change. However, we cannot instantly accept this unless we can trust the results of an experiment. To trust results, the experiment must be designed to be reproducible, repeatable, and valid. These characteristics ensure that any conclusions drawn are 'evidence-based', reliable, and meaningful.

Theory in context

SHIFTING PARADIGMS IN BIOLOGY

Biological models and theories change when more evidence is gathered. For example, scientists used to assert that genes could only be passed down from parents. But in the 20th century, biologists discovered that bacteria could transfer genes horizontally between individuals, like swapping clothes. The fields of evolution and phylogenetics are still trying to include, understand, and adapt to this new understanding of genetic transmission.

The strongest evidence is derived from controlled experiments that use random sampling methods and have been reviewed and reproduced by colleagues in the scientific community. Other scientific investigations can provide evidence to draw conclusions from, but it may not be as reliable, as the methods used are typically not as reproducible, repeatable, and valid. Conclusions may also be drawn from **anecdotes** or opinions, though these are not considered reliable sources of evidence as they are subject to no or low replicability and large amounts of bias.

Drawing conclusions from evidence isn't always easy. There are two common mistakes that students make when drawing conclusions: assuming that 1) **correlation** means **causation** and 2) the same pattern will exist beyond the data you measured.

anecdote evidence involving a personal account or report of a previous experience that may provide a certain level of support for a position

correlation when there is a relationship between two variables

causation when change in one variable leads to reliable change in another

Correlation does not mean causation

Not all experiments will reveal a correlation between two variables – in fact you may find that the DV and the IV are unrelated. Furthermore, even if your data indicate that your IV is related to your DV in a consistent and measurable manner (e.g. if you increase the IV, the DV increases), this doesn't necessarily mean that the IV causes the change in DV. In other words, correlation of two variables does not mean that one causes the other.

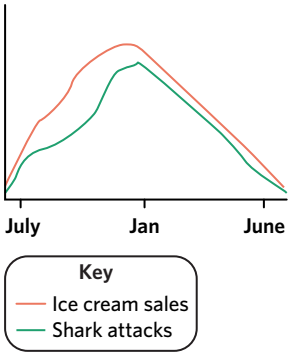


Figure 22 The number of ice cream sales and shark attacks are correlated, but one does not cause the other. It's more likely that an uncontrolled variable – for example, hot weather – explains the relationship (i.e. when it's hot, people are both more likely to eat ice cream and go to the beach, the latter likely increasing the number of shark attacks).

Data may not follow the same trend outside of the range you measure

In Figure 23, scientists measured the height of a seedling for ten days. Although a positive trend exists – indicating that seedlings get taller with time – we cannot assume that the growth will continue after day ten. Therefore, it is not correct to state that 'it will take the seedlings 20 more days to reach 9 cm'. One could, however, say that 'if the rate of growth continues in the same manner, then it will take the seedlings 20 more days to reach 9 cm'.

Similarly, the scientists did not collect data on day zero. So, when drawing a line of best fit, it is important not to force your line through zero. Drawing a trendline that is forced through zero results in a different slope (red dotted line) to the trendline that actually best fits their data (green line).

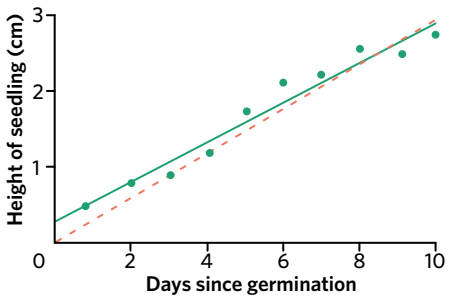


Figure 23 When drawing a trendline, avoid forcing your data through zero (red dotted line) as you end up with a different slope that doesn't accurately represent the data you collected (green).

Example

HOW POWERFUL IS A POWER NAP?

Let's see if we can draw some conclusions from our data on power napping and cramming. Here is our data:

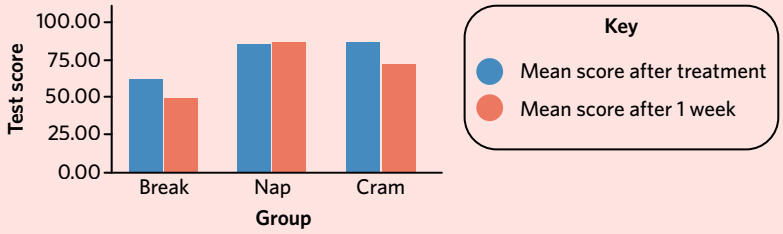


Figure 24 Transformed data showing that napping and cramming after learning improves student performance on tests more than taking a break, and that napping is better than cramming for long term memory formation

From these results, we can tell that both napping and cramming for one hour after a lesson are better study techniques than just taking a break – average students in these groups scored more than 20 points higher than control participants. After one week, students in the cramming group had forgotten more of the lesson than students in the napping group. In fact, the average test score for students in the napping group actually increased slightly. This tells us that for long term retention of knowledge, it is best to take a nap after studying. For short-term retention, you can either nap or study – both will help you achieve better scores than doing nothing.

Ok, so that's what our data tell us. Are there any reasons why this data might not be reliable? How could we address these limitations? Here are a few points that could be worth exploring:

Table 8 Limitations and potential solutions for the experiment 'how powerful is a power nap?'

Limitation	How to address
We used convenience sampling, so we can't assume the same pattern would exist in a different student population.	Collect a larger, random sample of students from lots of different schools. Or you could explain that it doesn't matter that you used convenience sampling, as you wish to determine the best study technique for you, and Biology students at your school are probably more similar to you than Biology students at a different school.
We never dictated in our methods what students should do in the week between tests – if some students studied and others didn't this would impact the results.	Ensure that all students do not study the test preparation material over the next week.
There were a number of variables that we did not control for in this investigation. For instance, did any individuals take medication, drink coffee, or have unusual sleeping routines? Were there any external life events that could have affected individual or group performance? If these variables were similar across groups, they shouldn't affect our results. But if one group is more affected than another, then our results may not be accurate.	Design a more controlled study, where participants are only included if they agree not to consume food or medicines that are stimulants or depressants. Alternatively, we could ask participants to track what they eat and how much they sleep during the study then retrospectively try to see if there are potential issues.
We haven't designed an investigation that helps us understand why napping cements learning.	To be sure that napping is the true underlying cause of improved memory, further investigations into the mechanism behind memory formation need to be undertaken.
There is a possibility that the napping group ended up with lots of high achieving students, and the awake break group was mostly composed of unmotivated students.	Ensure that the groups are composed of academically diverse students by using stratified sampling.

4. Communicate your findings

As we face global challenges like climate change, pandemics, and pollution, it is crucial that all citizens have basic scientific literacy. However, approximately forty per cent of Australians report being uninterested in, and disengaged from, science (Cormick, 2014). One of the major barriers to improving scientific literacy is that scientists often use complex, technical words and high levels of detail which can make science seem boring or difficult.

Professional science communicators emphasise that the best way to communicate your findings depends on your audience. For instance, to communicate your results to your teacher or supervisors, a formal laboratory report written according to standard scientific practice would be most appropriate. However, if you are trying to teach your siblings or parents about something you learned at school, you should avoid jargon and perhaps use drawings and examples to support your communication.

In this book, you'll find more information on specific communication techniques for assessments in Unit 4 Outcome 3 in the section called 'How to conduct a practical investigation '. In addition to these assessments, your teacher may ask you to complete practical reports on scientific investigations you conduct. To help you communicate your findings clearly, Table 9 outlines the typical conventions and formats for each section.

Lesson link

Check out the ***How to conduct a practical investigation*** guide which includes a visual example of a poster you might create for your SAC. This guide is found after Chapter 11.

Table 9 The components of a practical report, including the suggested length and tense of each section

Section	Section description	Suggested length	Suggested tense
Title	The title may be written as a question or statement that describes the main phenomenon you are trying to determine in your experiment. Examples include: • How does light intensity affect the rate of photosynthesis? • Does the theory of natural selection explain the increasing carp (*Cyprinus carpio*) population in the Murray River? • The impact of pH on the rate of enzyme-catalysed reactions • The isolation and characterisation of spermatogonial stem cells in the fat-tailed dunnart (*Sminthopsis crassicaudata*) • What does medical student study behaviour look like, and is it effective? • Bathing salmon in cold water is an effective treatment for removing skin parasites Note that if you are investigating a particular species you may wish to include the species name in the title.	One sentence	Present
Abstract	Abstracts are optional but recommended. In essence, the abstract is a short overview of the entire experiment. One formula you could use for writing an abstract is answering each of these questions in one sentence, then using linking words to make the paragraph flow: • What is the significance of the experiment? • What was the aim of the experiment? • What was your method? • What were your results? • Why are your results important? • Given these results, what should be researched next? Or, what are the broader implications of these results?	100–300 words	Past

con'd

Table 9 Continued

Section	Section description	Suggested length	Suggested tense
Introduction	The purpose of the introduction is to justify why you needed to perform your experiment. Introductions generally contain the following information (not necessarily in this order): • Background information. This may include: – Why the system or model is important to study › For example, photosynthesis is important to study as it plays a major role in controlling the levels of different gasses in our atmosphere. – The broader implications of answering your particular question – Any prior research that has been undertaken › This may include pilot studies your class undertook or research by other sources. – Any gaps in knowledge, and how your experiment could fill that gap • The aim of the experiment • The variables that are being tested • The hypothesis – As well as a justification for your prediction • The final sentence of the introduction is typically 'big picture', suggesting how what you discover could help the world or influence future research.	Variable – check with your teacher, but usually one to four paragraphs	Mostly present and future
Method	The purpose of a method is to outline all the materials and steps you took during an experiment. Like a cooking recipe, it must be very detailed so that someone else could read it and follow your steps exactly. You can usually write the method in steps and in third person, using short sentences and direct language. Make sure you: • Write the steps in order • Name any equipment used – You may wish to outline if/how the equipment was maintained or calibrated. • Draw and label any complex experimental setups • State what you measured and when	Usually no longer than half a page	Past
Results	The purpose of the results section is to present the key findings of the study in a clear and honest manner. You do not usually present raw data in the results section, but manipulate it into transformed data (e.g. table, line graph, bar graph) that best shows any trends, patterns, or relationships that exist. Each figure is accompanied by a brief (2–3 sentences) description of the key findings. If statistical analyses have been performed, they are presented here as well. Do not interpret or explain your findings in this section.	Variable – it depends on the number of figures and tables	Past
Discussion	The purpose of the discussion is to determine if the data obtained supports the hypothesis and to explore the implications of the findings. It is very important that you highlight any problems that arose during the experiment in the discussion, as well as any limitations of the data. One way you could structure a paragraph in your discussion would be to: • Restate one key result (e.g. the result from one figure) • State if the result supports or refutes the hypothesis • Discuss if your findings support or differ from prior research – Be sure to reference sources • Weigh up the strengths and weaknesses of the data to determine if the result can be trusted – Identify reasons why this result may be invalid or unreliable. Here, you could refer to: › Personal, systematic, or random errors › Precision, accuracy, and uncertainty of data › Problems with the experimental design › Other studies that contradict your data – Identify reasons why the results may be limited – what is the data not telling us that would be useful to know? – Suggest how the method could be changed to overcome any problems – Identify any strengths that support the validity, reliability, and scope of the results	At least one paragraph – usually three or four	Mostly present

cont'd

Table 9 Continued

Section	Section description	Suggested length	Suggested tense
Conclusions	The purpose of this section is to summarise your study. Generally, conclusions begin by stating whether the hypothesis was supported. They also may include: • Justification of why the hypothesis is supported/rejected • Summary of limitations and improvements • The broader implications of the results, for example – Future research – The impact on scientific knowledge – The impact on society/environment	One paragraph	A mix, but mostly present
Acknowledgements	Individuals involved in the experiment should be recognised for specific contributions.	One to three sentences (not included in word count)	Present
References	A list of references in a standard style (e.g. Harvard or APA) should be included. For more information on how to reference, please refer to the Strategies for Success lessons included in this book.	Typically anywhere from 2–20 references (not included in word count)	N/A

Example

HOW POWERFUL IS A POWER NAP?

The investigation we've been stepping you through for this lesson is not made up – there is strong evidence that suggests daytime naps improve long term memory formation more than cramming. You can read the original research here: academic.oup.com/sleep/article/42/1/zsy207/5146032

This is how we'd reference the article in the Harvard style:

Cousins, J., Wong, K., Raghunath, B., Look, C., Chee, M. (2019). The long-term memory benefits of a daytime nap compared with cramming. Sleep, 42 (1).

Theory summary

KSSs are a set of capabilities that help build scientific thinking. There are opportunities to develop, demonstrate, and test your KSSs throughout VCE Biology. Figure 25 summarises the questions you should ask to demonstrate KSSs in scientific investigations.

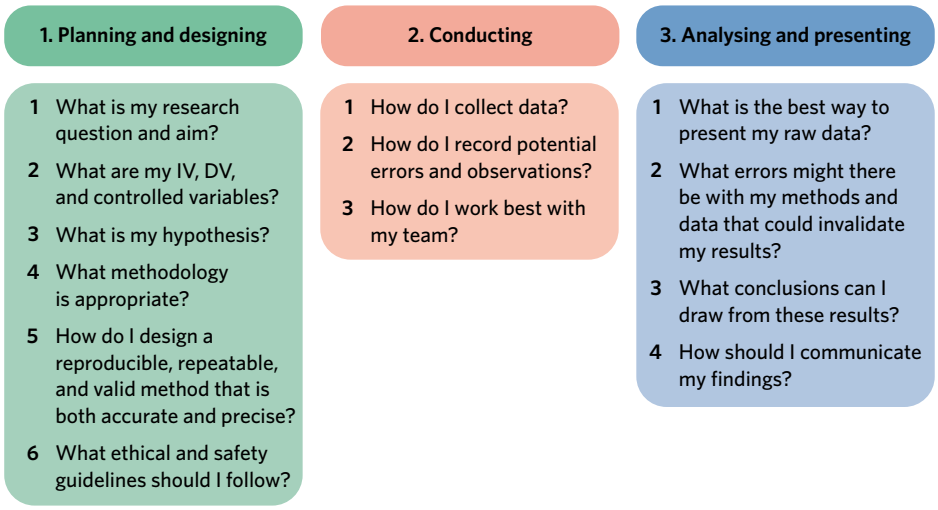


Figure 25 A summary of the questions to ask to demonstrate KSSs

The process of creating the HPV vaccine would have started with an observation about the nature of the virus – perhaps it is closely related to another virus, or it has a particular protein embedded in it's protective envelope. From there, the scientists would have constructed a research question, aim, and hypothesis about the nature of a HPV vaccine. They would have selected a methodology and developed a method for creating the vaccine, then tested it using random sampling, replication, and control and experimental groups, all the while attempting to minimise error.

To uphold ethical and safety guidelines, the vaccine would first be tested on cells and tissues, then animal subjects, and then, if it passed the previous trials, humans. The original HPV vaccine was tested on more than 20 000 females in 33 countries and 4 000 males in 18 countries before it was approved for general commercial use. Usually it takes more than 10 years to invent and approve a new drug, and only ~ 1 in 5 000 drugs that are 'invented' end up making it to market. What happens with the other 4 999? The results may not have supported the hypothesis that the drug would be effective, the method may have been unreproducible, the results may have been inaccurate or imprecise, the side effects may have made the drug unethical to sell – any number of things may have gone wrong. Luckily, rigorous testing using KSSs means that these ineffective or potentially dangerous drugs don't make it to pharmacies.

1A QUESTIONS

Theory review questions

Question 1

KSSs are

A the set of capabilities that people demonstrate when undertaking scientific investigations.

B biological theories and knowledge that must be memorised for the exam.

Question 2

An example of a testable, specific, and realistic research question is

A 'Does garlic inhibit the growth of the bacteria *Staphylococcus epidermis*?'

B 'How does garlic affect the growth of the bacteria *Staphylococcus epidermis*?'

Question 3

Which of the following options outlines all true statements about variables in experiments?

	Independent variable	Dependent variable	Controlled variable	Uncontrolled variable
A	manipulated	measured	a group in which the IV is not manipulated	a factor that might influence the results
B	measured	manipulated	kept constant	neither measured nor kept constant
C	manipulated	measured	kept constant	neither measured nor kept constant
D	measured	manipulated	measured	not measured but kept constant

Question 4

Control groups are important because they

A help us to make assumptions beyond the sample population.

B reveal if any factors besides the IV are influencing the results.

Question 5

Which of the following is true regarding replication? (*Select all that apply*)

I Replication decreases the influence of outliers on results, and proves that the same result can be achieved multiple times.

II Replication improves the reliability and validity of experiments, as it shows data are not due to random chance.

III Replication reduces the impact of random error, but cannot reduce systematic errors.

IV Replication, repeatability, and reproducibility are different words for the same thing.

V Replication always makes measurements more accurate and precise.

VI Replication never affects accuracy or precision.

Question 6

Fill in the blanks in the following sentences.

_____ errors decrease the precision of results. _____ errors decrease the accuracy of results. One way to increase _____ is to ensure all instruments are calibrated correctly. One way to increase _____ is to use appropriately sized measuring equipment.

Question 7

Which of the following is an example of a strategy to minimise risk in an experiment that involves growing plants?

A avoid using hazardous chemicals

B allergies of individuals in the class

C following Bunsen burner safety procedures

D sanitising equipment and benches after lab work

Question 8

Order the types of evidence from most to least reliable for drawing scientific conclusions.

I opinion

II anecdote

III primary data from a controlled experiment

IV primary data from an unreplicated case study

SAC skills questions

Case study analysis

Use the following information to answer Questions 9–12.

Scott and Mark Kelly are identical Caucasian male twin astronauts who participated in NASA's first ever twin study on the physical, molecular, and physiological effects of long-term space flight. Scott Kelly spent an entire year onboard the International Space Station whilst Mark Kelly remained on Earth.

Scott and Mark Kelly had both previously been on three short-medium length space expeditions (less than 300 days) prior to this study.

Both brothers had blood and urine samples taken routinely at the same time over the course of the previous months prior to Scott's departure, as well as routinely during the 12-month period Scott was in space, and for 6 months after the expedition. While living in space, Scott followed a strict diet and exercise regime like all astronauts, however, Mark did not have this restriction placed on him while living on Earth.

Question 9

Identify the control in this experiment.

A Scott Kelly

B Mark Kelly

Question 10

Which of the following is not a limitation of NASA's study design?

A The sample size is too small.

B Only Caucasian males were used in the study.

C NASA used twins which limits the genetic diversity of their samples.

D Mark and Scott Kelly had both previously been to space, and therefore may have some preexisting adaptation.

Question 11

Identify an uncontrolled variable in this study.

A timing of blood and urine tests

B location of participants

C diet of participants

Question 12

Why did tests continue to proceed six months after the expedition?

A to detect any new adaptations that arose from the expedition

B to detect how long it took to adjust back to normal conditions on Earth

Exam-style questions

Within lesson

Question 13 (1 MARK)

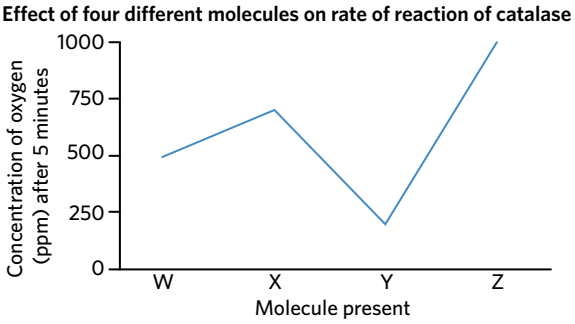
A student investigated the effect of the presence of four different molecules, W, X, Y, and Z, on the rate of reaction of catalase, an enzyme which converts hydrogen peroxide into water and oxygen. The production of oxygen was recorded over a five-minute interval. The final concentration of oxygen was recorded. The data collected is shown in the table.

Molecule present	Concentration of oxygen (ppm) after five minutes
W	500
X	700
Y	200
Z	1000

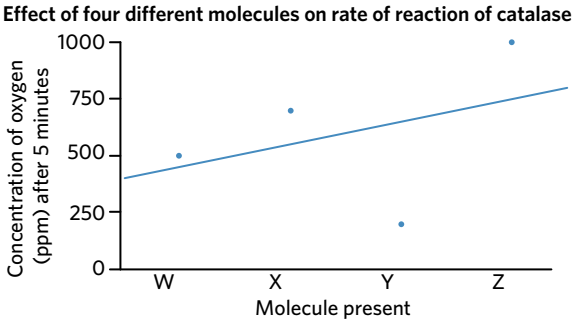
The student presented the results as a graph.

Which one of the following graphs is the best representation of the results?

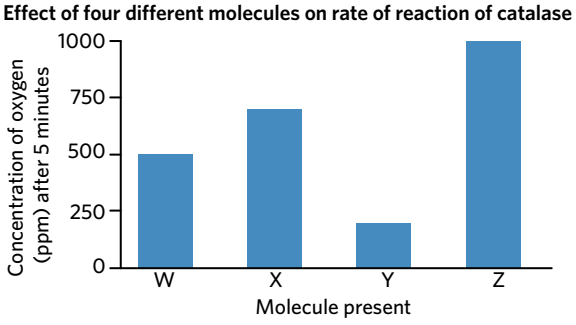
A



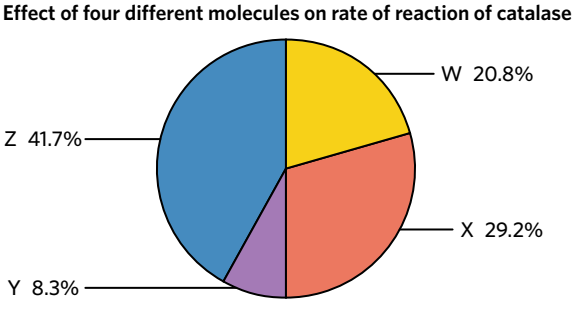
B



C



D



Adapted from VCAA 2020 Section A Q40

Question 14 (1 MARK)

Which of the following statements is correct?

A Precision is how close the measurement is to its true value.

B The true value is any measurement taken in an experiment.

C Accuracy is how closely each replicate is to other measurements.

D Validity is whether a measurement records what it is supposed to.

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q11e

Question 15 (12 MARKS)

Some plants are resistant to attack by insects because they produce a protein that poisons the larval stage of some insects that feed on them. The production of the protein is under the control of a gene found in the plant. A particular species of crop plant that does not usually produce the protein was genetically engineered to contain this gene. Such plants are referred to as genetically modified (GM) plants. These GM plants produce the insecticide protein.

Two farmers have properties next door to each other and grow the same cereal crop.

- Farmer X wishes to grow GM crops that are resistant to attack by insects.
- Farmer Y wishes to continue to grow non-GM crops.

Farmer Y was concerned that pollen from farmer X's GM crop could fertilise her non-GM plants, causing the next generation of Farmer Y's crops to produce the insect-poisoning protein.

The farmers agreed to carry out field trials to establish whether leaving a gap between crops reduced the likelihood of cross pollination. A number of trials were planted so that the results of one trial did not interfere in any way with the results of another. The percentage of seeds produced at various positions as a result of cross-pollination was measured for each trial. The outline of these trials and the results gathered are shown in the following table.

		Percentage of cross-pollination	
		at edge of non-GM crop	10 metres into non-GM crop
Trial 1 GM | non-GM	no gap between plots	10	2
Trial 2 GM non-GM	5 metres between plots	1	0.5
Trial 3 GM non-GM	7 metres between plots	1	0.3

a State the independent and dependent variables in the field trial. (1 MARK)

b Was a control group used in this experiment? Explain your response and, if a control group was not used, describe what an appropriate control group would be for this experiment. (2 MARKS)

c From the data, what conclusions can be drawn about cross-pollination and the gap between crops? (3 MARKS)

d Farmer X was dissatisfied with the results of the trial, and insisted that they undertake another trial with replication.

i Explain why this is a good suggestion. (1 MARK)

ii Draw and explain an experimental setup the farmers could use in a field trial with replication. (2 MARKS)

e In an attempt to minimise error, a number of trials were planted at different times so that the results of one trial did not interfere in any way with the results of another. Explain one potential problem with this experimental design. (2 MARKS)

f Eventually, the farmers decided to plant their crops 5 m away from each other, agreeing that this should keep the amount of cross-pollination low. After a few years, Farmer Y's initially non-GM crops were 50% GM. Despite this, Farmer Y was not displeased because her crops were growing far better than usual. Identify one ethical issue with the situation. (1 MARK)

Question 16 (7 MARKS)

An experiment was carried out by students to test the effect of temperature on the growth of bacteria. Bacterial cells were spread onto plates of nutrient agar that were then kept at three different temperatures: –10 °C, 15 °C, and 25 °C. All other variables were kept constant. The experiment was carried out over four days. The nutrient agar was observed every day at the same time and the percentage of nutrient agar covered by bacteria was recorded. At the conclusion of the experiment, the results were recorded in a table.

Time (days)	**Percentage of nutrient agar covered by bacteria at three different temperatures**		
	–10 °C	15 °C	25 °C
0	0	0	0
1	0	5	10
2	0	10	20
3	0	15	40
4	0	20	60

a Identify the independent and dependent variables. (2 MARKS)

b State a hypothesis that is supported by these results. (1 MARK)

c Suggest two variables that would have to be kept constant in this experiment. (2 MARKS)

d Two of the students, Mimi and Diego, wanted to reduce the possibility of personal errors affecting the results. Mimi said that they could do this by getting multiple students to estimate the percentage of nutrient agar covered by bacteria, then taking the average. Diego thought it would be better to include a negative control group. Name the student who is correct, and explain your choice. (2 MARKS)

Adapted from VCAA 2019 Section A Q7

1B ETHICS IN BIOLOGY

Have you ever heard of The Fountain of Youth? It is an old, mythical story about a magic spring of water that can restore our youth. People have been writing about it for thousands of years but, until now, the idea of escaping death was squarely science fiction.

In the last twenty years, scientists have learned more about the specific genes involved in the aging process, and claim to be getting closer to unlocking the secrets of their regulation and control. Not only to slow the aging process, but also to increase our health and make us live longer as young, healthy adults. Some scientists have said we could live as long as 1 000 years and could be playing sports and running around for a lot of that. The focus now is on developing a safe and reliable medication to control the regulation of these anti-aging genes, a feat which some scientists from leading universities around the world suggest is as little as ten years away!

But the question is: how should we feel about all this? Should we meddle with the natural biology of our cells, or is living longer not all it's cracked up to be? How can we even begin to make a decision here, and feel confident that we're acting in the right way?

Lesson 1B

In this lesson you will be learning about the relevance of ethics in biology, including what defines an ethical issue and how to exercise judgement in real-life ethical dilemmas.

◀ *Prerequisite knowledge* — *Future applications* ▶

Years 7–10
You would have started thinking about ethics, especially when performing practical investigations using live organisms.

Year 11
You would have been introduced to the approaches to bioethics and bioethical concepts and applied this to the knowledge you learned, especially for concepts such as stem cell research or genetic testing.

Year 12
You are required to apply ethical frameworks when designing experiments, conducting your own investigations, analysing data, and identifying issues relating to the application of scientific knowledge. You will also get to practice evaluating and analysing ethical concepts in the question section of every lesson in this textbook, as well as a number of ethical deep dives at the end of chapter SAC practices.

Study design dot point

Over the course of your assessments and exam, you will be required to employ ethics when answering questions and approaching problems. For this reason, each lesson in this book includes questions that pertain to the KSSs you learned about in lesson 1A, and require you to apply the bioethical concepts and approaches you will learn about in this lesson. You can think of these as part of your bioethical 'toolkit' as a VCE Biology student.

Key knowledge units

Bioethical issues	0.0.0.14
Approaches to bioethics	0.0.0.15
Ethical concepts	0.0.0.16

Bioethical issues 0.0.0.14

OVERVIEW

Ethics is a way of thinking about right and wrong that helps guide our actions and decision-making. In the world of science, ethics is taught and developed to allow scientists to make informed judgements about how best to act in the interests of others.

THEORY DETAILS

What is ethics and why is it important?

Ethics is a field of knowledge that deals with our personal understanding of right and wrong. At its simplest, ethics can be thought of as a working system of moral principles that help us question our actions and those of others while defending our own values, beliefs, and principles.

ethics a field of knowledge that helps individuals exercise moral judgment and determine what is right and wrong

Applied ethics is important in helping us bridge the gap between abstract theories we might learn in the classroom and concrete situations we might face in the world. It is an attempt to implement ethical theories and moral principles to guide decision making in particular contexts and problems. The ethical situations we are faced with often arise when different stakeholders (e.g. scientists, organisations) have different opinions on what is right or wrong and must choose between alternative points of view when deciding how to act.

applied ethics the application of ethical theories to real-life moral problems and contexts

Why is ethics important in biology?

Science is rarely an individual endeavour. Instead, it involves constant interactions with others, whether that be colleagues, employers, or the public. An important part of 'doing science' is not just the theories and concepts you learn, but also how you apply that knowledge in a way that maximises ethical outcomes. This requires scientists to engage in **metathinking** – that is, thinking about the way they think – to ensure that they are aware of the outcomes of their actions and employ different strategies for reaching important decisions.

metathinking the practice of reflecting upon and evaluating the way we think, including the different strategies and tools for problem-solving and learning

It may be easy to assume that this ability to think critically and act ethically becomes part of a scientist's 'toolkit' incidentally through natural means such as their upbringing, education, and community involvement. However, this 'incidental learning' is not enough. Instead, ethics is deeply embedded and integrated within scientific education, and involves active learning and practice on the part of the developing scientist. Scientists use these ethical frameworks they learn to help guide their decision-making process and justify their actions. As such, the application of ethical understanding is an important feature that runs throughout the entirety of the VCE Biology course.

What is a bioethical issue?

Biologists frequently use **bioethics** in their work across interdisciplinary fields, including biotechnology, environmental conservation, and healthcare research. In the course of this work, biologists are often confronted with **bioethical issue**s, which are specific ethical dilemmas pertaining to biology (Figure 1).

bioethics the study of ethical issues pertaining to biology and medicine

bioethical issue an ethical dilemma pertaining to biology that typically involves a decision-making process between two or more choices or options for an action

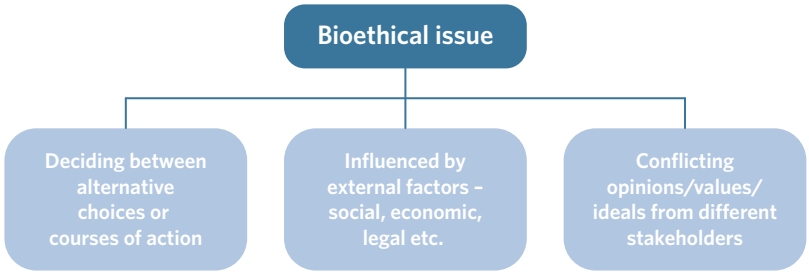


Figure 1 Some of the common features of a bioethical issue

We can recognise a bioethical issue because it will typically involve a decision-making process between two or more options for action, each of which will require some form of ethical justification – 'I should do X in this scenario, as it means that Y will occur'. The options available in these scenarios are usually in conflict with one another and can be evaluated using different **approaches to bioethics** and **ethical concepts**. The approaches and concepts help the individual consider the social, economic, legal, and political factors that might be relevant when deciding what is right (ethical) and wrong (unethical) (Table 1).

bioethical approach a decision-making framework that helps guide ethical behaviour

ethical concept a specific perspective or lens used to consider multiple angles of an ethical dilemma

Table 1 Some current areas of research that raise bioethical questions. Note that for each of these issues, multiple different ethical justifications can be identified. For example: 'we should use artificial intelligence in biology as it allows us to better track disease spreading patterns' vs 'we should not use artificial intelligence for disease-tracking as we are still not capable of containing its scope'.

Biological discipline	Bioethical issue
Biotechnology	• The use of artificial intelligence in biology, including disease-tracking software and facial recognition • The use of bioengineering in biology, such as the creation of synthetic vaccines, or replacement organs • The potential applications of stem cell research, including disease management and human enhancement
Healthcare	• The use of human embryos to research new therapies for diseases • The implications of prenatal testing for genetic defects during pregnancy • Whether or not organ donation ought to be voluntary, or if it should be state-imposed • How best to care for individuals at the end of their life, including the availability of euthanasia • The correct allocation of medical resources, including the dedication of physician time to hospital patients • The privacy of medical data, including the extent to which personal health data is shared with governments and insurers
Environmental conservation	• The potential of 'de-extinction' processes to bring back extinct species like mammoths • The potential for ecosystem management, and the best way to maintain the health of endangered species • How best to tackle climate change, including funding for research and changes to the way energy companies operate • How to balance the impact of industry and agriculture on natural environments, including laws that aim to control deforestation and habitat removal

Theory in context

ARE PATENTS HARMING BIOMEDICAL RESEARCH AND DEVELOPMENT?

A patent is a legally enforceable right to an invention that gives its owner the ability to exclude others from making, using, or selling that discovery. In the world of science and medicine, patents are useful in protecting the innovation of manufacturers by assigning them legal ownership of their developments, such as vaccines and medical equipment.

Nonetheless, it is unclear whether patents stimulate and promote important research and development, or whether they hinder it. Manufacturers argue that the protection of a patent provides an incentive to invest time and resources into further development, and allows for increased financial returns to improve resourcing and help progress further research. On the other hand, some critics argue that patents stifle free research by preventing others from accessing patented equipment or methods that might be helpful in their studies. This can lead to skyrocketing costs for the general public, as pharmaceutical companies might decide to heavily inflate the cost of their own prescription medications.

Approaches to bioethics 0.0.0.15

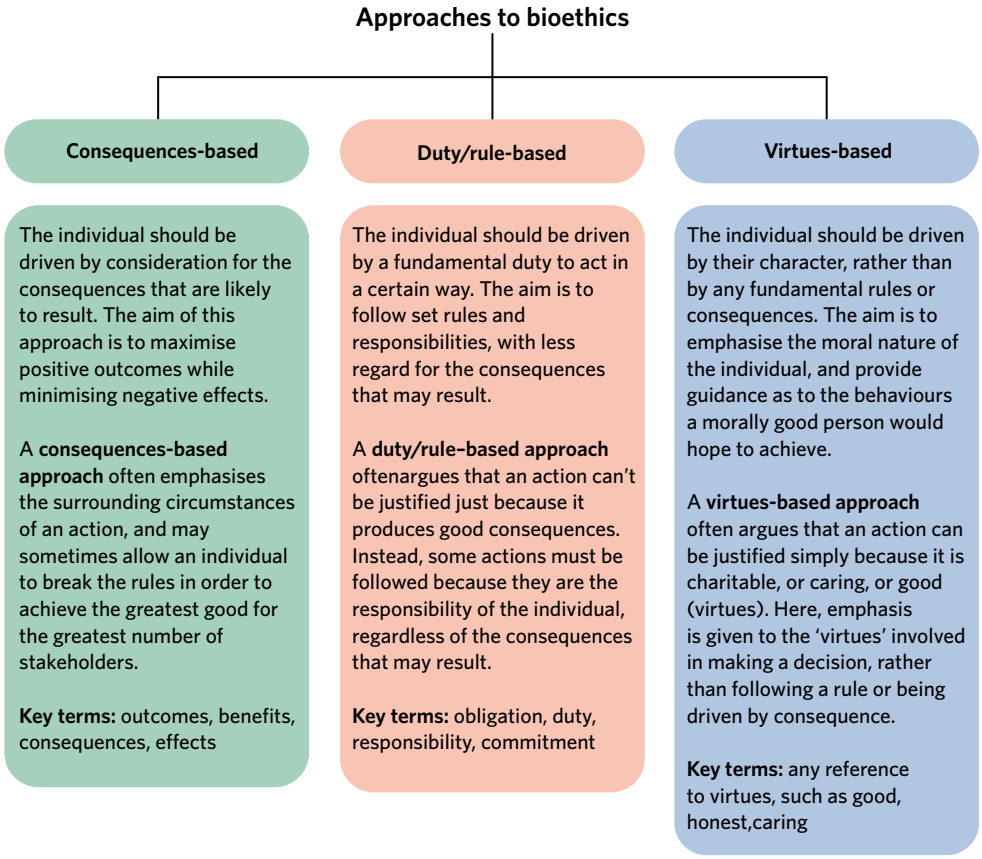
OVERVIEW

There are many different approaches that can be used when exploring bioethical issues. In VCE Biology, you are required to use three approaches in particular: consequences-based, duty/rule-based, and virtues-based. These approaches act as frameworks for addressing bioethical concerns and serve many purposes, including identification, exploration, consideration, decision-making, and reflection.

THEORY DETAILS

What is an approach to bioethics?

Because bioethics is often 'applied', scientists need specific tools to help them make informed ethical decisions. One type of tool scientists can use are approaches to bioethics, which are decision-making frameworks that help guide ethical behaviour. There are three specific approaches to bioethics that you need to be aware of in VCE Biology (Figure 2). Depending on the bioethical issue being considered, you may be able to use one or more of the following approaches to help inform your decision-making process.



consequences-based approach an approach to bioethics that aims to maximise positive outcomes while minimising negative outcomes

duty- and/or rule-based approach an approach to bioethics that promotes the responsibility of the agent above all else, and places importance on the duty of each individual

virtues-based approach an approach to bioethics that emphasises the individual goodness of the agent, and promotes acting in accordance with the values of a 'moral' person, such as honesty and compassion

Figure 2 The three major approaches to resolving bioethical issues. Notice that each approach has a different main focus – consequences, responsibility, and virtues. It can be helpful to use key 'buzz' terms when discussing each approach, as this demonstrates to your examiner that you can effectively separate the approaches in your mind and discuss their individual focuses.

Theory in context

CORONAVIRUS 2020 – THE APPROACHES IN ACTION

To consider these approaches in action, let us examine a bioethical issue during the early parts of the coronavirus pandemic, where Italian hospitals were faced with the ethical decision of who to treat and who to turn away. In such a situation, different ethical approaches may point to different courses of action.

As of March 1 2020, Italy had a total number of 1 701 cases of coronavirus nationwide. However, within the space of only 30 days, the number had sky-rocketed to 110 574 cases. This dramatic increase meant that hospitals and doctors could not treat everyone, leaving them with an incredibly hard decision: 'who do we treat first and how do we decide who to turn away?'

The ethical ramifications of such a decision were immense, as people who were denied treatment were sometimes at an increased risk of dying. Denying patients' access to treatment seems to stand in direct opposition to 'professional ethics' and therefore requires ethically trained professionals to try and develop the most moral course of action in the extraordinary circumstances.

As such, the Italian College of Anesthesia, Analgesia, Resuscitation, and Intensive Care (SIAARTI) published guidelines that doctors and nurses were asked to follow. The guidelines were rigorously examined against a range of bioethical considerations, including a consequences-based approach, which sought to maximise favourable outcomes for the largest number of people. This involved allocating care to patients with the highest chance of survival, specifically prioritizing young and otherwise healthy individuals while turning away older patients. How else might the SIAARTI have acted, and how might each approach point to a different course of action?

cont'd

Theory in context

CORONAVIRUS 2020 – CONTINUED

A consequences-based approach might argue that each hospital should aim to maximise good for the most number of people. If it turned out, as was the case for most Italian hospitals, that the demand for care outweighed their resources, then maximising good outcomes might mean focusing those resources on patients who are likely to best recover and respond to treatment. In this case, that means focusing treatment on young and otherwise healthy individuals, and less so on older patients who are perhaps less likely to survive.

A duty/rule-based approach might argue that as doctors and healthcare providers, each individual has a moral obligation to provide treatment to whoever is in need. Perhaps this would mean focusing resources in a first-come-first-serve manner, where care is distributed sequentially based on when a patient arrives at the hospital. A duty/rule-based approach might suggest that this is the fairest means of distributing care, and that hospitals have a duty to act indiscriminately rather than turning away patients based on their age.

A virtues-based approach might argue that doctors ought to follow the moral guidance of their own value judgements as to who is most in need. That is, the distribution of care should be left to the moral virtues of a good doctor – such as kindness, fairness, and good judgement. In this case, a hospital would not follow a certain rule (such as first-come-first-serve), nor focus on maximising consequences (though this may well be an outcome). Instead, each doctor is seen as their own moral agent deciding how best to distribute resources.

Figure 3 The approaches in action

In this case, the recommendations of the SIAARTI and the response of Italian hospitals most closely aligned with a consequences-based approach. However, it is important to understand that the three approaches rarely act in isolation. That is, it is rare for the approaches to sit distinctly in their own box. Instead, each approach will often act in conjunction with others to inform an individual as to the best or most ethical course of action in the circumstances.

Memory device

It is helpful to think of bioethical approaches as tools in a toolbox. Sometimes, a single hammer will be enough for a job, such as hammering a nail. Other times, however, the job might be more complex and require not only a hammer but also a saw and a wrench. The same is true of the three approaches. We are required to consider each in our quest to make ethical decisions and judgements.

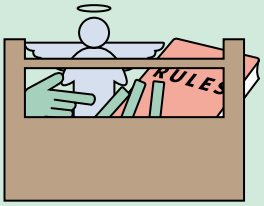
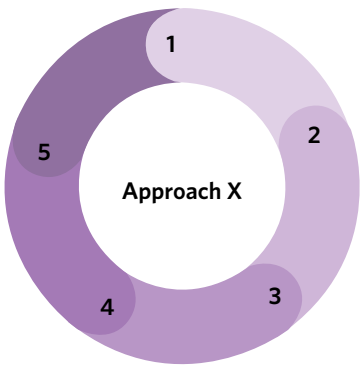


Figure 4 Bioethical approaches are like tools in a toolbox.

Why are approaches to bioethics important?

The three approaches we have looked at serve as broad frameworks for considering a bioethical issue and help guide us when considering the potential of different outcomes. Figure 5 represents some of the ways a bioethical approach might be useful when considering a bioethical issue. Ultimately, each approach can serve a range of important functions, and they provide the individual with useful guidelines when approaching a particular dilemma or issue.



1 **Identifying the issue**
Why is this an important bioethical concern?
Why are we considering this?

2 **Exploring the issue in context**
What situational factors surround the issue?
What do we need to be aware of?

3 **Considering different perspectives**
Who are the stakeholders involved in this issue?
What do they think and how might this affect them?

4 **Deciding on a position or course of action**
What is the most ethical way to proceed?
What position am I going to take on this issue?

5 **Reflecting on chosen course of action**
How did my position or course of action affect stakeholders?
What was the impact of my judgement?
Do I need to revise my position?

Figure 5 The role of a bioethical approach – identification, exploration, consideration, decision-making, and reflection. Note that we should be using all three approaches in conjunction wherever possible. Often this comes between steps 3 and 4, when it comes time to analyse the strength of different perspectives and come to a position or course of action.

Ethical concepts 0.0.0.16

OVERVIEW

There are five ethical concepts that are often used in conjunction with the three overarching approaches to bioethics. These concepts – integrity, justice, beneficence, non–maleficence, and respect – help inform the approaches spoken about in the previous section.

THEORY DETAILS

What are ethical concepts and why are they important?

As well as the three overarching approaches to bioethics, there are also a variety of ethical concepts which may be used in the exploration of bioethical issues. Each concept serves as a unique perspective or lens for considering different angles of an ethical dilemma and may be used either in isolation or in conjunction with each other alongside the three approaches we spoke about in the previous section.

There are five specific bioethical concepts that you need to be aware of in VCE Biology. Depending on the bioethical issue being considered, you may use one or more of the following concepts to help you analyse a bioethical issue:

- **Integrity** – the commitment to knowledge. This concept encourages individuals to act honestly and truthfully, especially when presenting their findings or results. Integrity prioritises an accurate understanding and representation of the facts, whether favourable or unfavourable to an individual's personal position, and encourages scrutiny and criticism.
- **Justice** – the commitment to fairness. This concept encourages consideration of different people's opinions and positions, especially those directly affected or marginalised by a course of action. Justice prioritises the fair distribution of resources, as well as equal access to the benefits of an action, policy, investigation, or research.
- **Beneficence** – the commitment to maximising benefits. This concept encourages individuals to act in a way that benefits others. Beneficence promotes the personal wellbeing and good of other persons, particularly direct stakeholders such as patients and research subjects.
- **Non–maleficence** – the commitment to minimising harm. This concept encourages individuals to act in ways that remove as much harm as possible. Indeed, while actions may always involve some degree of possible harm, non–maleficence prioritises minimising this harm, sometimes to the detriment of people's freedom of choice and autonomy.
- **Respect** – the commitment to consideration. This concept encourages individuals to consider the value of others, including their personal welfare, beliefs, freedom, and autonomy. Respect prioritises the freedom of others to make their own decisions and be protected from persecution or exploitation.

integrity an ethical concept that encourages a full commitment to knowledge and understanding as well as the honest reporting of all sources of information and results

justice an ethical concept that encourages fair consideration of competing claims, and ensures that there is no unfair burden on a particular group from an action

beneficence an ethical concept that seeks to maximise benefits when taking a particular position or course of action

non–maleficence an ethical concept that discourages causing harm – or when harm is unavoidable, ensuring that the harm is not disproportionate to the benefits from any position or course of action

respect an ethical concept that encourages the acknowledgment of the intrinsic value of living things, and considers the welfare, beliefs, customs, and cultural heritage of both the individual and the collective

The concepts in action

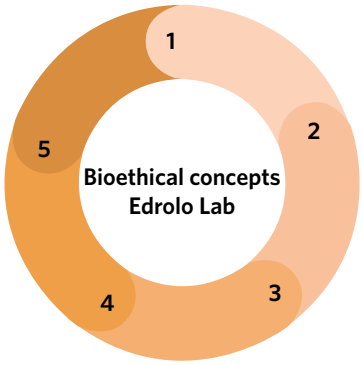
Here at Edrolo Lab, our team is developing a new vaccine that can help cure a terrible new disease known as 'Biologitis'. Throughout our research and trial stages, we were sure to act ethically and were informed at different times by the three approaches to bioethics. Now, it is time to release our vaccine and make it available to the public. To ensure we continue to behave ethically, we have considered the relevance of each of the five bioethical concepts (Figure 7).

Memory device

When using the five concepts to help evaluate a bioethical issue, it may be helpful to use the mnemonic memory device:

I Joke **B**ut **N**o-one **R**eacts

Figure 6 It's 'not funny' to forget the concepts



1 Integrity
Edrolo Lab decided to publicly release the research and results that went into developing the vaccine to allow for public scrutiny.

2 Justice
Edrolo Lab decided to provide free access to their vaccine for people in low socioeconomic communities.

3 Beneficence
Edrolo Lab decided to produce excess vaccines for storage in all hospitals in Victoria.

4 Non-maleficence
Edrolo Lab was sure to minimise unnecessary harm and distress of the animals used during the testing phase of their vaccine.

5 Respect
During human trials to check for vaccine efficiency, Edrolo Lab was sure to gain informed consent from all of their research participants.

Figure 7 The bioethical concepts in action. Can you identify other ways that each concept might be used to inform the actions of Edrolo Lab?

Theory in context

THE ISSUE OF GENETICALLY MODIFIED ORGANISMS (GMO)

A genetically modified organism (GMO) is an organism whose DNA has been altered using genetic engineering techniques. This often occurs in a laboratory, or in agriculture, and is typically used to favour the expression of some trait, such as pest/pesticide/disease/drought tolerance, higher yields, larger size, greater nutritional content, longer shelf lives, or brighter colour.

The use of GMOs for human and animal food is a bioethical issue. Some of the most common pros and cons associated with GMOs are summarised in Table 2.

Table 2 Common pros and cons associated with the GMO debate

Pros	Cons
• GM crops typically have better crop productivity than non-GM crops. This means that more food can be grown using less land, reducing habitat loss due to land clearing • GM foods can be made to have improved nutritional content, improving the health of individuals • GM crops can sometimes grow in more adverse conditions (e.g. drought-tolerant corn), protecting against famine and improving food security • Increased crop yields result in larger profits for farmers, while herbicide-tolerant crops reduce labour demands as farmers don't need to pull weeds by hand	• GM crops may lose their effectiveness if weeds or pests evolve resistance • Widespread use of GM crops could result in loss of genetic diversity within crop populations • Cross-pollination between GM crops and wild species or weeds may cause GM genes to spread accidentally • Some people consider GMOs to be unnatural, or like we are 'playing God' • Some people believe that genetically modifying animals for human benefit is inhumane – many anti-animal GMO arguments apply to animal agriculture in general • GM animals can have health issues

How might the bioethical concepts be used to inform this debate?

- **Integrity** – manufacturers might need to clearly label their products as GMOs, allowing consumers to be better informed.
- **Justice** – GMOs might create inequity between larger agricultural companies who have the resources to genetically alter their crops versus a small family farm that does not.
- **Beneficence** – there are positive health outcomes for people who consume GM foods that have been nutritionally enhanced.
- **Non-maleficence** – GMOs might cause unintended disruptions to the food web, such as insect-resistant crops that may alter population levels of different pests.
- **Respect** – it is important to promote the right of individuals to freely choose whether or not they use GMOs, and be provided with equal representation of alternatives.

By weighing up the considerations raised by each of the ethical concepts, scientists can figure out the best or most ethical course of action.

Theory summary

Ethics is a system of knowledge that deals with our personal understanding of right and wrong. In VCE Biology, we need to be aware of different strategies and tools to help us think and behave ethically. These include three bioethical approaches, and five bioethical concepts (Figure 8).

Each approach serves as an overarching framework for tackling a bioethical issue. The bioethical concepts may be used in conjunction with these frameworks, or indeed as standalone means for evaluating a position or course of action.

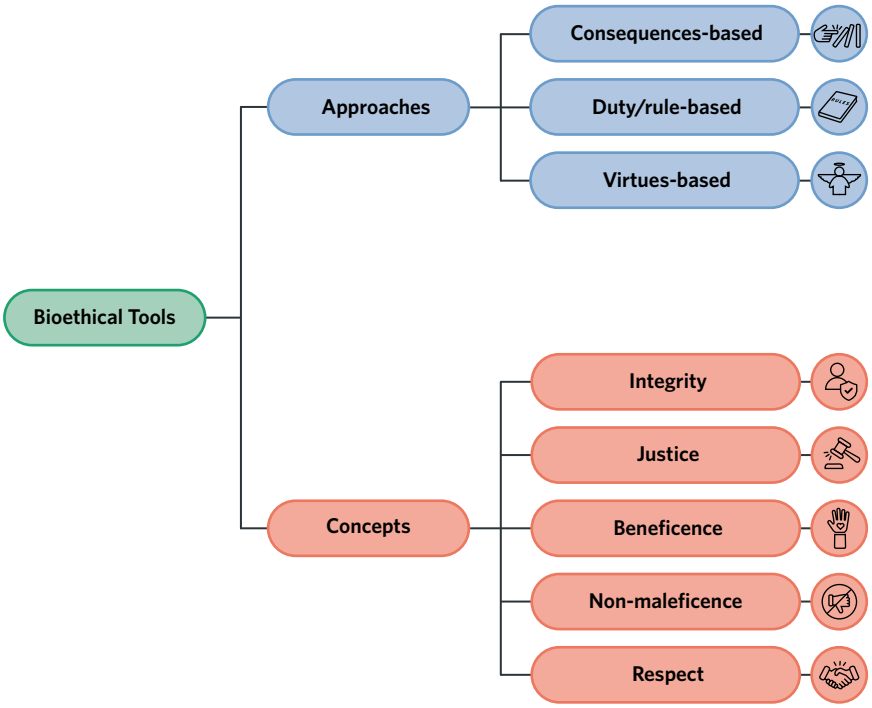


Figure 8 A summary of the bioethical toolkit

The prospect of an anti-aging pill that helps us live healthily for hundreds of years sounds like a no-brainer. But as with any biological research, we must remain in tune with the ethics and implications of all our decisions. For example, some people might argue that scientists have a duty/obligation to extend human life however possible, while others might point to unforeseen consequences on population levels, quality of life, corruption etc. Similarly, we must respect the sanctity of human life, but does that mean extending it by working against our inherent biology, or does it mean deferring to the power and autonomy of our cells? Who knows the right answer... but the approaches and concepts of bioethics can help us get closer to understanding all sides of the issue!

1B QUESTIONS

Theory review questions

Question 1

Fill in the blanks in the following sentences.

The study of ethical questions relating to biology and medicine is known as _____. It involves the practice of _____, which requires scientists to reflect on how they think and make decisions. The field is a smaller subsection of a larger field of study known as _____, which at its simplest is a working system of knowledge designed to help individuals evaluate their actions, values and beliefs.

Question 2

Which of the following best defines a bioethical issue?

A An ethical dilemma pertaining to biology that typically involves a decision-making process between two or more choices or options for action. These options are typically in conflict with one another and require some form of ethical justification.

B An approach to bioethics that aims to maximise positive outcomes while minimising negative outcomes. For example, we should make emergency vaccines readily available as soon as possible, as saving lives in the short term outweighs unknown side effects in the long term.

Question 3

The approaches to bioethics provide a framework for ethical thinking. Categorise the following statements as being **consequences-based**, **duty/rule-based**, or **virtues-based**.

I 'The decision to use human embryos for laboratory research should be left to the lab technicians themselves, as they are well-trained, intelligent, and compassionate.' _____

II 'We should make sure the vaccine goes through all trial rounds before releasing it to the public. Regardless of the rate of infection, it is our responsibility to ensure that the vaccine is safe.' _____

III 'We should ensure that all of the sensitive medical data of our clients is automatically deleted after six months. The dangers of being hacked outweigh the opportunities afforded by a large database.' _____

Question 4

Assume someone is in danger and in need of help. Which of the following responses best distinguishes a virtues-based approach from a duty/rule-based approach?

A A duty/rule-based approach suggests that to help the person is your responsibility, whereas a virtues-based approach suggests that to help them is to act with kindness and benevolence.

B A duty/rule-based approach suggests that to help the person is your responsibility, whereas a virtues-based approach suggests that to help them is to save their life and maximise well-being.

Question 5

Match the bioethical concept to its corresponding value statement.

Bioethical concept

- justice
- integrity
- respect
- beneficence
- non-maleficence

Value statement of bioethical concept

I _____ scientists ought to share their research with the public, even when their research disproves their hypothesis

II _____ scientists ought to consider how their research can be accessed by the public, and how it might benefit some groups more than others

III _____ scientists ought to design their research and the outcomes that arise from it in a way that directly benefits as many stakeholders as possible

IV _____ scientists ought to consider the different values and beliefs of various communities and stakeholders when researching and designing their experiment

V _____ scientists ought to acknowledge that their research often comes with a certain degree of risk, and should therefore consider ways to avoid unnecessary dangers when designing and carrying out their experiment

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 6–10.

During the month of May 2021, the Indian Medical Association called on the Indian government to enact a full nationwide lockdown in response to the coronavirus pandemic, after official figures reported upwards of 350 000 new infections each day during the earlier parts of the month as well as nearly 250 000 deaths in total. These numbers were compounded by one of the most dire instances of disaster ethics in recent memory, where an unimaginably strained medical system was forced to turn away hundreds of thousands of patients, forcing them to locate and purchase their own medical supplies, including oxygen tanks and respirators.

The situation stood in direct contrast to that of many other countries, particularly smaller island nations like Australia, who averaged around 10–15 cases per day over the same month. This has left many of these isolated nations to consider how they might respond to raging waves of pandemic intensity elsewhere in the world – including travel bans prohibiting people from affected countries from entering their borders. Concern stems from the fact that the strain affecting India, a variant known as B.1.617, shows mutations that may help it evade antibodies from prior infection or vaccination.

The Indian government was called upon to implement a planned and pre-announced lockdown, as opposed to a scattering of regional restrictions. It also considered discontinuing political campaign rallies and other crowded gatherings, such as religious and cultural festivals, which had previously been unrestrained. Such events are not localised only to India – Saudi Arabia, for example, a major trading partner with India, who shares commercial interests as part of the bilateral Indo-Saudi relations, allowed the major annual pilgrimage to Mecca (known as Hajj) to take place in 2021.

Question 6

According to the information provided, which of the following provides the best definition for the term 'disaster ethics'?

A Disaster ethics refers to the economic impacts of natural disasters, such as viral outbreaks, floods and earthquakes.

B Disaster ethics refers to bioethical decisions regarding religious and cultural observance in times of disaster and public emergency.

C Disaster ethics refers to situations in which a crisis has caused a large number of people to need medical help, especially where a medical system is unable to provide the level of support needed.

D Disaster ethics refers to situations in which international aid is being withheld in areas where mismanagement has occurred, so as to prioritise national interests over the implementation of foreign aid.

Question 7

Which of the following best summarises the bioethical issue presented in the text as it relates to the administering of care to COVID-19 patients in India?

A The Indian healthcare system is considering more inventive ways to reach as many patients as possible due in large part to a strict national lockdown.

B The Indian healthcare system is being forced to consider how best to ration its administration of care due to the strain faced during the pandemic.

C The Indian healthcare system is considering how best to honour various religious and cultural observations and withhold care where appropriate.

Question 8

Consider the following arguments concerning disaster ethics and the rationing of medical care during times of emergency. Which of the options best summarises the argument from the perspective of a duty/rule-based approach to bioethics?

	Argument for rationing care	Argument against rationing care
A	It is the duty of the hospital to be as effective as possible with their care. Rather than extending admittance beyond their capabilities, it is best to provide adequate assistance where possible as this is the best way to save lives.	Regardless of how serious the disaster is, hospitals have a duty to overstretch themselves and admit anyone who needs care. It is the responsibility of the healthcare system to save lives above all else.
B	A hospital ought to only turn away patients where the benefits of that action, including maintaining levels of care for other patients, outweigh the negative consequences, such as loss of life.	A hospital ought never to turn away patients in a disaster, as the negative consequences that result, including definite loss of life and a loss of trust in the healthcare system, will always outweigh the benefits of rationing care.

Question 9

According to the text, smaller island nations are faced with a bioethical issue surrounding their emergency border policies. To best uphold the bioethical concept of justice, smaller island nations ought

A to enact a stricter border policy in times of disaster. This is to minimise any undue and/or unnecessary harm to their own populations.

B to enact a stricter border policy as the economic consequences of a new wave of COVID-19 outweigh the benefits of an open border.

C not enact a stricter border policy as this directly contradicts the free sharing of information, whether favourable or unfavourable, and promotes an unnecessary message of nationalism over humanitarian interests.

D not enact a stricter border policy given that this directly marginalises those groups affected by the disaster, and fails to uphold the otherwise fair distribution of resources and equal access to care wherever possible.

Question 10

During the disaster period, the Indian government decided to provide 'emergency approval' to a range of different vaccines, typically those already in use in other countries. Emergency approval is relied on in times of significant health crisis, where regulators will choose to loosen their typical scientific standards of safety in favour of allowing experimental vaccines that are still undergoing testing.

Which of the following options best summarises the decision-making process of the regulators in these scenarios?

A Consequences-based approach – instead of relying on usual requirements of 'substantive evidence for safety', regulators choose to admit the use of the emergency vaccines on the grounds that their benefits are likely to outweigh their risks.

B Integrity – the pharmaceutical industry is large and highly lucrative. For this reason, regulators have an impartial responsibility to the broader public to be transparent and to share all research and data from ongoing studies, so as to avoid misuse of power.

C Justice – vaccines cost a lot and can be too expensive for some patients. For this reason, regulators have a moral responsibility to ensure that vaccines are made readily available to all individuals, regardless of their socioeconomic status and ability to freely vaccinate.

D Duty/rule-based approach – regulators understand the long-term consequences of unproven drugs, antimicrobials, and other medical practices. For that reason, they make it their responsibility to uphold the strict standards of evidence when determining a drug's efficacy – a process that can take a decade.

Exam-style questions

Within lesson

Question 11 (5 MARKS)

A team of biologists have been asked to speak at a forum regarding the allocation of resources for important gene cloning technology. The technology has been shown to have potential benefits in greatly improving the lives of patients with various genetic diseases, such as cystic fibrosis or diabetes. The team decides to conduct a literature review, and have collated secondary data and viewpoints from the following sources:

- Diabetes Australia
- Cystic Fibrosis Community Care
- Thalassaemia and Sickle Cell Australia

a Describe what is meant by a literature review. In your answer, provide one benefit of using this scientific investigation methodology. (2 MARKS)

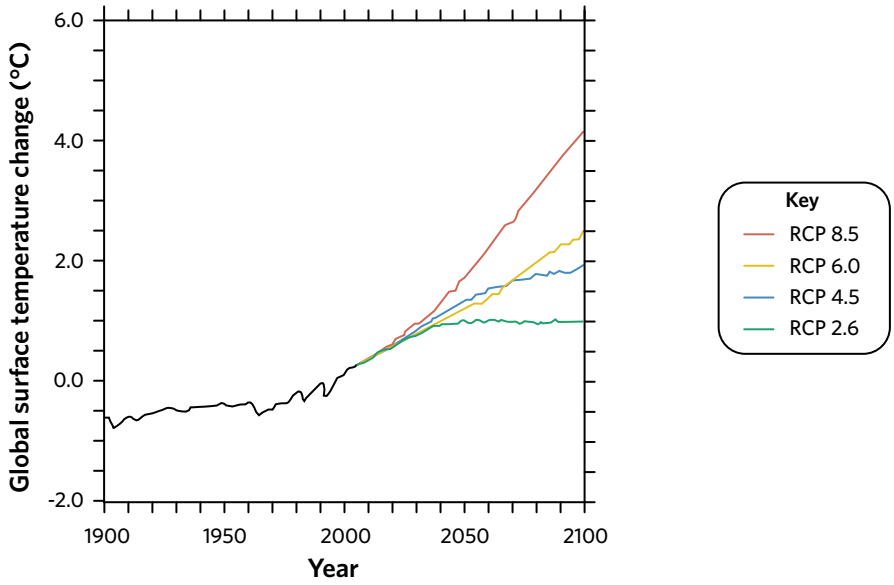
b The team used the information provided by these three companies to construct evidence-based arguments and draw conclusions. Provide one distinction between opinion and evidence. (1 MARK)

c Some audience members were critical of the team's findings. With reference to the bioethical concept of integrity, suggest one weakness of the data used by the team. (2 MARKS)

Question 12 (10 MARKS)

The Intergovernmental Panel on Climate Change (IPCC) is a cross-national body established by the United Nations and dedicated to objective, scientific dissemination of rigorous, peer-reviewed research into all aspects and issues pertaining to global warming. In 2018, the body published long-term climate change projections based on averages from a collection of 35 commissioned experiments conducted under the Coupled Model Intercomparison Project 5 (CMIP5).

When considering potential global warming trajectories, the IPCC makes reference to what is known as a 'Representative Concentration Pathway (RCP)', which is a measure of potential greenhouse gas concentrations. Each potential RCP is informed by various assumptions regarding economic activity, energy sources, population growth, and other socio-economic factors. Based on the projection averages of the CMIP5, the IPCC was able to model projected global surface temperature changes across a 200-year span (1900–2100) depending on varying RCPs.



a Based on the findings of the IPCC, what is the predicted global surface temperature change as of 2100 given an RCP of 8.5? (1 MARK)

b Based on the findings of the IPCC, what was the retrospective global surface temperature change as of the year 1900? (1 MARK)

c Based on the trend line seen during the period 2050–2100, comment on the significance of a global RCP of 2.6. (2 MARKS)

d This study employs various scientific investigation methodologies, including a literature review and modelling. With reference to the findings of the IPCC, distinguish between a literature review and modelling. (3 MARKS)

e Explain how the IPCC upholds the bioethical concept of integrity. (1 MARK)

f As many as 97% of climate scientists agree that global temperatures have risen since pre-industrial times and that human activity and lifestyle is a significant contributing factor. To reduce climate projections, the scientists argue that we first need to change our emissions habits, such as the extent of our fossil fuel usage. Many of these scientists also suggest that lower-socioeconomic countries close to the equator and near oceans are most likely to be affected by rising surface temperatures. Nonetheless, many sceptics of climate change argue that the warming projections are flawed and that the predicted changes to global surface temperature as a result of human activity are greatly exaggerated.

- **i** With reference to the information provided, how might the argument of climate change sceptics be informed by a consequences-based approach to bioethics? (1 MARK)
- **ii** With reference to the information provided, explain how future climate policies might be informed by the bioethical concept of justice. (1 MARK)

Rotate page

In science, I must make sure my experiment is:

Reproducible

Different scientists can get the same results when they follow the same method as the original scientists.

Repeatable

The original scientists can get the same results when they replicate the experiment.

Valid

The experiment measures what it claims to be measuring.

I can ensure this by designing an experiment that:

1. Tests the effect of **independent variable(s)** on **dependent variable(s)**.

The IV is the factor you control or change. The DV is the factor you measure. You want to test if/how your IV affects your DV. Both variables should be mentioned in your hypothesis, which should predict how the IV influences the DV.

The groups that have the IV applied to them are known as the experimental groups

2. Has a **control** group.

A control group is a group where the IV is not applied to the DV.

The results from control groups are compared to experimental groups, and any difference between the groups may be attributed to the IV (provided all potential uncontrolled variables are controlled against).

3. Has **replicates** of each experimental and control group.

Replication means you have multiple groups under the same conditions. This suggests that your results are less likely to be outliers or due to chance, and allows you to take the average of your replicates for each experimental group. If your experiment is well-designed, the values for different replicates of the same treatment should be similar.

4. Minimises potential for **uncontrolled variables** and sources of **error**.

Personal errors are mistakes or miscalculations made by the experimenter. They can affect accuracy, precision, validity, and/or reliability.

Systematic errors cause readings to differ from the true value by a consistent amount each time. They affect the accuracy of the experiment.

Random errors are unpredictable variations in the measurement process that result in a spread of readings. They affect the precision of the experiment.

Uncontrolled variables are potential variables besides the IV that may affect your results. You should remove them or control them by keeping them constant. At the very least, you should measure them so you know how they change.

5. Is informed by **bioethical** approaches and concepts.

Your experiment must follow the bioethical concepts of **integrity**, **justice**, **beneficence**, **non-maleficence**, and **respect**.

When analysing a bioethical issue, it is important to consider the three approaches to bioethics: **consequences-based**, **duty/rule-based**, and **virtues-based**.

6. Collects data that is **reliable**.

To collect reliable data: get a large, unbiased sample, be **accurate**, be **precise**, and minimise uncertainty in measurements.

An accurate measurement is one that is close to what is considered to be the 'true' value. Accuracy can be increased by reducing measurement/systematic error.

Precision refers to how closely multiple measurements are to one another. Precision can be increased by having a larger sample size.

7. Is **communicated** clearly, so other scientists can peer-review my work.

This means you need to present data in a meaningful way, give a clear method section so your work can be reproduced, and consider the appropriate safety and bioethical factors.

CHAPTER 1 SAC PRACTICE

SAC skills covered in this section:

✓ Bioethical deep dive ✓ Data analysis ✓ Scientific methodology comparison

DEALING WITH CHOLESTEROL (20 MARKS)

'Good' and 'bad' cholesterol

Cholesterol is a fatty substance found in the blood. Blood cholesterol is synthesised by the body and is important in both the digestion of lipids and the formation of hormones. However, excessive consumption of cholesterol from meat, dairy, and fried foods can lead to an increase in blood cholesterol, namely one major type of cholesterol known as low-density lipoproteins (LDL).

The majority of the body's cholesterol is made up of LDL, which is sometimes referred to as 'bad cholesterol'. This is because high levels of LDL can accumulate in the walls of arteries and increase the risk of stroke and cardiovascular disease. High-density lipoproteins (HDL), the 'good cholesterol', serve a reverse function to LDL and are able to absorb LDL and transport them to the liver where they can be broken down and eventually excreted.

The following table outlines the healthy, borderline, and high risk ranges of LDL and HDL in the blood of adults. (Note: 1 dL = 0.1 L and 1 mg = 0.001 g)

	Healthy level	Borderline level	High risk level
Low-density lipoproteins (mg/dL)	<130	130–159	>159
High-density lipoproteins (mg/dL)	>60	40–60	<40

1 Explain whether cholesterol is dangerous for the body. (2 MARKS)

2 A 60-year-old man recorded a blood cholesterol result of 140 mg/dL of LDL and 55 mg/dL of HDL. Explain what level his results are, and what course of action a doctor should recommend. (2 MARKS)

3 If a patient presented with 0.17 g/dL of LDL in their blood, what level are their results? (1 MARK)

Statin research

Statins are a class of drug that can lower cholesterol, and are used especially for those patients with readings of over 159 mg/dL of LDL. Statins are produced by many different major pharmaceutical companies around the world. In 2007, a study published by PLOS (Bero et al., 2007) evaluated the degree of bias introduced into a study based on how its funding was obtained. The studies in question were comparing the effectiveness of one statin drug to another, which could be a competitor's statin or an entirely different product altogether.

What this study found was that when a comparison study was pharmaceutical industry-sponsored, the main product being tested was up to 20 times more likely to produce favourable results compared to that of a government funded study.

4 Describe how privately funded studies may introduce bias into their results. (2 MARKS)

5 A student claimed that the Bero et al. study was focused on the integrity of statin research. With reference to your understanding of the bioethical concept of integrity, justify whether they are correct. (2 MARKS)

In comparison studies, there is often a group of patients issued the drug of interest, a group administered a comparison drug, and a group administered the placebo.

6 Explain two controlled variables that should be factored into comparison statin studies. (2 MARKS)

7 With reference to the dosages used in the study, explain one way in which bias could be introduced into a comparison study. (1 MARK)

Studying treatments of stroke

A stroke is caused when insufficient blood reaches part of the brain and, as a result, brain tissue dies. This can be caused by a blockage, known as an ischemic stroke, or through a vessel rupture causing bleeding in the brain, known as a haemorrhagic stroke. High levels of LDL increase your chance of an ischemic stroke, as the cholesterol creates a plaque in blood vessel walls. These can interact with blood cells and platelets, causing them to form a clot which then travels to the brain and blocks off the blood supply to a particular part of the brain.

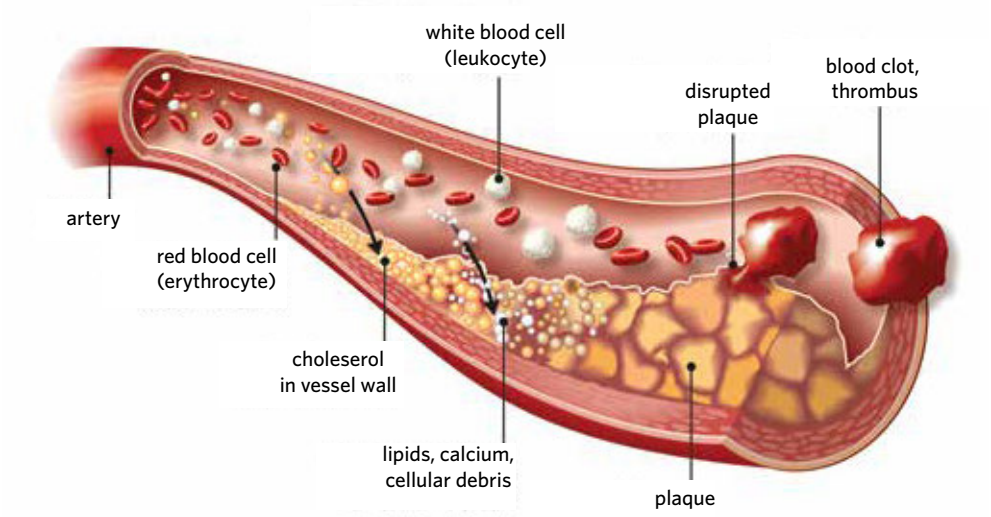


Image: Axel_Kock/shutterstock.com

Strokes are the third leading cause of death in Australia, and require a rapid medical response in order to reduce the potential for permanent effects on the brain. A key area of research relates to the impact of different drugs and/or procedures that can be administered to stroke patients to limit these life-threatening consequences.

To study the effects of different stroke treatments, scientists can use the following models:

- Model 1: human sample – working with hospitals to administer experimental treatments to patients admitted with a stroke and assessing the consequences.
- Model 2: animal sample – inducing a stroke in animals, then administering experimental treatments to the animals and assessing the consequences.

8 Explain which model is more valid for testing stroke treatments in humans. (1 MARK)

9 Both models are usually used in drug testing for stroke research. Explain in which order these two models should be conducted. (2 MARKS)

10 With reference to the bioethical concept of respect, outline why an animal sample model may be rejected by an ethics committee. (2 MARKS)

11 With reference to the bioethical concept of non-maleficence, justify why a doctor may reject administering experimental treatments on a patient admitted with a stroke. (2 MARKS)

12 Some may argue that a patient suffering a stroke should not be exposed to an experimental treatment as they cannot provide informed consent. Explain which bioethical concept this is relevant to. (1 MARK)

CHAPTER 1 EXAM PRACTICE

Section A (10 MARKS)

Question 1 (1 MARK)

Doctors tested a new medication, Medi-X, that controls blood pressure in pregnant women. 100 pregnant women aged between 25 and 35 years were divided into two groups of 50 patients. The first group received a pill containing Medi-X and the second group received an identical looking pill to Medi-X but it had no active pharmaceutical ingredients. Each patient was given one pill per day. All pills were the same colour and of equal mass.

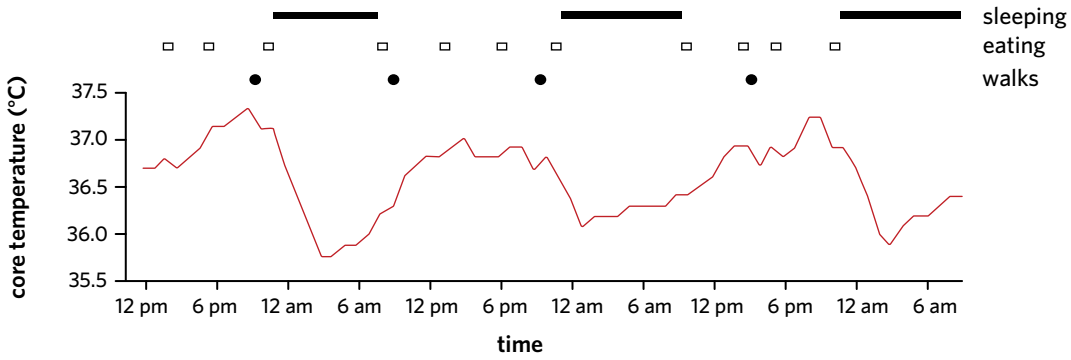
The dependent variable in this experiment was

A the composition of the given pill.
B the pregnant women aged 25–35 years.
C the blood pressure of the pregnant women.
D being given a pill of the same mass each day.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q27

Use the following information to answer Questions 2 and 3.

An experiment on the control of body temperature recorded the core temperature of a human subject, Jonah, living in one room for three days. The room temperature was kept constant at 20 °C. The results of the experiment are shown on the graph.



Question 2 (1 MARK)

The dependent variable in this experiment is

A time.
B Jonah.
C the activity of Jonah.
D the core body temperature of Jonah.

Adapted from VCAA 2012 Exam 1 Section A Q15

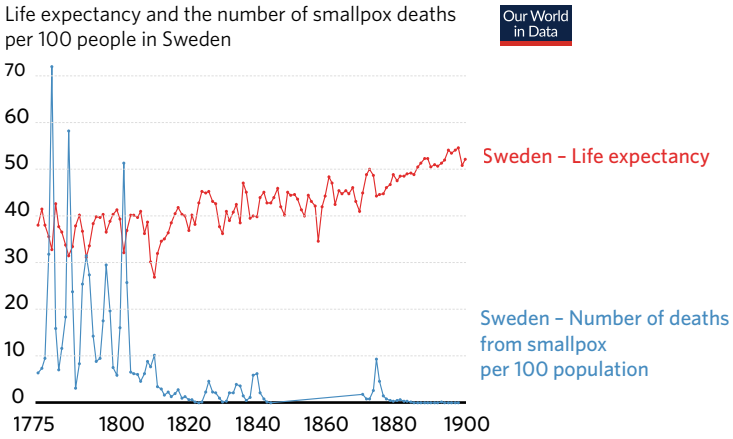
Question 3 (1 MARK)

From the graph, it can be concluded that

A Jonah always sleeps for the same duration each night.
B Jonah's core body temperature decreases during sleep.
C after eating, Jonah's core body temperature reaches its highest peaks.
D core body temperature is exclusively affected by the environmental temperature.

Question 4 (1 MARK)

Smallpox is a disease caused by the variola virus. The World Health Organisation (WHO) has officially declared that it has been eradicated. Data from Sweden displays the number of smallpox deaths per 100 people in the population and the life expectancy between 1775 and 1900.



It can be concluded from the data that

A in 1800, approximately 52 people died in Sweden from smallpox.

B overall life expectancy is dependent upon the number of smallpox infections.

C smallpox deaths reached their lowest point of 28 deaths per 100 people in 1807.

D between 1775–1900, the greatest life expectancy for Swedish people was 55 years.

Adapted from VCAA 2016 Section A Q22

Use the following information to answer Questions 5 and 6.

An experiment was conducted to test the following three hypotheses about the effect of the plant growth regulator indoleacetic acid (IAA).

- Hypothesis 1 – concentrations of IAA below 0.0001 parts per million stimulate shoot and root growth.
- Hypothesis 2 – concentrations of IAA above 0.0001 parts per million inhibit both shoot and root growth.

In the experiment, radish seedlings were grown in different concentrations of IAA, as indicated in the table.

Concentration of IAA (parts per million)	Stimulation (+)/ inhibition (−) of shoot growth (%)	Stimulation (+)/ inhibition (−) of root growth (%)
0	0	0
0.00001	+ 0.20	− 30
0.0001	+ 6	− 50
0.001	− 20	− 70
0.01	− 60	− 85
1	− 70	− 90
10	− 80	− 95
100	− 90	− 100

Question 5 (1 MARK)

Which one of the following is a reasonable conclusion to draw from the results of the experiment?

A Only Hypothesis 1 is supported.

B Only Hypothesis 2 is supported.

C Hypotheses 1 and 2 are both supported.

D Neither Hypotheses 1 or 2 are supported.

Adapted from VCAA 2016 Section A Q15

Question 6 (1 MARK)

Which sample serves as the experimental control?

A 1 part per million of IAA

B 0 parts per million of IAA

C 0.1 parts per million of IAA

D 100 parts per million of IAA

Question 7 (1 MARK)

Danielle performed an experiment on a stick of celery to see whether it needed light to grow. Each piece of celery was watered with 5 mL every day. Under these conditions, the amount of water provided to each piece of celery is referred to as a

A control.

B controlled variable.

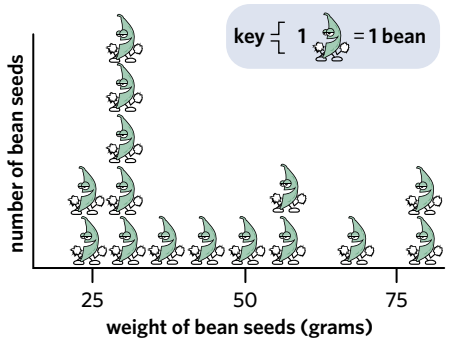
C dependent variable.

D independent variable.

Question 8 (1 MARK)

Farah recorded the weight of 15 bean seeds and graphically presented the data as shown.

The seeds came from bean plants grown in identical environmental conditions.



What conclusion can be drawn from these results?

A There is a spread of bean seed weight due to different growing conditions.

B The largest bean seed weighed greater than 100 grams.

C The average bean seed weight is close to 40 grams.

D The bean seeds at 80 grams are outliers.

Adapted from VCAA 2014 Section A Q26

Question 9 (1 MARK)

Tatiana set up an experiment in her school science laboratory to test the effect of different wavelengths of light on photosynthesis. Part of the experimental method directed Tatiana to 'add a few bunches of leaves' of an aquatic plant, *Elodea*, to a number of different test tubes. *Elodea* leaves naturally vary in size.

This experiment would be

A repeatable only.

B reproducible only.

C repeatable and reproducible.

D neither repeatable or reproducible.

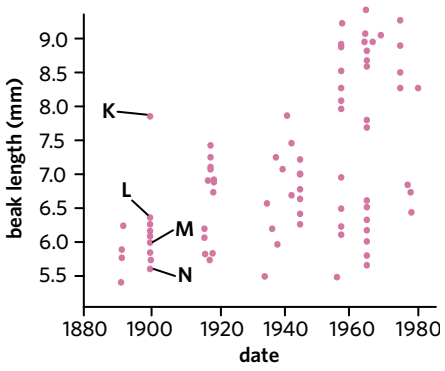
Adapted from VCAA 2014 Section A Q26

Question 10 (1 MARK)

Scientists have measured the beak length of the soapberry bug, *Jadera haematoloma*, over time. Their results are shown in the graph.

Which of the following points is most likely considered to be an outlier?

A point K
B point L
C point M
D point N



Section B (30 MARKS)

Question 11 (13 MARKS)

Four groups of students carried out an experiment testing the effect of glucose concentration on the fermentation rate of yeast. Higher temperature indicates higher rates of fermentation.

Note: glucose is an input in the fermentation reaction.

a Identify the independent and dependent variable. (2 MARKS)

b Define the purpose of a control and outline a possible control for this experiment. (2 MARKS)

c Before beginning the experiment, each group practised measuring the temperature of water and checked their thermometer against an electronic thermometer that gave a true measure of temperature.

The following results were obtained during the practice.

Group	Thermometer readings (°C)			Electronic thermometer readings (°C)
	1st measurement	2nd measurement	3rd measurement	
1	18.0	16.5	17.5	20.1
2	19.0	18.0	18.5	20.5
3	21.0	21.0	20.5	19.9
4	20.0	19.0	21.0	20.0

i Identify which group has the most precise results. Justify your response. (2 MARKS)

ii Identify which group has the most accurate results. Justify your response. (2 MARKS)

iii Explain how testing the thermometers increases the reliability of the experimental results. (2 MARKS)

iv State whether the data obtained is qualitative or quantitative data. Outline a difference between the two types of data. (2 MARKS)

d After completing their experiments, one group realised that their results did not support their hypothesis. With reference to the bioethical concept of integrity, outline what course of action they should take. (1 MARK)

Adapted from VCAA 2018 Section A Q11

Question 12 (11 MARKS)

The effect of different concentrations of sucrose solution on the average height of groups of tomato plants was tested. Six groups containing 40 plants each were left to grow for 20 days. Each plant had an initial height of approximately 2 cm. Each group was watered daily with 5 mL of water.

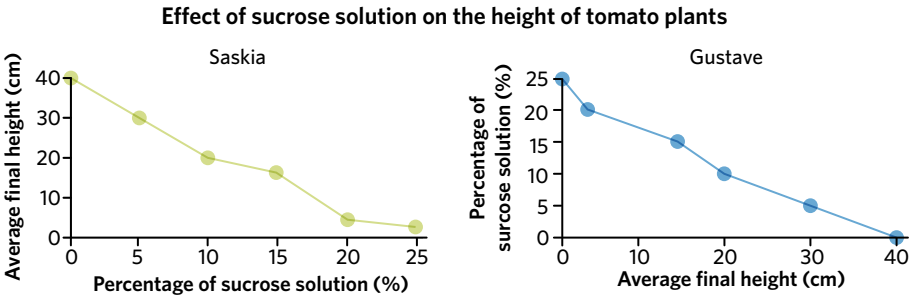
a Identify the independent and dependent variables. (2 MARKS)

b List three variables that were controlled to ensure the experiment produced valid results. (3 MARKS)

c The concentration of sucrose solution for each group is shown in the table. The heights of the plants were measured after the 20 days and averaged for each group.

Plant group	Percentage of sucrose concentration (%)	Average final height (cm)
A	0	40
B	5	30
C	10	20
D	15	15
E	20	5
F	25	2

i Saskia and Gustave both attempted to graph their results.



Identify who has created the more correct graph. Justify your response. (2 MARKS)

ii Explain why groups of plants were used in the experiment rather than individual plants. (2 MARKS)

d Consider any experiment. Explain the difference between the accuracy and validity of measurements. (2 MARKS)

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q36

Question 13 (6 MARKS)

From June 2019 through to February 2020, Australia faced a prolonged bushfire season which impacted 19 million hectares of land. It is estimated that up to three billion vertebrates were present in areas affected by fire, many of which would have been killed. Of those three billion vertebrates, it is predicted that 143 million mammals were affected.

a Explain how ecologists may be able to estimate the number of organisms affected by the bushfires. (2 MARKS)

b When sourcing a sample, it is important to ensure that the sample is unbiased and representative. What is meant by the terms 'unbiased and representative'? (2 MARKS)

c Judgement sampling is a technique where a researcher chooses which individuals to sample according to their needs. Outline a limitation of this technique. (2 MARKS)

UNIT 3

How do cells maintain life?

In this unit students investigate the workings of the cell from several perspectives. They explore the relationship between nucleic acids and proteins as key molecules in cellular processes. Students analyse the structure and function of nucleic acids as information molecules, gene structure and expression in prokaryotic and eukaryotic cells, and proteins as a diverse group of functional molecules. They examine the biological consequences of manipulating the DNA molecule and applying biotechnologies.

Students explore the structure, regulation, and rate of biochemical pathways, with reference to photosynthesis and cellular respiration. They explore how the application of biotechnologies to biochemical pathways could lead to improvements in agricultural practices.

Students apply their knowledge of cellular processes through investigation of a selected case study, data analysis, and/or a bioethical issue. Examples of investigation topics include, but are not limited to: discovery and development of the model of the structure of DNA, proteomic research applications, transgenic organism use in agriculture, use, research, and regulation of gene technologies, including CRISPR-Cas9, outcomes and unexpected consequences of the use of enzyme inhibitors such as pesticides and drugs, research into increasing efficiency of photosynthesis or cellular respiration, or impact of poisons on the cellular respiration pathway.

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UNIT 3

AOS1

What is the role of nucleic acids and proteins in maintaining life?

In this area of study students explore the expression of the information encoded in a sequence of DNA to form a protein and outline the nature of the genetic code and the proteome. They apply their knowledge to the structure and function of the DNA molecule to examine how molecular tools and techniques can be used to manipulate the molecule for a particular purpose. Students compare gene technologies used to address human and agricultural issues and consider the ethical implications of their use.

Outcome 1

On completion of this unit the student should be able to analyse the relationship between nucleic acids and proteins, and evaluate how tools and techniques can be used and applied in the manipulation of DNA.

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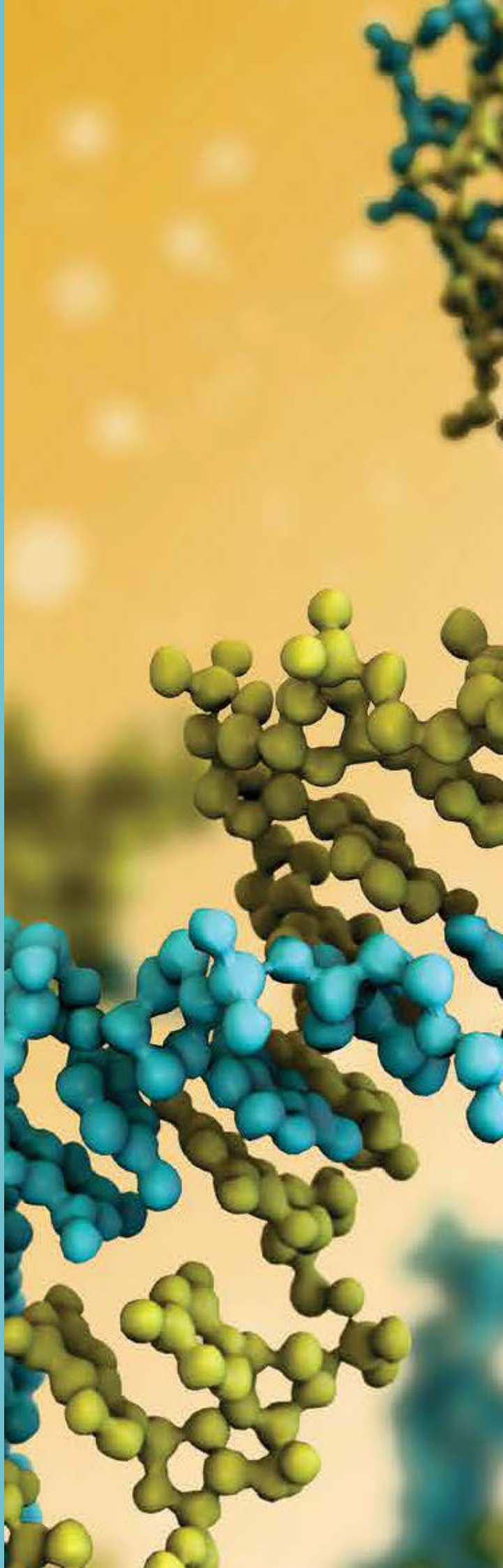


CHAPTER

2

Nucleic acids and proteins

Key knowledge

- nucleic acids as information molecules that encode instructions for the synthesis of proteins: the structure of DNA, the three main forms of RNA (mRNA, rRNA, and tRNA), and a comparison of their respective nucleotides
- the genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells, and translation by ribosomes
- the structure of genes: exons, introns, and promoter and operator regions
- the basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of a regulatory process
- amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein
- proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways
- the role of rough endoplasmic reticulum, Golgi apparatus, and associated vesicles in the export of proteins from a cell via the protein secretory pathway

2A PROTEIN STRUCTURE AND FUNCTION

Have you been thinking of going to the gym? Want to start working out? Well, if you have, you've probably realised that in order to maximise your gains, you need to start loading up on protein powder – after all, that's what all the professionals do. But how does protein powder actually help gain muscle? Does it have any other functions?

Image: Evgeniy Losev/Shutterstock.com

Lesson 2A

In this lesson you will learn about the functional diversity of proteins, including how their function is determined, and the molecular structure of proteins.

◀ *Prerequisite knowledge*

Year 11
Ribosomes, which can either be free-floating within a cell's cytosol or attached to the rough endoplasmic reticulum, are organelles present in nearly all living cells, serving as the site of protein synthesis.

Year 11
Many of the channels and carriers embedded within the plasma membrane are composed of proteins.

Future applications ▶

Lesson 2D
Gene expression involves the production of proteins via the processes of transcription and translation.

Chapter 3
Enzymes are proteins that serve as biological catalysts that increase the rate of chemical reactions within the body. Their structure and folding can be influenced by many factors, such as temperature and pH.

Study design dot points

- amino acids as the monomers of a polypeptide chain and the resultant hierarchical levels of structure that give rise to a functional protein
- proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways

Key knowledge units

The functional diversity of proteins	3.1.6.1
Amino acid structure	3.1.5.1
Protein structure	3.1.5.2

The functional diversity of proteins 3.1.6.1

OVERVIEW

Proteins are a diverse group of molecules that perform many different functions in cells, ranging from structural support in skin and hair to catalysing chemical reactions.

THEORY DETAILS

Proteins, also known as **polypeptides**, are one of the four types of biomacromolecules. They are large complex structures which are crucial to the functioning and development of all living organisms, serving a variety of different functions. While not an exhaustive list, some of the functions of proteins are outlined in Figure 1.

protein a biomacromolecule made of amino acid chains folded into a 3D shape

polypeptide a long chain of amino acids. Proteins can be made of one or many polypeptides

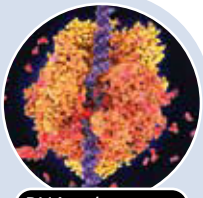
Due to the functional diversity of proteins, they are of particular interest to researchers. This is especially so because proteins rarely act in isolation, but rather, they often act together to form complex structures and processes. Therefore, the **proteome**, which refers to the entire set of proteins expressed by an organism at a given time, is a topic of significant interest.

proteome all the proteins that are expressed by a cell or organism at a given time

(a)

Function:
Enzymes

Explanation:
Enzymes are organic catalysts that speed up chemical reactions

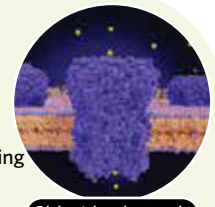

RNA polymerase

Examples:
- Catalase: breaks down hydrogen peroxide into water and oxygen
- Amylase: a digestive enzyme that breaks down starch into maltose
- RNA polymerase: catalyses the formation of mRNA from DNA

(b)

Function:
Transport

Explanation:
Typically embedded in membranes, controlling the entry and exit of substances from a cell


Chloride channels embedded within the plasma membrane

Examples:
- Chloride channels
- Glucose channels
- Sodium-potassium pumps

(c)

Function:
Structural

Explanation:
Support cell and tissue shape


The fibrous, elongated structure of keratin

Examples:
- Keratin: a tough protein found in skin, hair, and nails
- Elastin: found in elastic connective tissues such as the skin
- Collagen: found in connective tissues such as tendons and ligaments

(d)

Function:
Hormones

Explanation:
Many **peptide hormones** are chemical messengers used to communicate and induce changes in cells

Examples:
- Insulin: regulates blood sugar levels
- Amylase: a digestive enzyme that breaks down starch into maltose
- Adrenaline: increases heart rate and expands airways

Function:
Receptors

Explanation:
Receive signal from the environment

Examples:
- Acetylcholine receptors
- Hormone receptors


An insulin receptor protein (blue) embedded in the plasma membrane and bound to the hormone insulin (orange)

(e)

Function:
Defence

Explanation:
Involved in the immune system by recognising and destroying pathogens


The structure of an antibody

Examples:
- **Antibodies** (immunoglobulins)
- Complement proteins

(f)

Function:
Motor/contractile

Explanation:
Involved in the contraction and movement of muscles, the movement of internal cell contents around the cytoplasm, and the movement of cilia and flagella


The motor protein kinesin transporting a large vesicle along a microtubule

Examples:
- Myosin and actin: work together to enable muscle contraction
- Kinesin: moves along microtubules, enabling mitosis and vesicular transport

(g)

Function:
Storage

Explanation:
Act as reserves for metal ions and other molecules throughout organisms


Ferritin storing iron

Examples:
- Ferritin: storage of iron
- Casein: storage of amino acids, carbohydrates, and minerals

Images: (a, b, d) Juan Gaertner, (c, g) StudioMolekuul, (f) SciePro/Shutterstock.com

Figure 1 The functional diversity of proteins

Examiners' tip

The VCAA has not expected students to memorise specific examples of proteins (e.g. keratin, collagen). However, students should appreciate the functional diversity of proteins and the fact that they are crucial to the functioning and development of all living organisms.

enzyme an organic molecule, typically a protein, that catalyses (speeds up) specific reactions

peptide hormone a protein signalling molecule that regulates physiology or behaviour

antibody a protein produced by plasma cells during the adaptive immune response that is specific to an antigen and combats pathogens in a variety of ways. Also known as **immunoglobulin**

Amino acid structure 3.1.5.1

OVERVIEW

Amino acids serve as the building blocks of proteins. Their chemical structure is composed of a central carbon atom, a carboxyl group, an amino group, an R-group, and a hydrogen atom.

THEORY DETAILS

In terms of the molecular structure of amino acids, each amino acid consists of a central carbon atom that is bonded to a hydrogen atom, a **carboxyl group** (COOH), an **amino group** (NH_2), and an **R-group** (Figure 2). Of the 20 different types of amino acids in existence, each has its own specific R-group. Therefore, the R-group uniquely determines the identity of a particular amino acid. This also explains why proteins are not only made up of carbon (C), hydrogen (H), oxygen (O), and nitrogen (N) atoms, but they can also be made up of other elements such as sulphur (S) depending on the R-group present.

carboxyl group the functional group on amino acid molecules that contains a hydroxyl group (OH) and an oxygen double-bonded to a carbon atom

amino group the functional group on amino acid molecules that is made up of one nitrogen and two hydrogens (NH_2)

R-group the variable portion of an amino acid molecule. It can be one of twenty variations and determines the identity of the amino acid

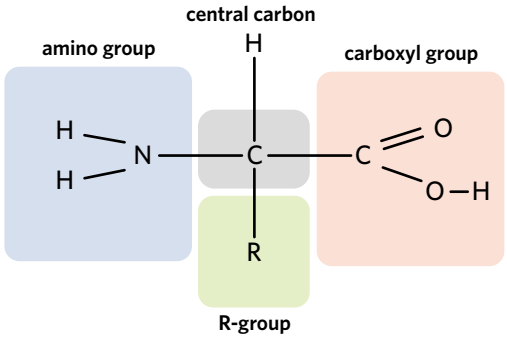


Figure 2 The basic structure of an amino acid

Additionally, each R-group has its own chemical properties, which can affect how different amino acids within a protein interact with each other. For example, an amino acid with a **hydrophobic** R-group is more likely to form bonds with other hydrophobic R-group amino acids than it would with an amino acid containing a **hydrophilic** R-group.

hydrophobic having a tendency to repel and be insoluble in water

hydrophilic having a tendency to be attracted to and dissolve in water

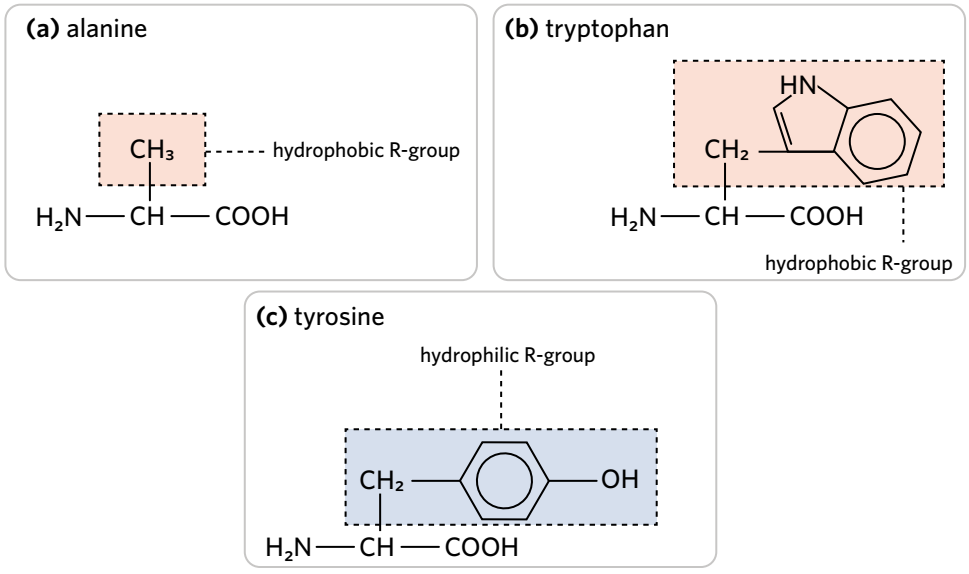


Figure 3 The structure of the amino acids **(a)** alanine, **(b)** tryptophan, and **(c)** tyrosine

When amino acids are joined together, they form a long chain known as a polypeptide chain, or protein. Because different amino acids have similar basic structures and can act as repeating subunits, they are known as **monomers**. When monomers are joined together, they form **polymers**. Therefore, polypeptides or proteins are the polymers of amino acids. The joining of amino acids together occurs at a cell's ribosomes via a **condensation reaction**, which results in the formation of **peptide bonds** between adjacent amino acids.

monomer a molecule that is the smallest building block of a polymer

polymer a large molecule that is made up of small, repeated monomer subunits

condensation reaction a reaction where two monomers join to form a larger molecule, producing water as a by-product

peptide bond the chemical bond linking two amino acids

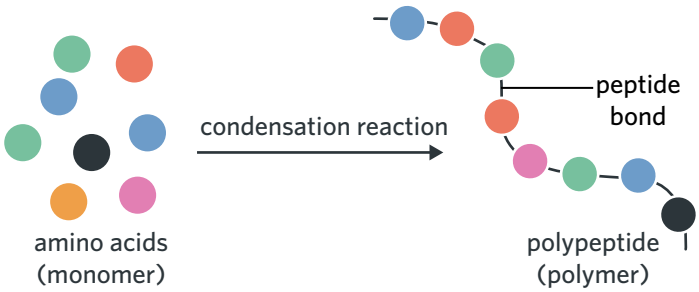


Figure 4 The formation of polypeptide polymers from amino acid monomers

> ***Examiners' tip***
>
> While the VCAA does not expect students to memorise the 20 different amino acids and their R-groups, it is expected that students will be able to draw a generalised diagram of an amino acid, which is depicted in Figure 2.

Protein structure 3.1.5.2

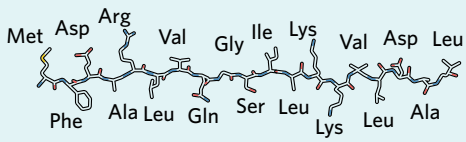
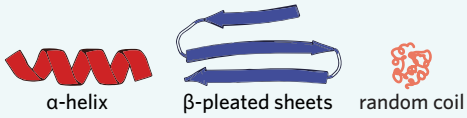
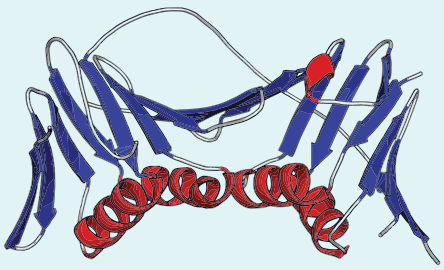
OVERVIEW

There are four levels of protein structure: the sequence of amino acids (primary), their arrangement into alpha-helices, beta-pleated sheets, or random coils (secondary), the functional 3D shape of the protein (tertiary), and the bonding of multiple polypeptide chains together (quaternary).

THEORY DETAILS

In order for a protein to function correctly, the polypeptide chain(s) produced must carefully fold into the correct shape. The four levels of protein structure describe how polypeptide chains fold to form this functional structure, beginning from the **primary structure** and becoming increasingly more complex to form **secondary** and **tertiary structures**. Some proteins can also have a **quaternary structure**.

Table 1 The different levels of protein structure

Structure level	Diagram
Primary The primary structure of a protein refers to the sequence (or order) of amino acids in a polypeptide chain.	
Secondary The secondary structure of a protein is formed when a polypeptide chain folds and coils by forming hydrogen bonds between amino acids of its different sections. When this occurs, structures such as **alpha (α) helices** and **beta-pleated (β) sheets** are formed. **Random coils** are irregular portions of secondary structure that join alpha-helices and beta-pleated sheets.	
Tertiary The tertiary structure refers to the overall functional 3D shape of a protein. For a protein to be functional, it must at a minimum have a tertiary structure. The tertiary structure of a protein is formed when the secondary structures further fold by forming interactions and bonds between amino acids and R-groups of its different sections. **Disulphide bonds** can also often form between cysteine amino acids due to the presence of sulphur atoms in their R-groups to further stabilise the protein's 3D structure.	

cont'd

primary structure the first level of protein structure, which refers to the sequence of amino acids in a polypeptide chain

secondary structure the level of protein structure where the amino acid chain forms either alpha-helices, beta-pleated sheets, or random coils

tertiary structure the functional 3D shape of a polypeptide chain

quaternary structure the level of protein structure where multiple polypeptide chains bond together, or other non-protein groups are added to form a fully functional protein

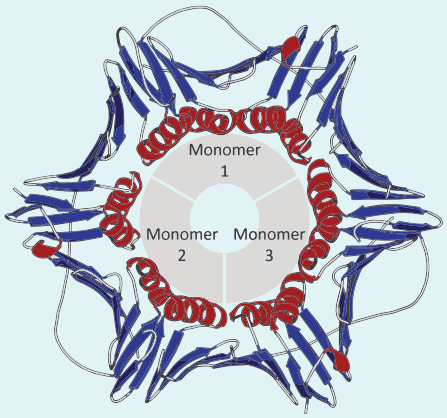
alpha helix an organised coiled secondary structure of proteins

beta-pleated sheet an organised folded secondary structure of proteins

random coil an irregular secondary structure of proteins that is neither an alpha helix nor a beta-pleated sheet

disulphide bond a strong covalent bond occurring between two sulphur atoms

Table 1 Continued

Structure level	Chain
Quaternary The quaternary structure is formed when two or more polypeptide chains with tertiary structures join together. Polypeptide chains with tertiary structure that have a **prosthetic group** attached are also considered to have a quaternary structure. However, it is important to remember that not all proteins will have a quaternary structure.	

prosthetic group a non-protein group bound to a protein. For example, a vitamin or ion

Theory in context

HAEMOGLOBIN

Haemoglobin, which is a protein responsible for carrying oxygen in red blood cells, is one example of a protein that has a quaternary structure.
It is composed of four polypeptide chains bonded together, and within each of these chains, there is also an iron ion embedded within a haem prosthetic group.

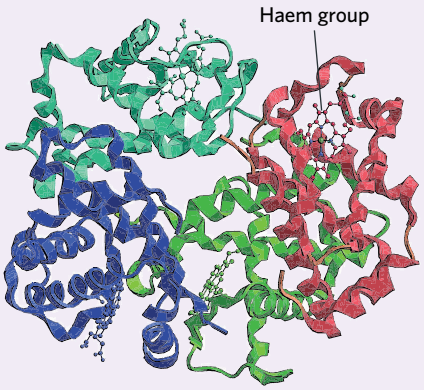


Image: Raimundo79/Shutterstock.com

Figure 5 The molecular structure of haemoglobin

Ultimately, the folding of a protein into its functional tertiary or quaternary structure relies on its primary structure. Depending on the sequence of amino acids, the R-groups will interact with each other differently, forming different bonds that favour folding into specific 3D structures. Therefore, the functional diversity of proteins arises due to the ability to create an unlimited number of complex combinations of amino acids that fold into polypeptides of varying shapes and sizes.

Additionally, since protein folding depends on the primary structure of a protein, if there are any changes to the original sequence of amino acids, a protein may no longer be able to fold correctly, preventing it from functioning normally.

Theory summary

Proteins demonstrate functional diversity through the various roles that they serve in living organisms. Amino acids are the monomers of proteins, and they join together via condensation reactions to form polypeptide chains. The primary level of protein structure refers to the sequence of amino acids, the secondary level of protein structure involves alpha helices, beta-pleated sheets, and random coils, the tertiary level is the functional 3D structure of a protein, and when there are two or more polypeptides in a protein it is said to have a quaternary structure.

Protein serves as a core component of muscle. Therefore, by increasing your protein intake, you can increase your muscle mass. But apart from contributing to muscle mass, protein also has a variety of other functions, such as its involvement in signalling and reception, transport, muscle contraction, storage, immunity, and structure.

2A QUESTIONS

Theory review questions

Question 1

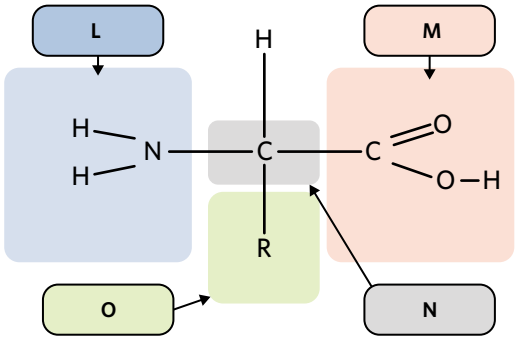
Proteins have a

A large range of different functions.

B limited number of functions.

Question 2

Label the parts of the amino acid.



Question 3

Which one of the following statements about amino acids is incorrect?

A The R-group is specific for each type of amino acid.

B The joining of amino acids occurs at the ribosomes.

C Amino acids are only composed of carbon, hydrogen, nitrogen, and oxygen atoms.

D Amino acids are monomers, which join together to form polymers known as polypeptides.

Question 4

Match the level of protein structure to its description.

Protein structure	Description
• tertiary	**I** _____ sequence of amino acids
• primary	**II** _____ functional 3D structure of the protein
• secondary	**III** _____ composed of two or more polypeptide chains
• quaternary	**IV** _____ formation of alpha helices and beta-pleated sheets

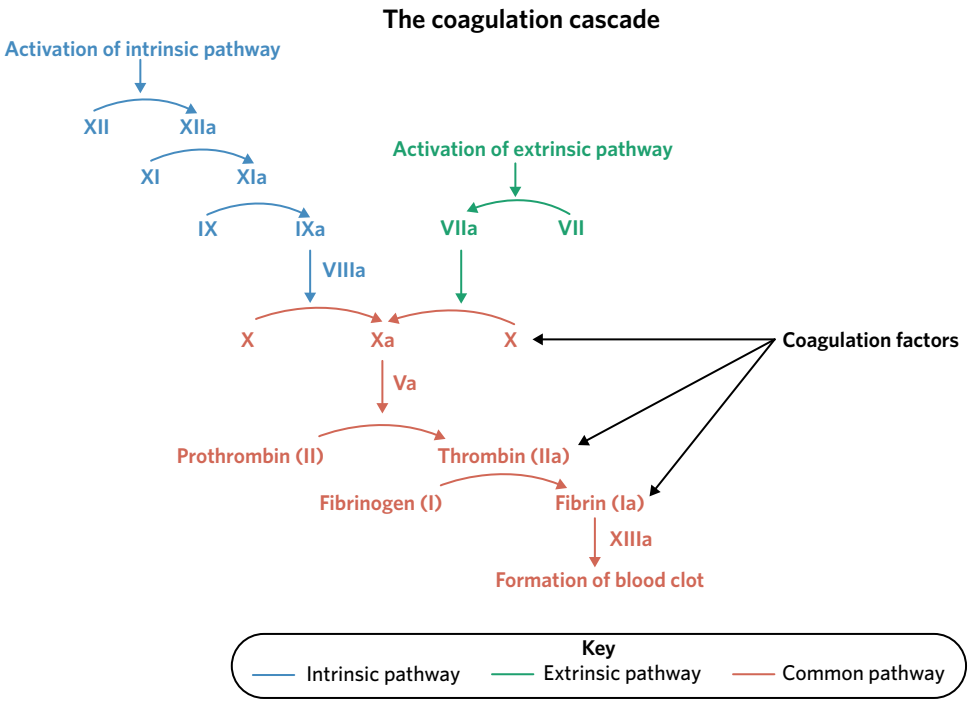
SAC skills questions

Data analysis

Use the following information to answer Questions 5–9.

In the human body, proteins rarely act in isolation, but rather, they come together to form large complex processes. For example, the ability of the body to form blood clots to prevent blood loss when blood vessels are damaged involves over 21 different proteins. These proteins are known as coagulation factors and are activated through a complex set of pathways known as the coagulation cascade, which helps form a blood clot.

There are two pathways involved in initiating coagulation: the intrinsic and extrinsic pathways. After either of these pathways is activated, they both converge into the common pathway to form a stable blood clot.



When doctors conduct coagulation tests, they measure how long each pathway takes to form a clot, allowing them to detect abnormalities in the coagulation cascade. For example, the activated partial thromboplastin time (APTT) measures the speed of the intrinsic and common pathways and the prothrombin time (PT) measures the speed of the extrinsic and common pathways.

Individuals diagnosed with haemophilia, a blood clotting disorder, have difficulty in forming blood clots, often due to an abnormal version of coagulation factor VIII. Therefore, they often suffer from spontaneous bleeding and bruising. Their coagulation test is characterised by a prolonged activated partial thromboplastin time.

Blood test results involving two different individuals			
Test	**Individual A (male)**	**Individual B (female)**	**Normal reference values**
Prothrombin time (seconds)	12.8	11.5	10–13
Activated partial thromboplastin time (seconds)	44.9	30.9	25–36
Red blood cell count (million/mm^3)	4.7	2.9	Male: 4.3–5.9 Female: 3.5–5.5
Haemoglobin level (g/dL)	14.5	12.5	Male: 13.5–17.5 Female: 12.0–16.0

Question 5

To gain an understanding of how coagulation factors interact with each other, researchers would be most interested in studying

A individual coagulation factors.

B multiple coagulation factors.

Question 6

An abnormal coagulation factor VIII could be caused by

A malfunctions in other coagulation factors which lead to the production of an abnormal coagulation factor VIII.

B an altered primary structure for this protein, which causes different bonds and interactions between nearby R-groups, leading to a different tertiary structure.

Question 7

Haemophilia is characterised by a

A decreased extrinsic and common pathway time.
B prolonged extrinsic and common pathway time.
C decreased intrinsic and common pathway time.
D prolonged intrinsic and common pathway time.

Question 8

In the table provided, the individual suffering from haemophilia is most likely

A individual A.
B individual B.
C neither individual A nor B.

Question 9

Based on the blood test results

A individual B suffers from a high haemoglobin level.
B individual A suffers from a high haemoglobin level.
C individual B suffers from a low red blood cell count.

Exam-style questions

Within lesson

Question 10 (1 MARK)

The proteome is

A all the proteins in a cell, tissue, or organism.
B the complete set of chromosomes found inside a gamete.
C the set of genes that code for all the proteins in an organism.
D the entire set of proteins expressed by an organism at a given time.

Adapted from VCAA 2018 Section A Q2

Question 11 (1 MARK)

Consider the structure and functional importance of proteins. Which one of the following statements about proteins is false?

A The tertiary structure of a protein can be stabilised by disulphide bridges.
B Two proteins with different amino acid sequences will likely have different functions.
C A change in the secondary structure of a protein will affect the biological function of the protein.
D Proteins with a quaternary structure are always more active than proteins without a quaternary structure.

Adapted from VCAA 2017 Section A Q1

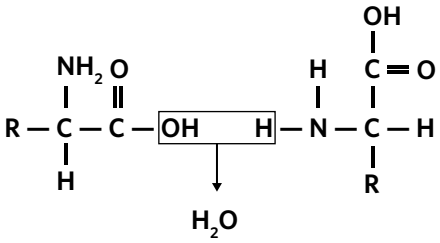
Question 12 (1 MARK)

For a protein to be functional, it must have a

A tertiary structure.
B primary structure.
C secondary structure.
D quaternary structure.

Use the following diagram to answer Questions 13 and 14.

The following diagram represents the joining of two organic monomers.



Question 13 (1 MARK)

The diagram represents monomers of an organic molecule being joined together. The monomers are

A amino acids.

B nucleic acids.

C monopeptides.

D monosaccharides.

Question 14 (1 MARK)

The 'R' symbol on the monomer represents

A one of 25 possible amino acids.

B the chemical element rubidium.

C a variable group specific to the amino acid.

D the continuation of the carbon-hydrogen-nitrogen chain.

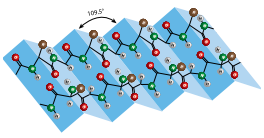
Adapted from VCAA 2018 Section A Q3

Question 15 (3 MARKS)

The diagrams represent four levels of structure with respect to the folding and assembly of a protein. The diagrams are not to scale.

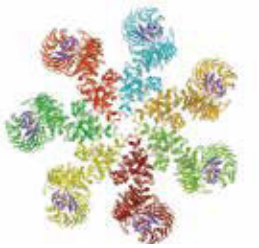
W

X



Y

Z



Images (left to right): ibreakstock, magnetix, chromatos, Raimundo79/Shutterstock.com

a Identify which diagram represents each structural level of a protein. (1 MARK)

b Describe how the functional 3D structure of a protein is formed. (1 MARK)

c Suggest how the functional diversity of proteins arises. (1 MARK)

Adapted from VCAA 2015 Section B Q1

Question 16 (5 MARKS)

Oxytocin is a peptide hormone that has an important role in social bonding, childbirth, lactation, and sperm movement. It is produced in an area of the brain known as the hypothalamus and released by a nearby gland called the posterior pituitary gland.

a Name the bond that joins the monomers of oxytocin. (1 MARK)

b Draw and label the general structure of the oxytocin monomer. (2 MARKS)

c Oxytocin is a relatively simple peptide hormone and is composed from a single chain of nine amino acids joined together. Identify and describe the level of protein structure that oxytocin folds into. (2 MARKS)

Adapted from VCAA 2017 Section B Q1

Multiple lessons

Question 17 (1 MARK)

All specialised cells that secrete protein molecules uniquely

A have an extensive endoplasmic reticulum.

B have a flexible plasma membrane.

C have large vacuoles for storage.

D contain numerous chloroplasts.

Adapted from VCAA 2015 Section A Q5

Question 18 (1 MARK)

Consider the diagram of the plasma membrane.

Identify which of the following molecule(s) are made up of many amino acids.

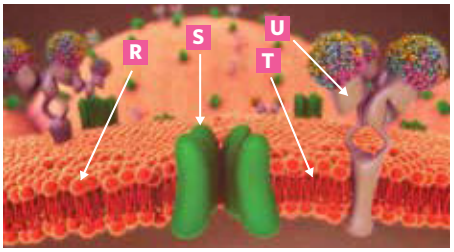


Image: sciencepics/Shutterstock.com

A S

B R

C R & T

D S & U

Adapted from VCAA 2017 Section B Q1

Key science skills and ethical understanding

Question 19 (10 MARKS)

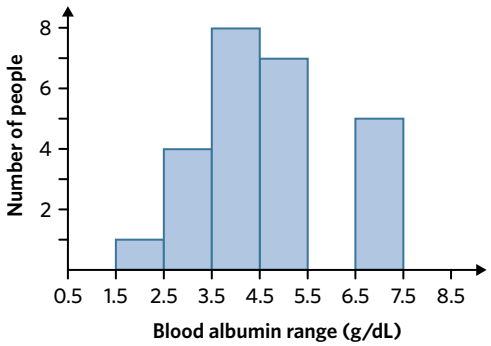
Albumin is a globular protein involved in many different processes within the body, one of which is the transport of substances around the body. It has many hydrophilic R-groups on the outside and many hydrophobic R-groups facing the interior of the protein. It is also highly insoluble in lipids. The given image depicts the structure of albumin.

a Explain why albumin is highly insoluble in lipids. (2 MARKS)

b Albumin normally constitutes 50% of human plasma protein, making it important for regulating blood pressure. Identify two other functional roles of proteins in living organisms. (2 MARKS)

c While low albumin levels are often caused by liver diseases, malnutrition, and burns, high albumin levels are often caused by dehydration. Albumin in the urine can be indicative of kidney disease.

A doctor working for Médecins Sans Frontières at a refugee camp was concerned about the blood albumin levels of her patients. She took blood samples from each of her patients to run a test for blood albumin, and documented these results. The normal range of albumin is 3.5 to 5.5 grams per decilitre (g/dL).



i According to these results, how many of her patients have abnormal albumin levels? (1 MARK)

ii During the test, someone used an uncalibrated scale to measure the weight of the albumin in each blood sample. Identify the type of error that has occurred. Justify your response. (2 MARKS)

d In Melbourne, another doctor measured blood albumin levels in one patient from several blood samples taken during a single visit.

Sample	Blood albumin level (g/dL)
1	5.75
2	3.21
3	4.12
4	4.25

i Explain whether these results are precise. (1 MARK)

ii Based on the bioethical concept of non-maleficence, suggest whether the doctor should have taken multiple blood samples from the patient. (2 MARKS)

2B NUCLEIC ACIDS

Shopping at IKEA is always an enjoyable experience, providing an immersive adventure within their almost endless and sprawling warehouse. But after you've laid your eyes on one of their Swedish furniture masterpieces and bought it, you're faced with the challenging task of assembling it. But you, knowing that you're a genius, realise that assembling IKEA furniture is super easy. Who needs the instructions right? How hard could it be?

Image: Prachana Thong-on/Shutterstock.com

Lesson 2B

In this lesson you will learn about the functional and structural differences between the two types of nucleic acids – DNA and RNA.

◀ *Prerequisite knowledge*

Year 11
DNA is tightly packaged around histone proteins to form chromosomes within the nucleus of a cell.

Lesson 2A
DNA is responsible for storing the information required to make proteins, determining the sequence of amino acids, and allowing for the huge functional diversity of proteins.

Future applications ▶

Lesson 2D
Nucleic acids are fundamental to the production of proteins, serving a role in the transcription of DNA into mRNA, the formation of ribosomes, and the assembly of amino acids into polypeptides.

Chapter 4
Researchers are capable of manipulating DNA using a variety of different mechanisms, allowing them to edit an organism's genome and produce genetically modified organisms.

Study design dot point

- nucleic acids as information molecules that encode instructions for the synthesis of proteins: the structure of DNA, the three main forms of RNA (mRNA, rRNA, and tRNA) and a comparison of their respective nucleotides

Key knowledge units

Introduction to nucleic acids	3.1.1.1
DNA	3.1.1.2
RNA	3.1.1.3

Introduction to nucleic acids 3.1.1.1

OVERVIEW

Nucleic acids are polymers of nucleotide monomers, which not only store genetic information, but also form molecules that aid in the production of proteins.

THEORY DETAILS

Found in every living organism on Earth, **nucleic acids** are large **polymers** composed from **nucleotide monomers** that store genetic information and help produce the proteins required for survival. There are two types of nucleic acids – **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**. While there are several distinct differences between DNA and RNA nucleotides, at a fundamental level, they both follow the same basic structure (Figure 1).

nucleic acid the class of macromolecule that includes DNA and RNA. All nucleic acids are polymers made out of nucleotide monomers

polymer a large molecule that is made up of small, repeated monomer subunits

Every nucleotide includes:

- a phosphate group
- a five-carbon (pentose) sugar
- a nitrogen-containing base.

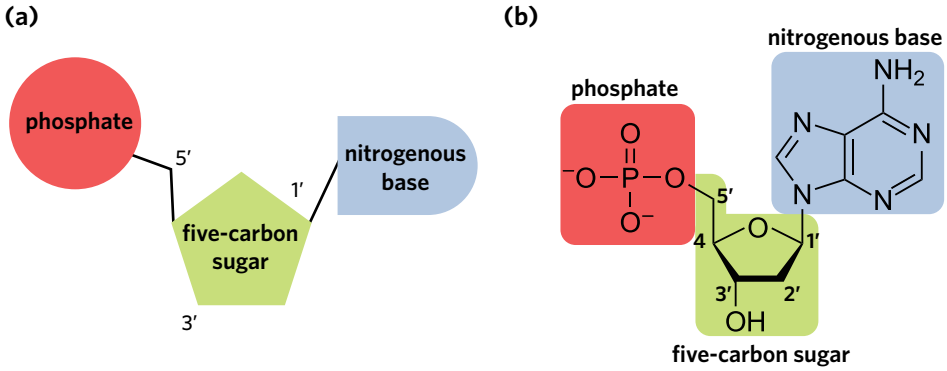


Figure 1 **(a)** The basic structure of a nucleotide and **(b)** the chemical structure of a DNA nucleotide

Within the five-carbon sugar, each carbon is assigned a number in a clockwise direction, with the first carbon being labelled 1' (one prime) and the last carbon being labelled 5' (five prime). The three carbons of particular interest include:

- 1' which attaches to the nitrogenous base
- 3' which attaches to the phosphate of the following nucleotide
- 5' which attaches the five-carbon sugar to the phosphate group of the nucleotide.

Therefore, the 3' and 5' ends of nucleotides are significant in contributing to the directional nature of nucleic acids (Figure 2).

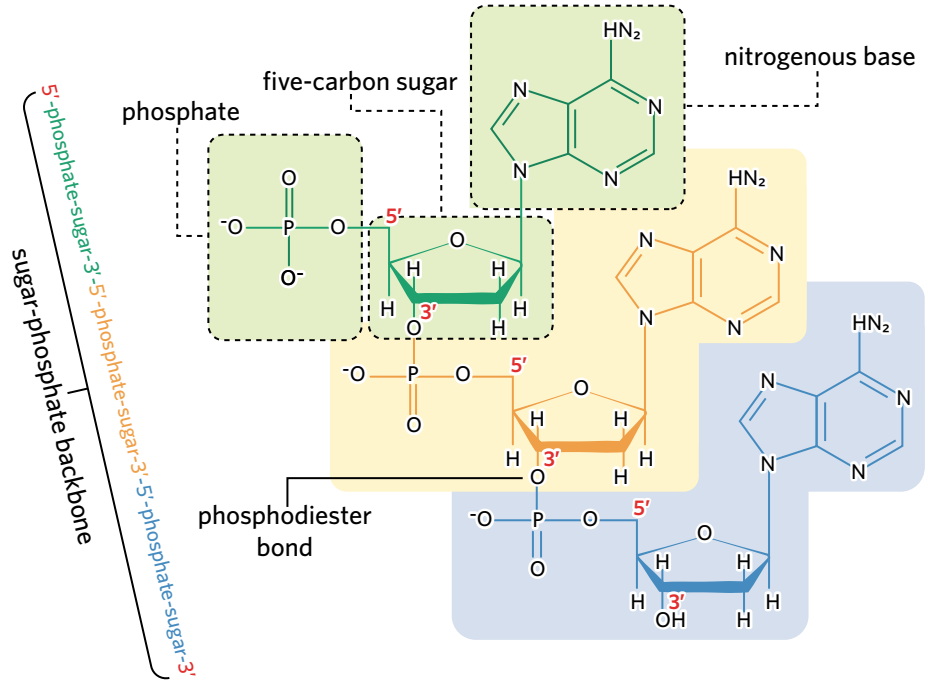


Figure 2 A polymer of nucleotides joined together

When many nucleotides bond together, they form a polynucleotide chain. The bonds joining nucleotides are strong covalent bonds known as **phosphodiester bonds**, which form via **condensation reactions** and exist between the sugar group of one nucleotide and the phosphate group of another. The linkage of sugars and phosphate groups is commonly referred to as the **sugar-phosphate backbone** of nucleic acids.

nucleotide the monomer subunit of nucleic acids. Made up of a nitrogen-containing base, a five-carbon sugar molecule (ribose in RNA and deoxyribose in DNA), and a phosphate group

monomer a molecule that is the smallest building block of a polymer

DNA (deoxyribonucleic acid) a double-stranded nucleic acid chain made up of nucleotides. DNA carries the instructions for proteins which are required for cell and organism survival

RNA (ribonucleic acid) a single-stranded nucleic acid chain made up of nucleotides. Includes mRNA, rRNA, and tRNA

Theory in action

Check out scientific investigation 2.1 to put this into action!

phosphodiester bond a strong covalent bond linking a five-carbon sugar to a phosphate group

condensation reaction a reaction where two monomers join to form a larger molecule, producing water as a by-product

sugar-phosphate backbone a strong covalently linked chain of five-carbon sugar molecules and phosphate groups in a nucleic acid chain

DNA 3.1.1.2

OVERVIEW

Deoxyribonucleic acid (DNA) consists of two strands of nucleotides bonded together via complementary base pairing, forming a double-helix which runs in an antiparallel fashion.

THEORY DETAILS

Inside the nucleus of human eukaryotic cells, DNA is packaged into 46 **chromosomes**, each of which contains tens of thousands of different **genes**. Each of these genes carries the instructions required to make a protein. Therefore, as DNA determines the structure of a protein, and proteins play a vital role in the structure and function of cells and tissues, DNA is essential for life.

chromosome a structure made of protein and nucleic acids that carries genetic information

gene a section of DNA that carries the code to make a protein

Examiners' tip

Be aware that DNA is found in places besides the nucleus. For example, the mitochondria and chloroplasts have their own DNA. Additionally, as prokaryotes don't have nuclei, their circular chromosome is located within the nuclear region of the cytoplasm.

The complete set of DNA in an organism is referred to as the **genome**. In order for life to continue, DNA, and by extension the traits it codes for, must be heritable and passed down from parents to their children.

genome the complete set of DNA housed within an organism

antiparallel a characteristic of DNA strands describing how each strand runs in an opposite direction to the other. One strand runs in a 3′ → 5′ direction and the other runs in a 5′ → 3′ direction

complementary base pairing describes which nucleotides can form hydrogen bonds with each other. C pairs with G, A pairs with T (or U in RNA)

Structure of DNA

DNA is composed of two polynucleotide chains which run **antiparallel** to each other, meaning that while one strand runs in a 3' to 5' direction, the other runs in a 5' to 3' direction. The two chains are subsequently joined together via the rules of **complementary base pairing**, which dictates the pairs of nucleotides that can join together to form hydrogen bonds with each other. Each DNA nucleotide is composed of a phosphate group, a deoxyribose sugar, and one of four possible nitrogenous bases – adenine, thymine, cytosine, or guanine (Figure 3). The base pairing rules are:

- adenine (A) will always form a pair with thymine (T)
- guanine (G) will always form a pair with cytosine (C).

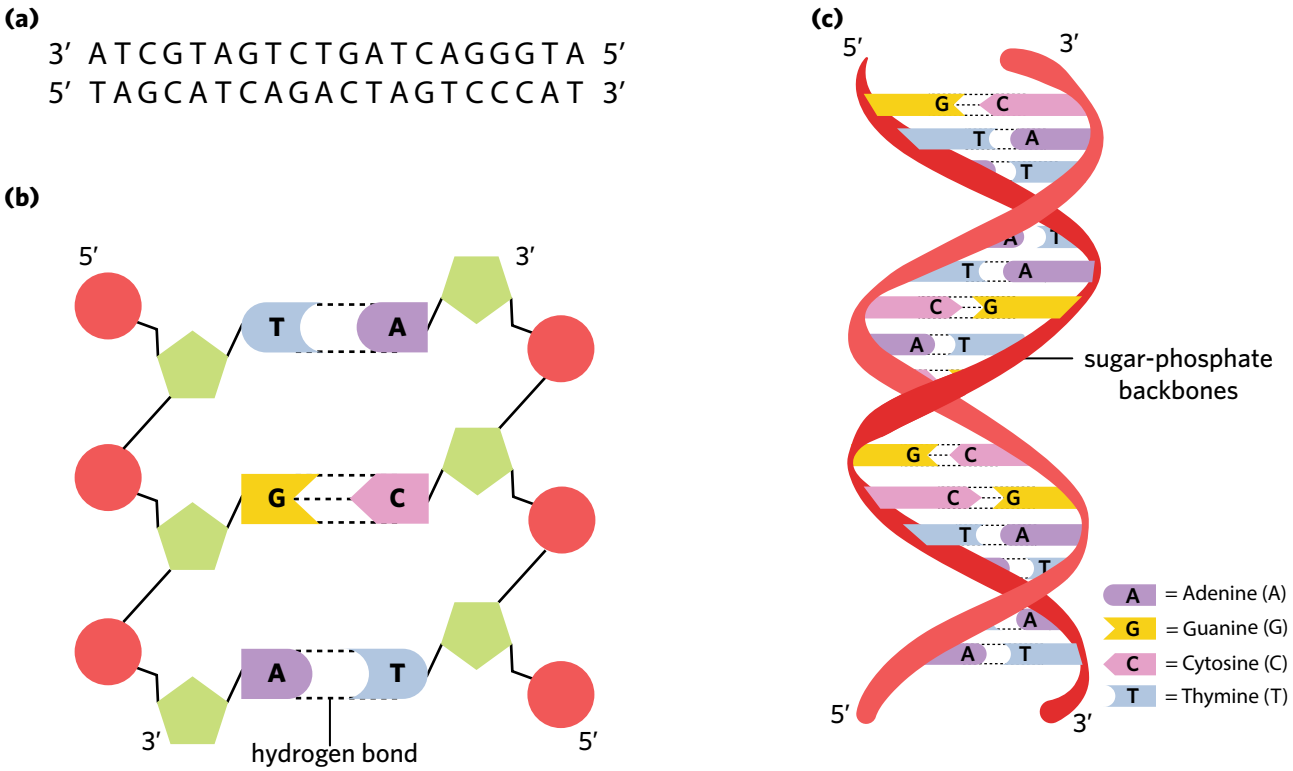


Figure 3 **(a)** DNA can be represented using the letters corresponding to each nitrogenous base and **(b)** with complementary base pairing, it forms a double stranded molecule **(c)** which is wound as a double helix.

By understanding complementary base pairing, it is possible to predict the nucleotide sequence of a strand of DNA given the nucleotide sequence of the complementary strand. Furthermore, using the same rules, it is also possible to deduce that in a double-stranded DNA molecule, there will always be equal numbers of A and T nucleotides, and equal numbers of G and C nucleotides.

Given the sheer length of DNA (the human nuclear genome is approximately three billion base pairs long or 1.8 m in length), DNA needs to be compressed and stored effectively. To do this, the two strands of DNA twist around each other, forming a **double helix**, which can help compress and store DNA. In **nuclear DNA**, this helix structure also coils around proteins known as histones, which then condense further to form tightly packed chromosomes (Figure 4).

double helix the structure of double-stranded DNA in the nucleus of eukaryotic cells, where each DNA strand wraps around a central axis

nuclear DNA DNA that is located in the nucleus of a cell

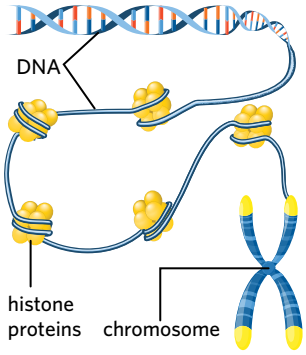


image: Designua/Shutterstock.com

Figure 4 The packaging of nuclear DNA into chromosomes

RNA 3.1.1.3

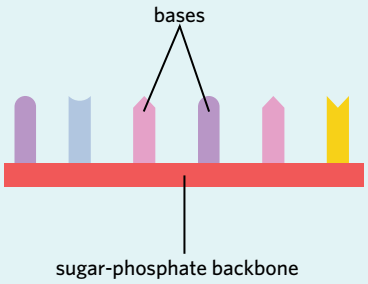
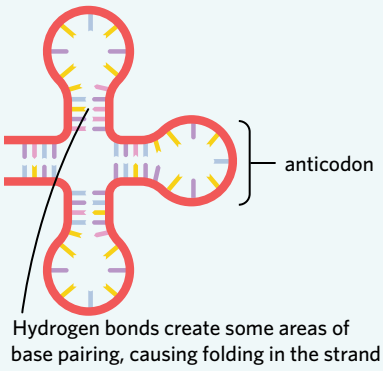
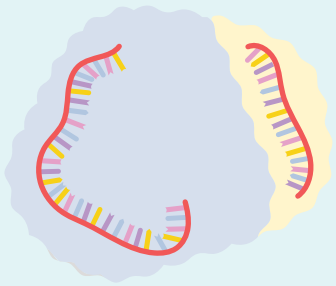
OVERVIEW

Ribonucleic acid (RNA) is a single strand of nucleotides that comes in a variety of different forms and is found in many different parts of the cell.

THEORY DETAILS

While RNA serves many different functions within cells, it is primarily involved in the synthesis of proteins. There are several different types of RNA, including **messenger RNA (mRNA)**, **transfer RNA (tRNA)**, and **ribosomal RNA (rRNA)** (Table 1).

Table 1 The three types of RNA and their corresponding function and structure

RNA	Function	Diagram
messenger RNA (mRNA)	Carries genetic information from the nucleus to the ribosomes for protein synthesis	
transfer RNA (tRNA)	Delivers specific amino acids to the ribosome after recognising specific nucleotide sequences on mRNA	
ribosomal RNA (rRNA)	Serves as the main structural component of ribosomes within cells	

messenger RNA (mRNA) RNA molecules that are produced during transcription and carry genetic information from the nucleus to the ribosomes

transfer RNA (tRNA) RNA that recognises specific codons on the mRNA strand and adds the corresponding amino acid to the polypeptide chain during protein synthesis

ribosomal RNA (rRNA) RNA that is a key structural component of ribosomes, which assemble proteins

Lesson link

The roles of mRNA, tRNA, and rRNA will be further explored in ***lesson 2D***, which delves into the formation of proteins through the processes of transcription and translation.

Structure of RNA

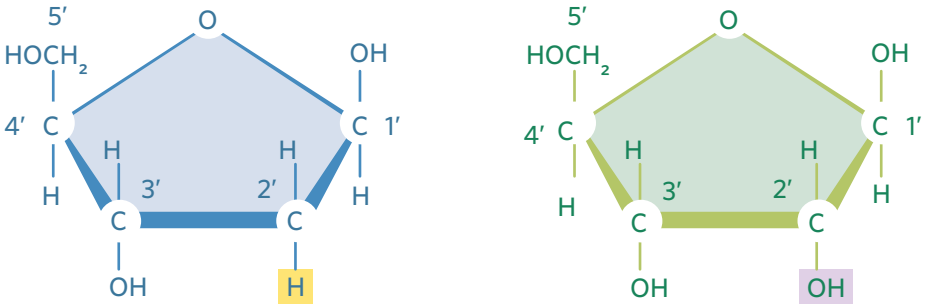
The structure of RNA is relatively similar to that of DNA. However, instead of a deoxyribose sugar, RNA contains a ribose sugar, and instead of thymine, RNA contains another nitrogenous base known as uracil. RNA is also single-stranded instead of double-stranded. Additionally, while DNA is inherited from generation to generation, RNA is typically synthesised on demand. These differences are summarised in Table 2.

Table 2 The differences between DNA and RNA

	DNA	RNA
Structure	Double-stranded	Single-stranded
Sugar	Deoxyribose sugar	Ribose sugar
Nucleotides	Adenine, thymine, cytosine, guanine	Adenine, uracil, cytosine, guanine
Lifetime	Inherited, long-term storage	Temporary, short-lived molecules

While RNA is single-stranded, the principle of complementary base pairing still exists and can help RNA fold into many different structures. In RNA, adenine pairs with uracil (A–U) and guanine pairs with cytosine (G–C).

The main difference between ribose sugar and deoxyribose sugar is the presence or absence of an oxygen atom at the 2' position of the five-carbon sugar. This can be easily remembered through the extended names of DNA and RNA, with deoxy- signifying the absence of oxygen (Figure 5).



Deoxyribose **Ribose**

Figure 5 The difference between deoxyribose and ribose sugar

> ***Examiners' tip***
>
> DNA tends to be double stranded (dsDNA) and RNA tends to be single stranded (ssRNA). However, like most things in biology, there are always exceptions to the rule. Some bacterial viruses (such as those in the Microviridae family) contain single-stranded DNA (ssDNA) and other viruses, including rotaviruses, contain double-stranded RNA (dsRNA).
>
> When asked to differentiate between DNA and RNA, if possible, you should rely on the nucleotides present in a strand, as DNA will contain thymine whilst RNA will contain uracil. To double check, you can also look at the five-carbon sugar found within each nucleotide – DNA contains one less oxygen molecule than RNA on the 2′ carbon.

Theory summary

Nucleic acids are responsible not only for carrying genetic information, but also for synthesising proteins. There are two types of nucleic acids – deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which are each composed of a phosphate group, a five-carbon sugar, and a nitrogenous base. Differences between DNA and RNA include the sugar molecule present, the nitrogenous bases present, and whether they form single or double strands (Table 3).

Table 3 Similarities and differences between DNA and RNA

	DNA	RNA
Similarities	• nucleotides follow the same basic structure (phosphate group, five-carbon sugar, nitrogen-containing bases) • contain the nucleotides adenine, guanine, and cytosine • contain a sugar phosphate backbone • follow the complementary base pairing rule: C pairs with G, A pairs with T (or U)	
Differences	• nucleotides contain a deoxyribose sugar • contains the base thymine (T) • double-stranded • inherited/long-term storage	• nucleotides contain a ribose sugar • contains the base uracil (U) • single-stranded • temporary molecules

There's a reason for IKEA printing instructions – and wasting paper is certainly not one of them. Instructions are included so that you can accurately and efficiently assemble your IKEA furniture. Just like our cells, nucleic acids, and in particular DNA and RNA, form the instructions for the production of proteins, and without them life would not be able to exist.

Image: Nattapat.J/Shutterstock.com

2B QUESTIONS

Theory review questions

Question 1

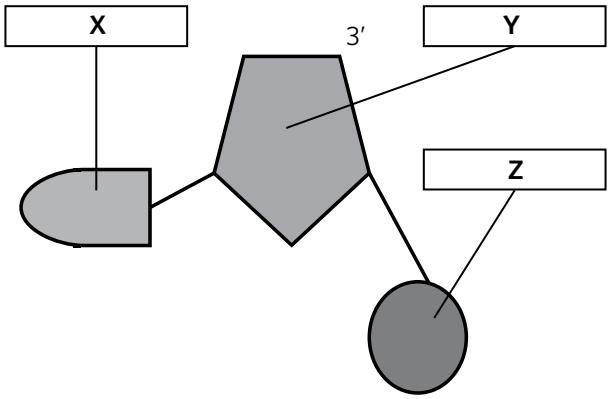
Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- uracil
- ribose
- thymine
- guanine
- cytosine
- polymers
- monomers
- deoxyribose
- single-stranded
- double-stranded

Nucleic acids are _____of nucleotide _____. While DNA is composed of a _____ sugar, RNA is composed of a _____ sugar. Additionally, DNA contains the nitrogenous base _____, whereas RNA contains the nitrogenous base _____. DNA is also a _____ molecule, allowing it to form a double helix, whereas RNA is a _____ molecule.

Question 2

Label the parts of the nucleotide.



Question 3

Which one of the following sequences correctly represents DNA?

A	3′ A A A T C G T C A T 5′ 3′ T T T A G C A G T A 5′
B	3′ G G T A A A T T T T G U A 5′ 5′ C C A T T T A A A A C A T 3′
C	3′ A A T G C T A T G C A T C G A T C C 5′ 5′ T T A C G A T A C G T A G C T A G G 3′
D	5′ A A T G C G C T G C U T C A T G T T A A G 3′ 5′ T T A C G C G A C G A A G T A C A A T T C 5′

Question 4

Match each type of RNA to its appropriate description.

RNA	Description
• mRNA	**I** _____ serves as the main structural component of ribosomes within cells
• rRNA	**II** _____ carries genetic information from the nucleus to the ribosomes for protein synthesis
• tRNA	**III** _____ delivers specific amino acids to the ribosomes after recognising specific nucleotide sequences

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–8.

In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Physiology or Medicine for the discovery of the structure of DNA as well as its shape as a double helix. However, the absence of Rosalind Franklin, another researcher who heavily contributed to the discovery, is certainly notable. Unfortunately, her pivotal role in contributing to the discovery has been largely forgotten, shrouding the work in controversy.

In fact, the discovery of the structure of DNA would not have been possible if it were not for the work of Rosalind Franklin. Her work and expertise in the field of X-ray crystallography, which is a technique used to photograph the molecular structure of compounds, provided the crucial X-ray photographs that were used to interpret and determine the structure of DNA. While Franklin was not able to fully interpret the photographs herself, Watson and Crick obtained the photographs through unconventional methods without asking for her permission, effectively stealing her work. Then, through their interpretation of her work, they were finally able to determine the structure of DNA.

Unfortunately, Franklin passed away in 1958 from ovarian cancer and due to the stringent rules of the Nobel Prize, recipients must be alive to receive the award. Due to an unfortunate series of events and her premature death, she was not given the credit that she fully deserved for her contribution to the discovery of the structure of DNA.

Question 5

Rosalind Franklin could not be awarded the Nobel Prize because

A to be awarded the Nobel Prize, the recipient must be alive.

B her contribution to the discovery of the structure of DNA was unrecognised.

Question 6

Reference to the double helix shape of DNA refers to it being a

A double-stranded molecule.

B single-stranded molecule.

Question 7

Without complementary base pairing, DNA would not be able to form a

A chain of nucleotides.

B double helix.

Question 8

The use of Franklin's X-ray crystallography photographs by Watson and Crick without her permission primarily violates the bioethical concept of

A non-maleficence.

B beneficence.

C integrity.

D respect.

Exam-style questions

Within lesson

Question 9 (1 MARK)

A particular DNA double helix is 100 nucleotide pairs long and contains 40 cytosine bases. The number of adenine bases in this DNA double helix would be

A 10.

B 20.

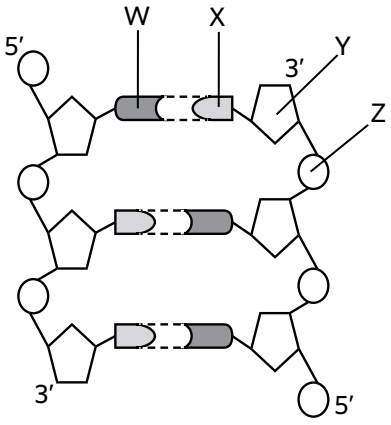
C 40.

D 60.

Adapted from VCAA 2012 Exam 1 Section A Q5

Use the following information to answer Questions 10 and 11.

The following diagram represents a chain of nucleic acids.



Question 10 (1 MARK)

A nucleic acid is made up of nucleotides which are linked by a sugar-phosphate backbone.

According to the diagram, structure(s)

A Y and Z must make up the sugar-phosphate backbone.

B Y and X must make up the sugar-phosphate backbone.

C Y is a ribose sugar molecule.

D X is a phosphate group.

Adapted from VCAA 2015 Section A Q3

Question 11 (1 MARK)

If the nucleotide structure W is the base thymine, then

A sub-unit X must be the base uracil.

B sub-unit X must be the base adenine.

C sub-unit Y must be the base cytosine.

D sub-unit X must be the nucleotide adenine.

Question 12 (1 MARK)

Which one of the following rows correctly describes a difference between DNA and RNA?

	DNA contains	RNA contains
A	the same number of guanine and thymine nitrogen bases	a different number of guanine and thymine nitrogen bases
B	ribose sugar	deoxyribose sugar
C	the nitrogen base thymine	the nitrogen base uracil
D	no hydrogen bonding between strands	hydrogen bonding between complementary strands

Adapted from VCAA 2017 Sample Exam Section A Q2

Question 13 (1 MARK)

A gene involved in the function of the male reproductive system has been sequenced. A small section of this gene is shown in the diagram.

The sequence of nucleotides on the complementary sequence of DNA would be

A GCACUCCGGU.

B CGTGAGGCCA.

C GCACTCCGGT.

D TCCAGAATTG.

Question 14 (1 MARK)

A particular mRNA strand is 50 nucleotide bases long and contains 10 adenine bases. The number of thymine bases in this mRNA strand would be

A 0.

B 10.

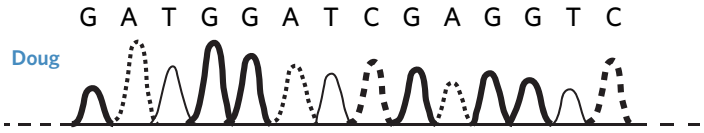
C 40.

D 50.

Adapted from VCAA 2012 Exam 1 Section A Q5

Question 15 (1 MARK)

A fragment of DNA on chromosome 7 from a person, Doug, is sequenced. The nucleotide sequence is shown.



For the sequence of nucleotides shown, the total number of cytosine bases on the complementary strand would be

A 0.

B 2.

C 6.

D 8.

Adapted from VCAA 2012 Exam 2 Section A Q15

Multiple lessons

Question 16 (1 MARK)

Genes

A are made up of the five nucleotide bases: adenine, thymine, cytosine, guanine, and uracil.

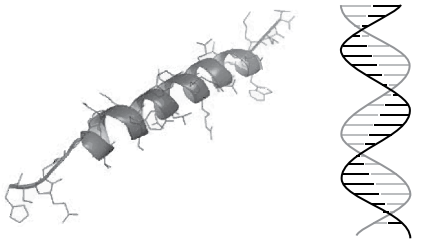
B contain the genetic information required to make proteins.

C can only be found in the nuclear DNA of a eukaryotic cell.

D are made up of amino acid monomers.

Use the following information to answer Questions 17 and 18.

The diagrams represent two of the four major groups of biomacromolecules.



Group A Group B

Question 17 (1 MARK)

Each monomer of a macromolecule from Group A is made up of a

A carboxyl group, an R group, and an amino group.

B carboxylic acid, an R group, and an amino acid group.

C ribose sugar, a phosphate group, and a nitrogen-containing base.

D deoxyribose sugar, a phosphate group, and a nitrogen-containing base.

Adapted from VCAA 2016 Section A Q3

Question 18 (1 MARK)

A feature that can be seen in the diagram of the macromolecule in Group B is

A its deoxyribose subunits.

B the double-helical structure of DNA.

C the complementary base pairing of C–G and A–U.

D the antiparallel arrangement of two complementary strands of amino acids.

Adapted from VCAA 2015 Section A Q4

Question 19 (1 MARK)

Nitrogen is an essential component of many of the molecules needed for growth and reproduction. Some bacteria live in the root systems of certain plant species, taking the nitrogen from our atmosphere and producing nitrogen-containing compounds (such as ammonia). These compounds can then be taken up by the plants and used to produce molecules that are essential for life. Soils lacking in these bacteria and nitrogen-containing fertilisers may become nitrogen deficient. Plants growing in these soils may be unable to produce sufficient levels of

A carbohydrates.

B nucleic acids.

C fatty acids.

D cellulose.

Adapted from VCAA 2012 Exam 1 Section A Q6

Question 20 (4 MARKS)

Researchers studying macromolecules found two tightly packed polymers in the nucleus of an animal cell. A short section of these macromolecules is shown in the diagram.

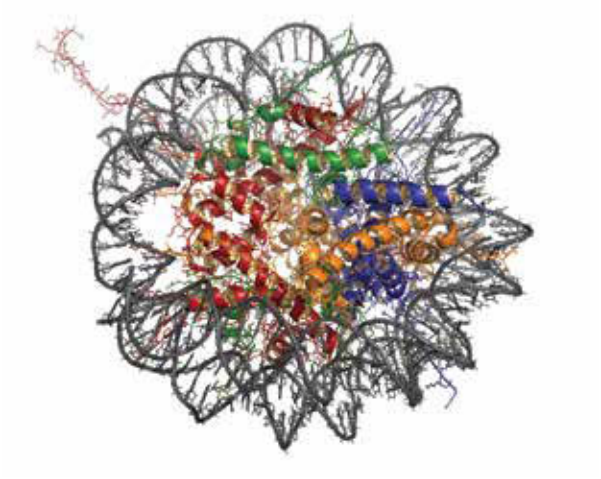


Image: StudioMolekuul/Shutterstock.com

a Name the two types of macromolecules shown in the diagram. (2 MARKS)

b Identify the monomers of the two types of macromolecules identified in part a. (2 MARKS)

Adapted from VCAA 2014 Section B Q9

Key science skills and ethical understanding

Question 21 (10 MARKS)

Two scientists were busy analysing two biological polymer samples.

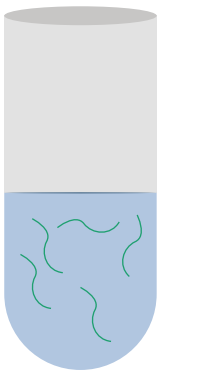
a All polymers are made up of repeating sequences of monomers. Scientists can determine the sequence of monomers in a sample by sequencing the sample.

i What information is obtained from protein sequencing? (1 MARK)

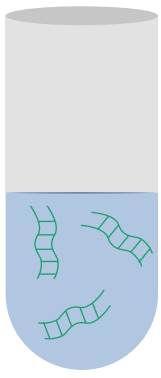
ii What information is obtained from gene sequencing? (1 MARK)

Adapted from VCAA 2017 Northern Hemisphere Exam 1 Section B Q7

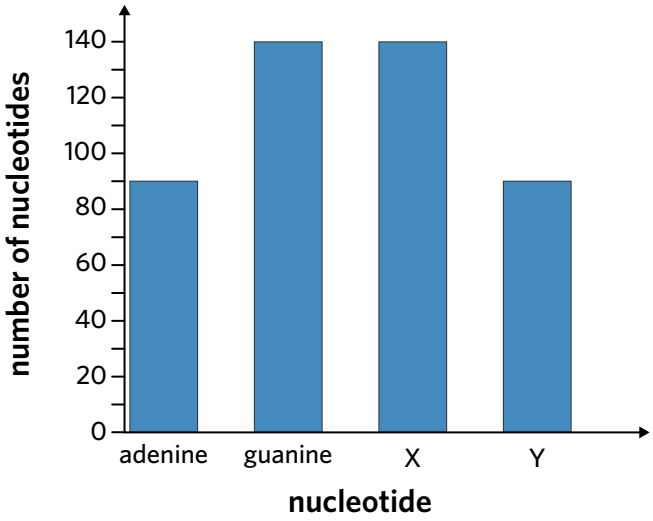
b Sample 1 only contained single-stranded sequences of DNA while sample 2 contained double-stranded sequences of DNA. After sequencing one of the samples, they plotted the number of individual nucleotides on a graph.



Sample 1



Sample 2



i Identify the nucleotides represented by the bars labelled X and Y on the graph. (1 MARK)

ii Scientist A argued that the graph shows data from Sample 1, while Scientist B argued that it is more likely that the graph shows data from Sample 2. Which scientist is correct? Explain your reasoning. (2 MARKS)

iii What is the length of the DNA strand in the sequenced sample? (1 MARK)

iv Draw a labelled diagram of a single monomer of the macromolecule found in Sample 2. (2 MARKS)

Adapted from VCAA 2014 Section B Q9

c When obtaining DNA samples from other individuals, consent must be given prior to the extraction of DNA. Identify the bioethical concept which researchers must follow in order to satisfy this requirement. Justify your response. (2 MARKS)

2C GENES

01000101 01100100 01110010 01101111 01101100 01101111 00100000 00100011 00110001.

Zeros and ones form the foundation of all your electronic devices through what is known as the binary code. The binary code consists of different sequences and combinations of zeroes and ones that can be translated into specific functions. Can you decipher the code written above? But more importantly, is there a similar phenomenon occurring in our own cells? How do our cells store genetic information? Is there another code that can be used?

Image: SVshot/Shutterstock.com

Lesson 2C

In this lesson you will learn how the genetic code enables nucleic acids to encode the information required for protein synthesis as well as the general structure of genes.

◀ *Prerequisite knowledge*

Lesson 2A
The genetic code provides a series of rules followed by living organisms via the production of proteins.

Lesson 2B
Genes are sections of DNA that carry the instructions required to make a protein. Important properties of DNA include its double-stranded helical structure, as well as its directional nature.

Future applications ▶

Lesson 2D
Gene expression involves the production of functional proteins through the processes of transcription, RNA processing, and translation.

Lesson 9A
Genes can often be altered through the occurrence of mutations, which involves permanent changes to the DNA sequence of genes, potentially leading to abnormally functioning proteins.

Study design dot points

- the genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells, and translation by ribosomes
- the structure of genes: exons, introns, and promoter and operator regions

Key knowledge units

DNA to protein	3.1.2.1
Gene structure	3.1.3.2

DNA to protein 3.1.2.1

OVERVIEW

Protein synthesis relies on the existence of the genetic code, which is a series of rules that determine how genetic information is transcribed and translated into functional proteins.

THEORY DETAILS

Cells produce proteins by reading and interpreting the genetic information stored within **genes** through a series of processes known as **transcription**, RNA processing (post-transcriptional modifications), and **translation**. During transcription and RNA processing, the DNA sequence of the gene is copied into RNA nucleotides in the form of **mRNA**. The mRNA is then decoded during translation to specify the sequence of amino acids required for the polypeptide chain. These processes are only possible due to the existence of the **genetic code** (Figure 1).

gene a section of DNA that carries the code to make a protein

transcription the process whereby a sequence of DNA is used as a template to produce a complementary sequence of mRNA

translation the process where an mRNA sequence is read to produce a corresponding amino acid sequence to build a polypeptide

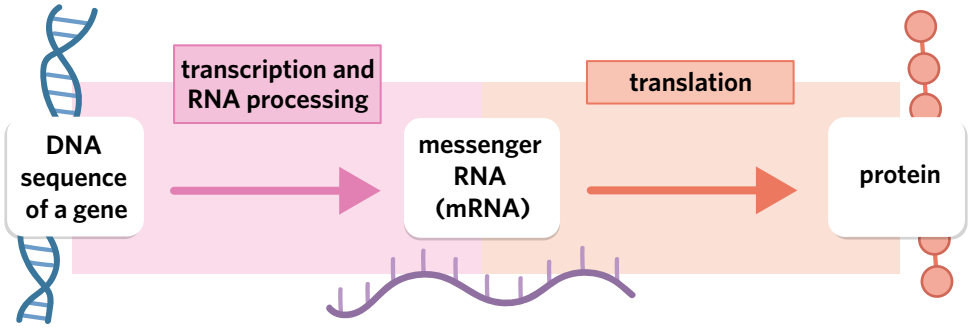


Figure 1 Protein synthesis involves the processes of transcription, RNA processing, and translation.

The genetic code is a series of rules that define how genetic information stored within nucleotides is transcribed and translated into functional proteins. The foundation of the genetic code relies on the grouping of adjacent nucleotides into groups of three. In DNA, a group of three adjacent DNA nucleotides is known as a **triplet**, and when a DNA triplet is transcribed into an mRNA molecule, the three nucleotides become known as a **codon**.

Codons and triplets are crucial to the production of proteins, as each triplet or codon codes for a specific amino acid in the final polypeptide chain. There are also specific triplets and codons that instruct the cell to start and stop protein synthesis. In doing so, they determine which nucleotides are transcribed and translated into a functional protein and explain why the order of nucleotides or codons essentially determines the order of amino acids in a protein (Figure 2).

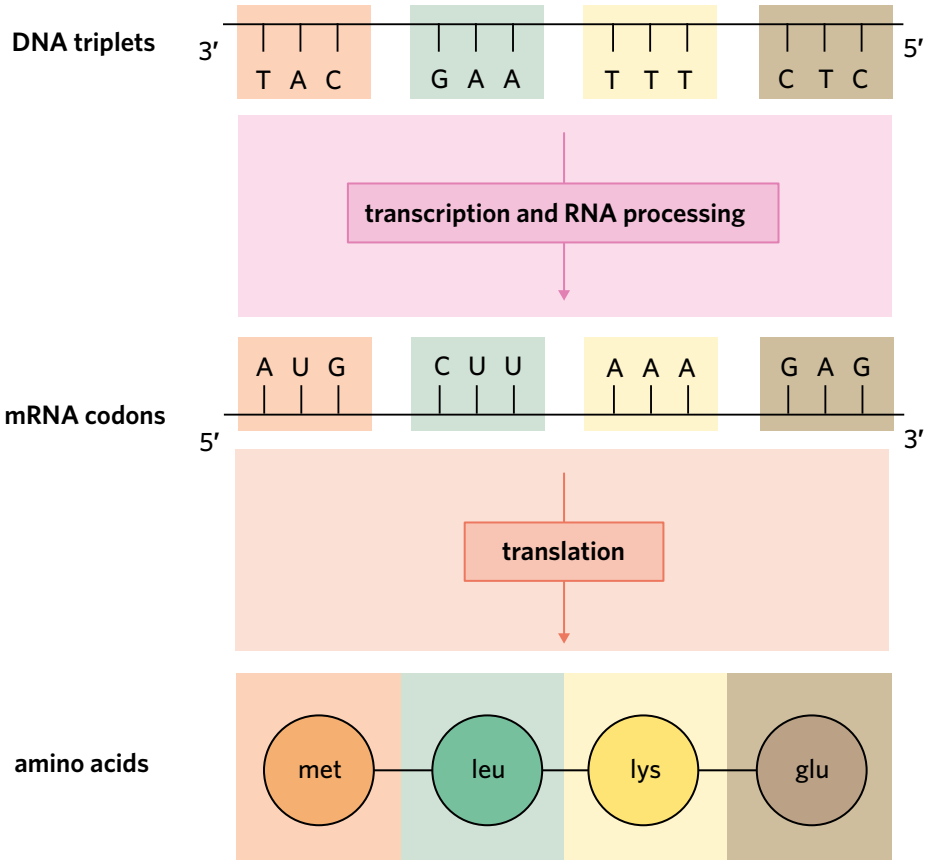


Figure 2 Triplets and codons are groups of three nucleotides, coding for specific amino acids.

To determine the amino acid coded for by a specific codon, a codon table can be used (Figure 3). When using a codon table, simply begin at the left-hand side, which refers to the first base of the codon. Next, observe the top of the table, which refers to the second base of the codon, and finally, observe the right-hand side, which refers to the third base of the codon.

Also note that while the **start codon** (AUG) codes for the amino acid methionine, signalling the initiation of translation, the **stop codons** (UAA, UAG, UGA), which signal for the termination of translation, do not code for a specific amino acid.

messenger RNA (mRNA) RNA molecules that are produced during transcription and carry genetic information from the nucleus to the ribosomes

genetic code the set of rules by which information is encoded in genetic material

Lesson link

The processes of transcription, RNA processing, and translation are explored in further detail in ***lesson 2D***.

triplet the sequence of three nucleotides in DNA coding for one amino acid

codon the sequence of three nucleotides in mRNA coding for one amino acid

start codon the sequence of three nucleotides in mRNA that signals the start of translation

stop codon the sequence of three nucleotides in mRNA that signals the end of translation

Second Base									
First Base	U		C		A		G		Third Base
U	UUU	phe	UCU	ser	UAU	tyr	UGU	cys	U
	UUC		UCC		UAC		UGC		C
	UUA	leu	UCA		UAA	STOP CODON	UGA	STOP CODON	A
	UUG		UCG		UAG		UGG	trp	G
C	CUU	leu	CCU	pro	CAU	his	CGU	arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	ile	ACU	thr	AAU	asn	AGU	ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	lys	AGA	arg	A
	AUG	met (START CODON)	ACG		AAG		AGG		G
G	GUU	val	GCU	ala	GAU	asp	GGU	gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	glu	GGA		A
	GUG		GCG		GAG		GGG		G

Figure 3 The codon table provides the amino acid coded for by each specific codon.

Examiners' tip

The VCAA does not require students to memorise the codon table or remember all of the different amino acids. However, they do frequently supply the table on exams and ask students to extract information from it. The amino acid table can also be displayed for DNA triplets instead of RNA codons, requiring students to look for thymine instead of uracil.

Aside from the fact that the genetic code is based on groupings of three adjacent nucleotides, some other properties of the genetic code are summarised in Table 1.

Table 1 Properties of the genetic code

Property	Description	Image
Universal	Nearly all living organisms use the same codons to code for specific amino acids.	codon → amino acid UUA → leucine
Unambiguous	Each codon is only capable of coding for one specific amino acid. For example, the codon UUA only codes for the amino acid leucine.	
Degenerate	While each codon only codes for one amino acid (unambiguous), each amino acid may be coded for by multiple different codons (degenerate). For example, both the codons UUA and UUG code for the amino acid leucine. This provides a degree of redundancy, where changes to the original DNA sequence through mutations may not necessarily lead to the insertion of a different amino acid.	codons → amino acid UUA, UUG, CUU, CUC, CUA, CUG → leucine
Non-overlapping	Each triplet or codon is read independently, without overlapping from adjacent triplets or codons.	mRNA: A U U C G A A A C 1 2 3

Gene structure 3.1.3.2

OVERVIEW

Genes can be composed of many different components, including a promoter region, introns, exons, termination sequences, and operator regions.

THEORY DETAILS

Within each gene there are several different regions, each with its own specific function. The purposes of these regions will become more apparent in lesson 2D, when we consider the processes of transcription, RNA processing, and translation in more detail (Table 2).

Table 2 The key regions of genes

Region	Description
Promoter	The **promoter** region is an upstream (5′ end) binding site for **RNA polymerase**, which is an **enzyme** responsible for transcription. When RNA polymerase binds to the promoter region of a gene, it allows for the transcription of that particular gene. Therefore, the promoter region effectively denotes the starting position and direction of transcription. In eukaryotes, the promoter region is often the sequence of bases 'TATAAA', commonly known as the **TATA box**.
Introns	**Introns** are regions of non-coding DNA that do not contribute to the final protein as they are removed during RNA processing (post-transcriptional modifications). Importantly, only eukaryotic genes contain introns – prokaryotic genes do not contain introns.
Exons	**Exons** are regions of coding DNA, which are transcribed and translated into the final protein. These can be found in both eukaryotes and prokaryotes.
Termination sequence	The **termination sequence** represents a sequence of DNA that signals for the end of transcription.
Operator	The **operator** region serves as the binding site for **repressor proteins**, which can then inhibit **gene expression**. This region is typically only found in prokaryotic genes, as eukaryotes have different regions for regulating gene expression.

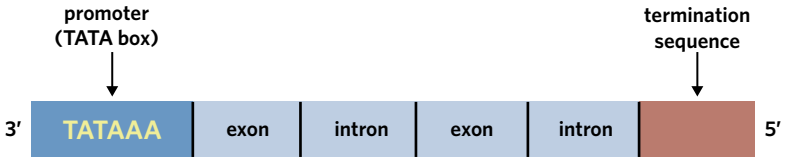


Figure 4 Eukaryotic genes often contain a promoter region, introns, exons, and a termination sequence.

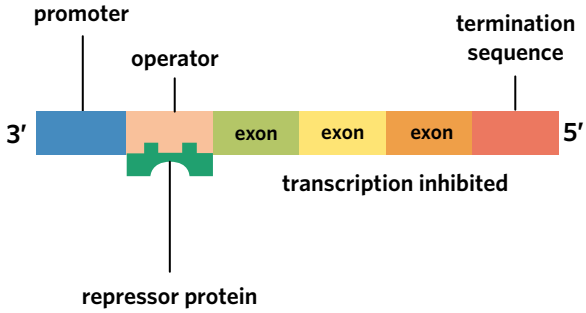


Figure 5 Prokaryotic genes often contain a promoter region, an operator region, exons, and a termination sequence.

promoter the sequence of DNA to which RNA polymerase binds

RNA polymerase the enzyme responsible for constructing a pre-mRNA sequence from a DNA sequence during transcription

enzyme an organic molecule, typically a protein, that catalyses (speeds up) specific reactions

TATA box a type of promoter region

introns non-coding regions of DNA that do not code for proteins. They are spliced out during RNA processing

exons regions of DNA that code for proteins and are not spliced out during RNA processing

termination sequence a sequence of DNA that signals the end of transcription

operator a short region of DNA that interacts with repressor proteins to alter the transcription of an operon

repressor protein a protein coded for by a regulatory gene that prevents gene expression by binding to its operator

gene expression the process of reading the information stored within a gene to create a functional product, typically a protein

Theory summary

The information required to produce proteins is stored within genes in the form of DNA triplets. Through the process of transcription, these DNA triplets are used as a template to produce mRNA codons. Then, through the process of translation, these mRNA codons are used to code for specific amino acids within a polypeptide chain. Key features of the genetic code include its universal, unambiguous, degenerate, and non-overlapping nature.

Lesson link

In ***lesson 2E***, the significance of the operator region and the repressor protein in regulating gene expression will be explored.

The genetic code is the mechanism through which our cells store genetic information. Using the different nucleotide bases – adenine, thymine, cytosine, guanine, and uracil – it is possible to construct varying sequences that come together to form triplets, codons, and anticodons. Then, through complementary base-pairing, each codon will code for a specific amino acid to form the final protein.

2C QUESTIONS

Theory review questions

Question 1

Fill in the blanks with the following terms.

- amino acid
- codon
- triplet

In the genetic code, three bases in a DNA sequence are known as a _____, while three bases in mRNA are known as a _____, and three bases in tRNA are known as an anticodon. To determine which triplets or codons correspond to which _____, a triplet or codon table can be used.

Question 2

Match the property of the genetic code to its description.

Property	Description
• universal	**I** _____ many different codons can code for the same amino acid
• degenerate	**II** _____ each codon is only capable of coding for a single amino acid
• unambiguous	**III** _____ each triplet or codon is read independently of adjacent triplets or codons
• non-overlapping	**IV** _____ nearly all living organisms use the same set of rules and codons to code for proteins

Question 3

Match the region of a gene to its description.

Region	Description
• exons	**I** _____ binding site of RNA polymerase, denoting the starting position of transcription
• introns	**II** _____ a sequence of DNA which signals for the end of transcription
• operator	**III** _____ binding site for repressor proteins
• promoter	**IV** _____ non-coding regions of DNA
• termination sequence	**V** _____ coding regions of DNA

Question 4

Categorise the following regions as **prokaryotic, eukaryotic,** or **both**.

I termination sequence _____

II promoter _____

III operator _____

IV introns _____

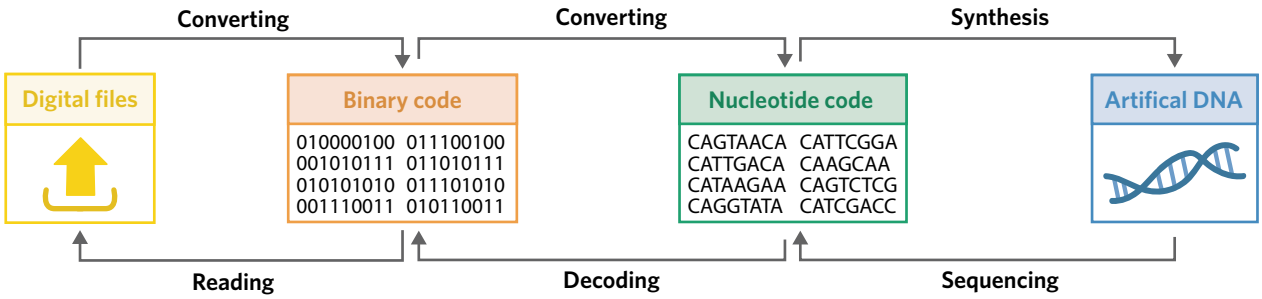
SAC skills questions

Case study analysis

Use the following information to answer Questions 5–8.

Since the beginning of the 21st century and the rise of the internet, the amount of content we consume and produce online is increasing at an exponential rate. Every minute in 2018, there were over three million Google searches, over four million videos watched on YouTube, and over 150 million emails sent. Currently, the only way of storing data is on physical hard drives, which require a significant amount of space and only last for around 100 years. Are there any alternative methods for storing data?

Fortunately for us, the answer to our data problem lies within our very own bodies! DNA, which has been used since the beginning of time to store the code for the production of proteins, has proven to be an extremely versatile and stable method of storing information. The significance of DNA as a source of data storage stems from its incredible data density, with estimates claiming that each gram of DNA could potentially house more than 250 million gigabytes of data. Scientists have already successfully stored an extract of the music from Super Mario Brothers in synthetic DNA. While the classic theme song can now be listened to by people living 100 000 years into the future, the cost of this technology is still quite high and it takes more time to extract the data in DNA compared to existing hard drives.



Question 5

Which type of data would be best to store on artificial DNA?

A Large media files, so people can watch movies whenever they want.
B Highly sensitive information that needs protection.
C Small files that are regularly accessed.
D Very large files that are rarely needed.

Question 6

The genetic code is grouped into groups of

A two nucleotides.
B five nucleotides.
C four nucleotides.
D three nucleotides.

Question 7

DNA is composed of the bases

A thymine, uracil, cytosine, guanine.
B adenine, thymine, uracil, cytosine.
C adenine, uracil, cytosine, guanine.
D adenine, thymine, cytosine, guanine.

Question 8

Which one of the following statements is incorrect?

A A protein could be used to convert the nucleotide code into artificial DNA.
B A zero or one would directly translate to an adenine, thymine, guanine, or cytosine.
C The artificial DNA would be lighter than the hard drive that the binary code is stored on.
D The artificial DNA that stores digital files would be universal, degenerate, unambiguous, and non-overlapping.

Exam-style questions

Within lesson

Use the following information to answer Questions 9–11.

Spinocerebellar ataxia type 1 is a condition that causes a loss of muscle coordination. It is caused by a mutation, which involves permanent changes to a DNA sequence, causing an increase in the number of repeats in the DNA triplet GTC. In the template strand of DNA, the regular gene sequence has between 25 and 36 GTC repeats, but in the mutant gene there are between 43 and 81 GTC repeats. The following codon table can be used to determine amino acids coded for by a nucleotide sequence.

1st position (5′ end)	2nd position				3rd position (3′ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Question 9 (1 MARK)

The corresponding codon belonging to the repeated DNA sequence is

A GTC.
B GUC.
C CAG.
D CUG.

Question 10 (1 MARK)

The amino acid coded for by the template DNA sequence GTC is

A Asn.
B Gln.
C Val.
D His.

Question 11 (1 MARK)

Thirty GTC repeats would code for

A 30 amino acids.

B 20 amino acids.

C 10 amino acids.

D 90 amino acids.

Adapted from VCAA 1998 CAT3 Article 1

Question 12 (1 MARK)

Bacteria, viruses, humans, and koalas all use the same triplet code to produce proteins. Which property of the genetic code does this refer to?

A universal

B degenerate

C unambiguous

D non-overlapping

Multiple lessons

Question 13 (7 MARKS)

Myosin is a contractile protein involved in the movement of muscles. It functions via the conversion of chemical energy in the form of ATP into mechanical energy, thereby causing muscle contraction. Myosin is a protein composed of many different polypeptide chains. The following codon table can be used to determine amino acids coded for by a nucleotide sequence.

1st position (5′ end)	2nd position				3rd position (3′ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

a The following mRNA sequence describes a small segment of one of the polypeptide chains of myosin – AUG CUU AUU ACU GGG GAG UCU GGU GCC.

i Using the table provided, what is the amino acid sequence that the mRNA sequence codes for? (1 MARK)

ii What is the corresponding DNA template strand to the mRNA sequence? (1 MARK)

b Identify the level of protein structure that myosin folds into. Justify your response. (2 MARKS)

c Identify three key differences in structure between mRNA and DNA. (3 MARKS)

Question 14 (5 MARKS)

A recent study has suggested that mutations, which are permanent changes to a DNA sequence, occurring within the *p53* gene in humans represent a significant risk for the development of certain cancers.

a While mutations can often lead to the production of polypeptides with different amino acid sequences, sometimes mutations still produce the same amino acid. Explain how this may occur. (2 MARKS)

b There are many different regions within a gene that are crucial to its functioning.

i Describe the difference between introns and exons. (1 MARK)

ii Explain whether an operator region would be found within the *p53* gene of humans. (1 MARK)

iii Describe the purpose of the promoter region of a gene. (1 MARK)

Adapted from VCAA 1999 CAT3 Article 4

Key science skills and ethical understanding

Question 15 (8 MARKS)

Mutations, which involve permanent changes to a DNA sequence, are primarily responsible for the development of cancers. This is because when mutations occur, they affect the DNA sequence of a gene, leading to the production of different amino acids and potentially causing proteins to malfunction. While mutations often occur spontaneously, there are also many environmental factors that can cause them, such as UV radiation. For example, UV radiation from the sun has been attributed to a high rate of skin cancer.

In an effort to investigate the effect of UV radiation on the development of skin cancer, researchers recruited a small group of mice. The group of mice were divided into two groups, with one group exposed to UV radiation while the other group lived in the dark. After several weeks, they were assessed for the formation of skin cancer.

a Identify the dependent and independent variables in the researchers' experiment. (2 MARKS)

b Identify a possible hypothesis for the experiment. (1 MARK)

c Identify two factors that should be controlled within the experiment. (2 MARKS)

d Suggest how the researchers could increase the precision of their experiment. (1 MARK)

e Describe the relevance of the bioethical concept of beneficence to the experiment conducted by the researchers. (2 MARKS)

2D GENE EXPRESSION

Never been to a restaurant before? Well lucky for you, here's a simple guide on what to do when you visit a restaurant:

- *Decide what to eat by looking at the menu.*
- *Call the waiter and let them know what you'd like to order.*
- *The waiter will write down your order and pass it on to the chef.*
- *The chef will receive your order and begin making your food.*
- *The waiter will bring the cooked food to your table.*

Something similar to this process is happening inside the cells in your body. Who's the waiter of the human body? Who's the chef of the human body? Is there somewhere angry patrons can write reviews about the service they receive?

Image: Semen Kuzmin/Shutterstock.com

Lesson 2D

In this lesson you will learn how genes are expressed to produce proteins through the processes of transcription, RNA processing, and translation.

◀ *Prerequisite knowledge*

Lesson 2A
Proteins are crucial to the functioning of the body and have a diverse range of roles. They are the product of translation.

Lesson 2C
The genetic code represents a series of rules used by living organisms to transcribe and translate genetic material into proteins.

Future applications ▶

Lesson 2E
Regulatory genes are involved in the production of repressor molecules that can either inhibit or allow the expression of genes under certain circumstances.

Lesson 2F
Proteins, which are the final products of gene expression, can be exported out of cells via the protein secretory pathway so that they can be used elsewhere in the body.

Study design dot point

- the genetic code as a universal triplet code that is degenerate and the steps in gene expression, including transcription, RNA processing in eukaryotic cells, and translation by ribosomes

Key knowledge units

Gene expression	3.1.2.1
Transcription	3.1.2.2
RNA processing	3.1.2.3
Translation	3.1.2.4

Gene expression 3.1.2.1

OVERVIEW

The production of functional gene products such as proteins or non-coding strands of RNA is known as gene expression.

THEORY DETAILS

Gene expression is a complex series of events which results in the formation of functional gene products such as proteins or non-coding strands of RNA. It is through gene expression that it is possible for living organisms to produce the proteins and products crucial for maintaining life.

gene expression the process of reading the information stored within a gene to create a functional product, typically a protein

The stages of gene expression involved in the production of proteins include (Figure 1):

- **transcription**, which involves the copying of DNA into **pre-mRNA**
- RNA processing, which modifies the pre-mRNA molecule to produce **mRNA**
- **translation**, which involves the decoding of the mRNA strand into a polypeptide chain.

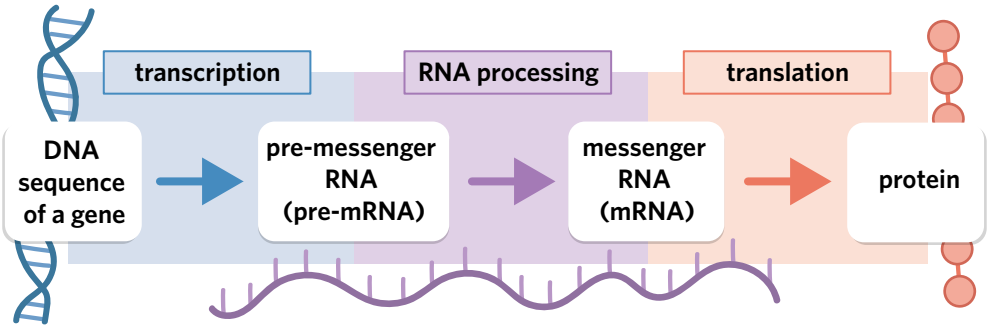


Figure 1 The processes of gene expression including transcription, RNA processing, and translation

In the production of other RNA molecules such as **tRNA** and **rRNA**, only the transcription and RNA processing stages occur. This is relatively intuitive, in that RNA is not composed of protein, and therefore do not require translation. When producing these strands of RNA, different **enzymes** are used, and therefore instead of mRNA, other forms of RNA are produced.

Transcription 3.1.2.2

OVERVIEW

Transcription is the first stage of gene expression and involves the creation of a pre-mRNA molecule by converting the genetic information found in DNA into RNA.

THEORY DETAILS

In eukaryotes, DNA is large and cannot leave the nucleus. The process of transcription creates an intermediary molecule known as mRNA, which serves as a copy of DNA in RNA that can leave the nucleus and transport the code for protein around the cell. As DNA is stored within the nucleus of eukaryotes, the process of transcription must also occur within the nucleus. In prokaryotes, however, because DNA is free-floating within the cytoplasm due to the absence of a nucleus, DNA is transcribed into mRNA within the cytoplasm.

It is also important to remember that the primary product of transcription in eukaryotes is known as pre-mRNA, which undergoes further modifications during RNA processing to become mRNA. The process of transcription can be broken down into three stages – initiation, elongation, and termination (Table 1).

Table 1 The general stages of transcription

Stage	Description
Initiation	To begin transcription, specific proteins called **transcription factors** bind to the **promoter** region to initiate transcription. With the help of transcription factors, **RNA polymerase** binds to the promoter region. This signals for the weak hydrogen bonds between the two strands of DNA to break, resulting in the bases of each strand being exposed and the DNA helix being unwound and unzipped. RNA polymerase is then able to start transcription.
Elongation	RNA polymerase moves along the **template strand** of DNA, reading the nucleotide sequence and uses free-floating complementary RNA nucleotides to produce a new single-stranded RNA molecule known as pre-mRNA. The pre-mRNA molecule is synthesised in a 5′ to 3′ direction, so new RNA nucleotides are added to the exposed 3′ end. This pre-mRNA strand has a complementary nucleotide sequence to the DNA template strand. The strand of DNA that is not read by RNA polymerase is called the **coding strand**. As the coding strand is also complementary to the template strand, the coding strand is identical to the pre-mRNA strand (except the pre-mRNA includes uracil instead of thymine).
Termination	Transcription ends when RNA polymerase reaches the **termination sequence** of a gene, signalling the end of transcription. RNA polymerase then detaches, releasing the pre-mRNA molecule and the DNA molecule winds up again into a double helix. The pre-mRNA molecule is then processed to become mRNA, carrying the message for protein synthesis from DNA in the nucleus to the **ribosomes** located in the cytosol or attached to the rough endoplasmic reticulum of the cell.

transcription the process whereby a sequence of DNA is used as a template to produce a complementary sequence of mRNA

precursor messenger RNA (pre-mRNA) the immediate product of transcription of a DNA sequence. Requires modifications before it can undergo translation

messenger RNA (mRNA) RNA molecules that are produced during transcription and carry genetic information from the nucleus to the ribosomes

translation the process where an mRNA sequence is read to produce a corresponding amino acid sequence to build a polypeptide

transfer RNA (tRNA) RNA that recognises specific codons on the mRNA strand and adds the corresponding amino acid to the polypeptide chain during protein synthesis

ribosomal RNA (rRNA) RNA that is a key structural component of ribosomes, which assemble proteins

enzyme an organic molecule, typically a protein, that catalyses (speeds up) specific reactions

transcription factor proteins that bind to the promoter region and control the functioning of RNA polymerase

promoter the sequence of DNA to which RNA polymerase binds

RNA polymerase the enzyme responsible for constructing a pre-mRNA sequence from a DNA sequence during transcription

template strand the strand of DNA transcribed by RNA polymerase to produce a complementary pre-mRNA strand

coding strand the strand of DNA not transcribed by RNA polymerase, contains an identical sequence to the mRNA strand produced (except thymine is replaced with uracil in mRNA)

termination sequence a sequence of DNA that signals the end of transcription

ribosome an organelle made of rRNA and protein that is the site of protein synthesis. Can be free in the cytosol or attached to the rough endoplasmic reticulum

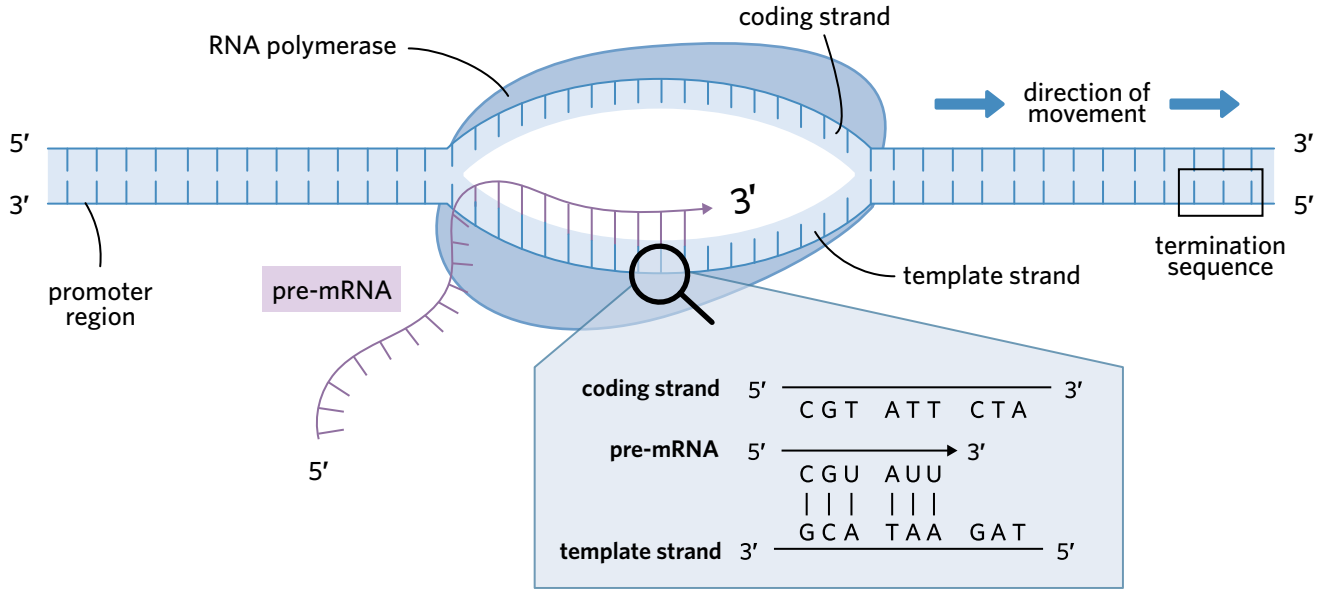


Figure 2 DNA is transcribed by RNA polymerase into a pre-mRNA molecule

Examiners' tip

The VCAA does not assess transcription in terms of the initiation, elongation, and termination stages. Instead, those three stages are simply a framework for memorising the process of transcription.
When asked to outline the process of transcription on past VCAA Biology exams (e.g. 2016 Section B Q6b, 2013 Section B Q6ai) the following points were required:

- DNA unwinds/unzips
- RNA polymerase catalyses transcription through the joining of complementary RNA nucleotides
- transcription of the DNA template strand into pre-mRNA occurs
- pre-mRNA is complementary to the DNA template strand
- in the pre-mRNA, adenine (A) pairs with uracil (U), not with thymine (T).

RNA processing 3.1.2.3

OVERVIEW

RNA processing, also known as post-transcriptional modifications, involves the modification of the pre-mRNA molecule into an mRNA molecule that can be used in translation.

THEORY DETAILS

Following transcription, the pre-mRNA molecule must undergo RNA processing, also known as post-transcriptional modifications, before being sent to the ribosomes for translation. RNA processing only occurs in eukaryotic cells, taking place in the nucleus. After RNA processing, the pre-mRNA molecule simply becomes known as an mRNA molecule or a mature mRNA molecule. The processing modifications include:

- the addition of a **5' methyl-G cap** and a **3' poly-A tail**
- the removal of **introns** and the **splicing** of **exons** together.

Examiners' tip

Frequently, you will hear the process of turning DNA into a protein as only transcription and translation. This is because RNA processing, or post-transcriptional modifications, can often be classified as part of the transcription stage of protein synthesis.

5′ methyl-G cap a molecule added to the 5′ end of pre-mRNA during RNA processing

3′ poly-A tail a chain of adenine nucleotides added to the 3′ end of pre-mRNA during RNA processing

introns non-coding regions of DNA that do not code for proteins. They are spliced out during RNA processing

splicing process where introns are cut out of a pre-mRNA molecule, and exons are joined together

exons regions of DNA that code for proteins and are not spliced out during RNA processing

Addition of a 5′ methyl-G cap and a 3′ poly-A tail

The addition of a methyl-guanine cap (methyl-G cap) at the 5' end and a chain of adenine nucleotides (poly-A tail) to the 3' end serve to stabilise the mRNA molecule, preventing it from degrading and allowing it to bind to ribosomes during translation.

Splicing

From lesson 2C, you should remember that introns are non-coding regions of DNA and exons are coding regions of DNA. The sections of pre-mRNA corresponding to introns must be removed and the regions corresponding to exons must be joined together. This occurs through the process of splicing via a complex molecule known as a **spliceosome**, which removes the introns and splices the exons together (Figure 3).

spliceosome the enzyme that removes introns from the pre-mRNA molecule and joins exons together during RNA processing

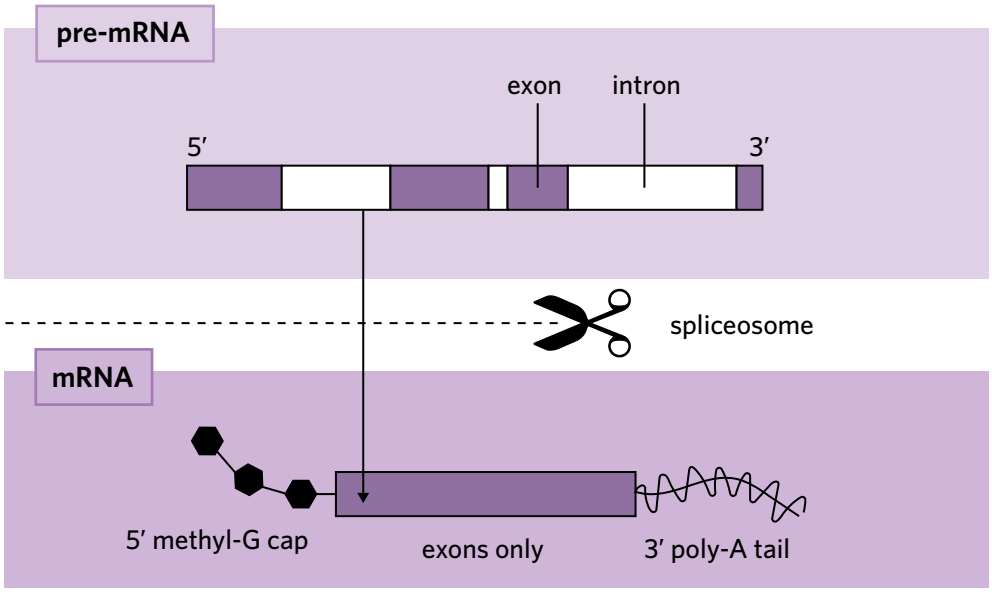


Figure 3 Transcribed pre-mRNA must undergo post-transcriptional modifications before it can be translated.

Examiners' tip

RNA processing only occurs within eukaryotic cells. An easy way to remember this is by recalling that while eukaryotic genes are composed of both introns and exons, prokaryotic genes are composed solely of exons. Therefore, prokaryotic mRNA can be directly translated without undergoing post-transcriptional modifications.

Alternative splicing

Sometimes, exons can also be removed during the splicing process. This means that a single pre-mRNA strand can produce many different mRNA molecules depending on which exons are spliced out or kept. This process is known as **alternative splicing** and allows for a single gene to give rise to many different mRNA strands and code for many different proteins (Figure 4).

alternative splicing the process where different exons may be spliced, resulting in a single gene producing multiple different mRNA strands

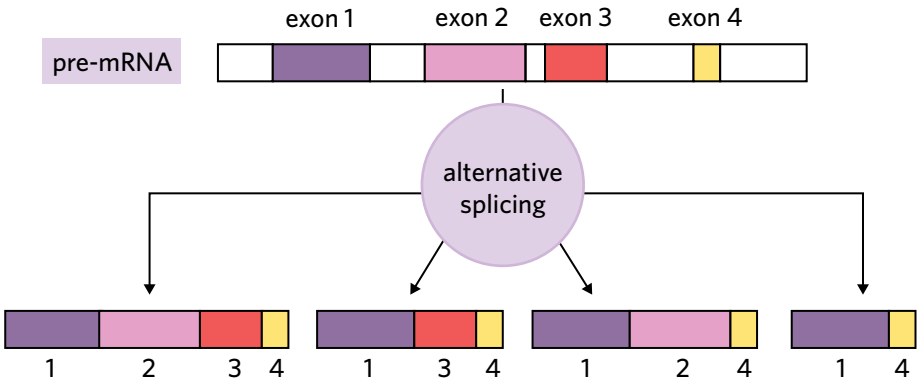


Figure 4 Alternative splicing can create many different strands of mRNA from a single gene.

Memory device

An easy way to remember the difference between introns and exons is that **ex**ons **ex**it the nucleus for translation, whereas **in**trons stay **in**side the nucleus.

Translation 3.1.2.4

OVERVIEW

Translation involves reading and converting the information carried in the mRNA molecule into a polypeptide chain.

THEORY DETAILS

After a pre-mRNA molecule is produced from a DNA template strand and undergoes post-transcriptional modifications, it is ready for translation. To undergo translation, the mRNA molecule exits the nucleus through a nuclear pore and travels to a ribosome either in the cytosol or attached to the rough endoplasmic reticulum (Figure 5). During translation, the mature mRNA molecule is decoded and translated into a sequence of amino acids, eventually forming a polypeptide chain.

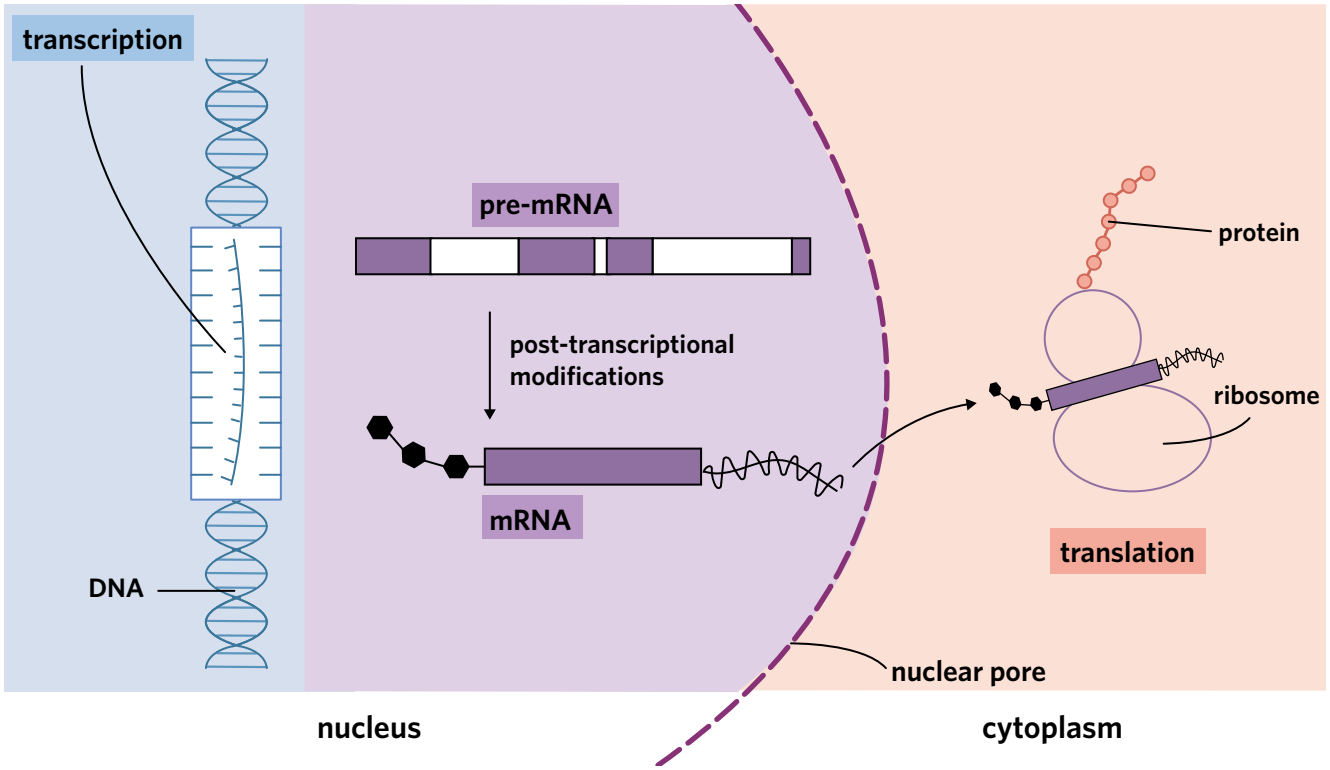


Figure 5 The mRNA molecule travels through the nuclear pore to reach the ribosome located in the cytosol or attached to the rough endoplasmic reticulum.

There are a number of key players involved in translation including mRNA, rRNA, tRNA, and amino acids. Translation can be broken down into three stages – initiation, elongation, and termination (Table 2).

Table 2 The general stages of translation

Stage	Description
Initiation	The 5′ end of the mRNA molecule binds to the ribosome and is read until the **start codon** (AUG) is recognised. Then, a tRNA molecule with a complementary **anticodon** (UAC) binds to the ribosome and delivers the amino acid methionine, signifying the commencement of translation.
Elongation	After the first amino acid is attached, the mRNA molecule is fed through the ribosome so that the next codon can be matched to its complementary tRNA anticodon. Then, complementary tRNA molecules deliver specific amino acids to the ribosome, which bind to adjacent amino acids with a **peptide bond** via a **condensation reaction**. The first tRNA molecule then leaves the ribosome and is free to pick up another amino acid, and the next mRNA codon is exposed for more tRNA-delivered amino acids to add to the growing amino acid chain.
Termination	The reading of mRNA, delivery of amino acids by tRNA, and the linking of amino acids in the polypeptide chain continues until the ribosome reaches a **stop codon** on the mRNA molecule. The stop codon signals the end of translation as there are no corresponding tRNA molecules. The polypeptide chain is then released by the ribosome into the cytosol or endoplasmic reticulum.

codon the sequence of three nucleotides in mRNA coding for one amino acid

start codon the sequence of three nucleotides in mRNA that signals the start of translation

anticodon the sequence of three nucleotides on a tRNA molecule that recognises a specific sequence of three nucleotides (codon) on an mRNA strand

peptide bond the chemical bond linking two amino acids

condensation reaction a reaction where two monomers join to form a larger molecule, producing water as a by-product

stop codon the sequence of three nucleotides in mRNA that signals the end of translation

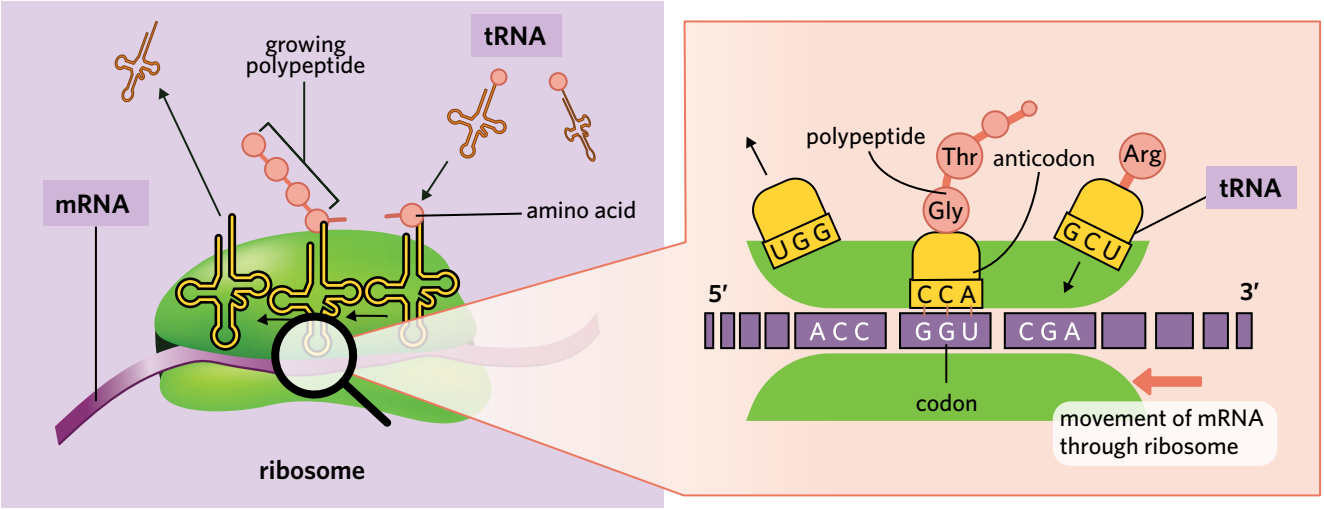


Figure 6 The translation of mRNA at the ribosome

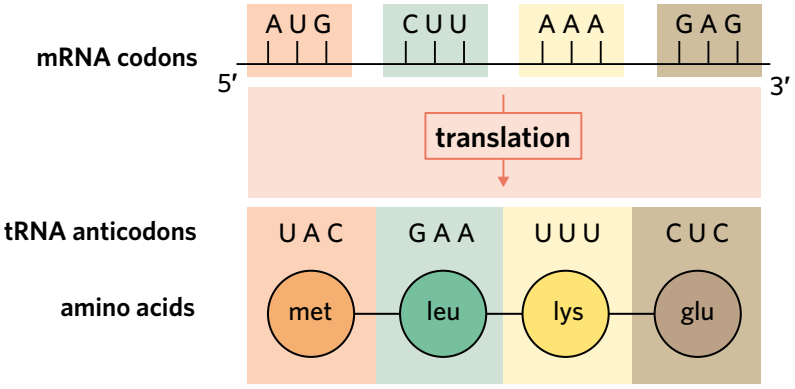


Figure 7 The tRNA molecules which carry specific amino acids have anticodons complementary to the mRNA codons.

Examiners' tip

The VCAA does not assess translation in terms of the initiation, elongation, and termination stages. Instead, those three stages are simply a framework for memorising the process of translation. When asked to outline the process of translation on past VCAA Biology exams (e.g. 2018 Section B Q1a, 2014 Section B Q7a), the following points were required:

- ribosome binds to and reads the mRNA molecule
- tRNA anticodons are complementary to the mRNA codons
- tRNA brings the corresponding amino acids to the ribosome
- adjacent amino acids are joined together into a polypeptide chain via a condensation reaction.

Following translation, the mRNA molecule can be reused to produce more polypeptides. At the endoplasmic reticulum and Golgi apparatus, each polypeptide chain is folded and modified into a fully functional protein, which can either remain in the cell for use, or it can be exported out of the cell via the process of **exocytosis**.

exocytosis a type of bulk transport that moves large substances out of a cell

Theory summary

Gene expression involves the formation of a functional gene product such as proteins or non-coding strands of RNA. Proteins are produced through the processes of transcription, which involves copying genetic information into the form of pre-mRNA, RNA processing, which involves modifications to the pre-mRNA molecule to become mRNA, and translation, which involves the decoding and interpretation of the mRNA molecule into a sequence of amino acids to form a polypeptide chain (Table 3).

Table 3 Summary of the three stages of protein synthesis

	Transcription	RNA processing	Translation
Key ideas	• RNA polymerase binds to the promoter region • DNA unwinds • RNA polymerase reads the DNA template strand and uses complementary RNA nucleotides to catalyse the formation of pre-mRNA • Transcription is terminated when the termination sequence is recognised	• Addition of a methyl-G cap to the 5′ end • Addition of a poly-A tail to the 3′ end • Introns removed and exons spliced together	• mRNA molecule binds to the ribosome • tRNA anticodons complementary to mRNA codons deliver corresponding amino acids to the ribosome • Adjacent amino acids are joined with peptide bonds via a condensation reaction to form a polypeptide • Translation ends when a STOP codon is recognised
Location	Nucleus	Nucleus	Ribosomes
Product	pre-mRNA from DNA template strand	mRNA from pre-mRNA	Polypeptide from mRNA

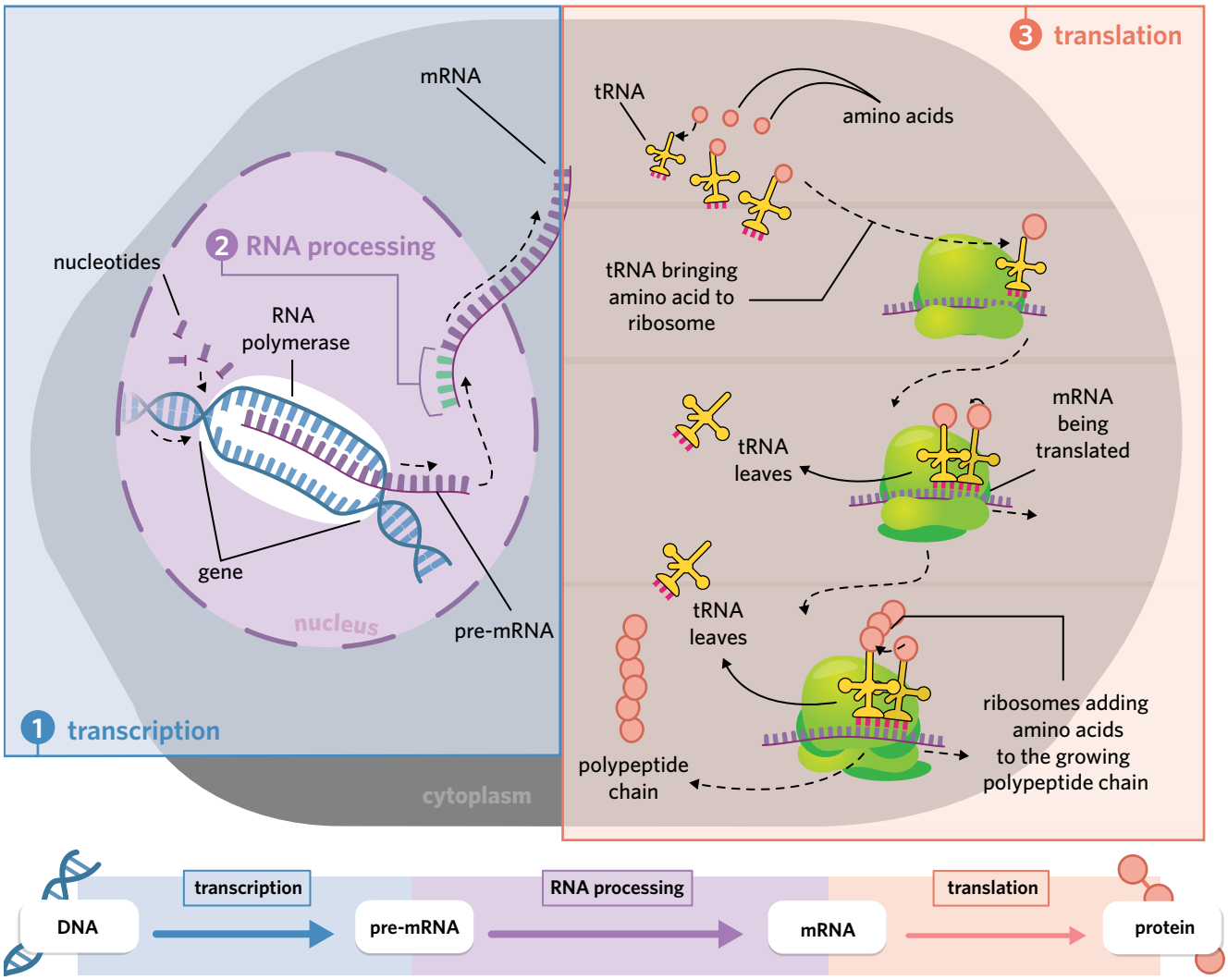


Image: VectorMine/Shutterstock.com

Figure 8 Summary of transcription, RNA processing, and translation

Just like the process of ordering food at a restaurant, your cells are constantly ordering proteins to be made. First, the order needs to be written down by the waiter in the form of mRNA through the process of transcription, before being sent off to the chef in the form of a ribosome to produce the final protein through the process of translation. Now, you'll not only be an expert at ordering food at restaurants, but you will also be well versed in the processes of transcription and translation!

Image: HAKINMHAN/Shutterstock.com

2D QUESTIONS

Theory review questions

Question 1

Gene expression involves the

A formation of functional gene products such as proteins or non-coding strands of RNA.

B process of translation, followed by transcription, and then RNA processing.

Question 2

The locations for the processes of transcription and translation occur in the

A nucleus and cytosol, respectively.

B cytosol and nucleus, respectively.

Question 3

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- template strand
- coding strand
- 3′ poly-A tail
- anticodons
- pre-mRNA
- codons
- triplets
- mRNA

To create protein from genetic information, a cell must undergo a series of steps. The first is transcription, where the DNA _____ is read and transcribed into _____. However, it must be processed before translation by splicing introns and the addition of a 5′ methyl-G cap and a _____. The mRNA can now be translated. This is possible due to its sequences of three nucleotides in the mRNA known as _____. At the ribosome, mRNA is used to create a protein.

Question 4

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

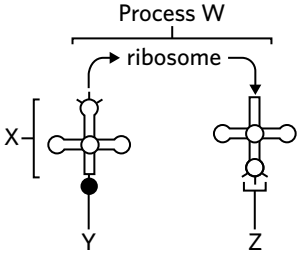
- START codon
- STOP codon
- anticodons
- ribosome
- nucleus
- codons
- triplets

Translation occurs at a _____, where the ribosome binds to the mRNA strand and reads it. The process is facilitated by tRNA molecules and their tri-nucleotide sequences known as _____. Amino acids delivered by tRNA molecules are linked into a polypeptide chain until a _____ is reached and translation is terminated.

Question 5

Label the parts of the following diagram from the list of terms.

- transcription
- translation
- amino acid
- anticodon
- protein
- mRNA
- codon
- triplet
- rRNA
- tRNA



SAC skills questions

Case study analysis

Use the following information to answer Questions 6–9.

In eukaryotes, there are many different mechanisms that exist to regulate the process of gene expression. These include the use of silencer regions within genes, which serve to downregulate gene expression by inhibiting transcription, and enhancer regions within genes, which serve to upregulate gene expression by activating transcription. The use of these mechanisms only applies to the production of mRNA – they have no effect on the length of time that an mRNA molecule can remain within the cytosol and be translated into proteins.

One of the primary methods of determining how long an mRNA molecule can remain within a cell depends on the length of its poly-A tail. This is because the poly-A tail is responsible for stabilising the mRNA molecule and protecting it against enzymatic attack and degradation. mRNA molecules with longer poly-A tails can persist for longer due to their increased resistance against degradation.

Another method of maintaining mRNA levels within a cell involves the use of microRNAs, which are another form of RNA. MicroRNAs are produced through the process of transcription, using a different type of RNA polymerase. When microRNAs are loaded into a complex known as an RNA-induced silencing complex (RISC), they are capable of binding to complementary strands of mRNA molecules within the cytosol and cleaving the mRNA molecule, rendering it obsolete.

Question 6

Enhancers are regions within genes involved in

A upregulating gene expression.

B downregulating gene expression.

Question 7

A shorter poly-A tail will result in

A a decreased rate of degradation of an mRNA molecule.

B an increased rate of degradation of an mRNA molecule.

C no change to the speed of degradation of an mRNA molecule.

D a significantly decreased rate of degradation of an mRNA molecule.

Question 8

The production of microRNAs would involve the process of

A transcription, as microRNAs are forms of rRNA.

B translation, as microRNAs are composed of protein.

C translation, as microRNAs involve the use of tRNA and rRNA molecules.

D transcription, as microRNAs are complementary to genes stored within the nucleus.

Question 9

MicroRNAs would not contain the nucleotide

A uracil.

B thymine.

C adenine.

D cytosine.

Exam-style questions

Within lesson

Question 10 (1 MARK)

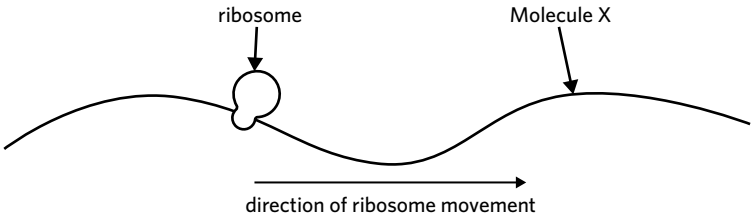
A molecule of messenger RNA could include the nucleotide sequence

A CTGTATUTA
B AGTGUACTT
C GTACGTAGG
D CUACGAGUU

Adapted from VCAA 2011 Exam 1 Section A Q4

Use the following information to answer Questions 11 and 12.

Ricin is a naturally occurring, powerful poison that affects eukaryotic organisms. Studies have concluded that ricin stops the movement of a ribosome along a specific molecule labelled Molecule X.



Question 11 (1 MARK)

Which monomer is Molecule X made of?

A fatty acids
B nucleotides
C amino acids
D carbohydrates

Question 12 (1 MARK)

At the stage shown in the diagram, Molecule X contains regions corresponding to

A exons only.
B introns only.
C both exons and introns.
D neither exons or introns.

Adapted from VCAA 2017 Northern Hemisphere Exam Section B Q23

Multiple lessons

Question 13 (1 MARK)

The genome of the Northern white-cheeked gibbon, *Nomascus leucogenys*, has been sequenced and compared to other primate species. The *N. leucogenys* genome would

A include the base uracil.
B include only the non-coding DNA sequences.
C be identical to the genome of the buffed-cheeked gibbon, *Nomascus annamensis*.
D contain the same nitrogenous base types to the genome of the black-crested gibbon, *Nomascus concolor*.

Adapted from VCAA 2015 Section A Q24

Question 14 (5 MARKS)

Consider the template strand of the hypothetical gene shown. The exons are in bold type.

3′ TAC ACC GCT TAT TTT **CAT** CTT **TCT** GCA TAG **GAT ATC 5′**

The DNA triplet TAC indicates START for transcription, and the mRNA codon that it produces will code for the amino acid methionine, which remains in the resulting polypeptide. The DNA triplets ATC, ATT, and ACT code for a STOP instruction.

a Identify the total number of amino acids present in the polypeptide expressed by this gene. Justify your response. (2 MARKS)

b The template strand is used to produce mRNA.

i What is the pre-mRNA strand that would be produced from this DNA sequence? (1 MARK)

ii What is the corresponding mRNA sequence following RNA processing? (1 MARK)

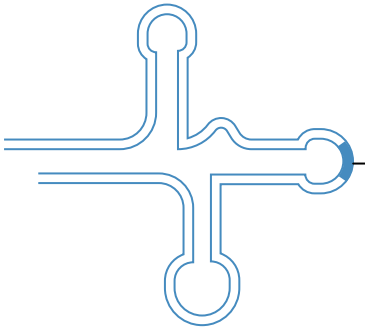
iii Using the table provided, what is the amino acid sequence produced when the mRNA molecule is translated? (1 MARK)

1st position (5′ end)	2nd position				3rd position (3′ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Iie	Thr	Asn	Ser	U
	Iie	Thr	Asn	Ser	C
	Iie	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Question 15 (4 MARKS)

The hormone insulin is a relatively small protein. Researchers studying the production of insulin in the cells of the pancreas noted that one of the early steps in this process is the formation of a polypeptide called preproinsulin. Researchers noted that the formation of this polypeptide requires different forms of Molecule Z shown.

Molecule Z



a Molecule Z is crucial to the functioning of cells.

i Identify the process Molecule Z is involved in. (1 MARK)

ii Describe the role of anticodons in this process. (2 MARKS)

b Proteins have four levels of protein structure. If preproinsulin has not been configured into alpha helices or beta-pleated sheets, what level of structure does it have? (1 MARK)

Question 16 (9 MARKS)

Scientists studying the nucleus of the fruit fly *Drosophila melanogaster* observed distinct types of nucleic acid chains. One type of nucleic acid chain was able to pass through the nuclear membrane and move to a ribosome. After the nucleic acid chain attached to the ribosome, a polymer was produced.

a What type of molecule is this nucleic acid chain? (1 MARK)

b Identify the enzyme responsible for the creation of the nucleic acid chain. (1 MARK)

c Describe the steps that occur within a cell that result in the production of this nucleic acid chain. (3 MARKS)

d What type of molecule is the polymer produced? (1 MARK)

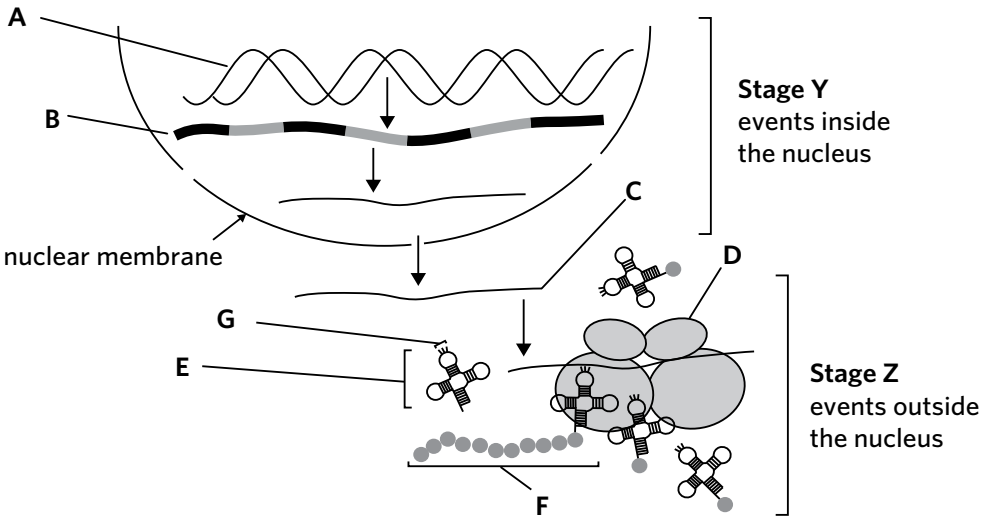
e Describe the steps that occur at the ribosome that convert this nucleic acid chain into the polymer. (3 MARKS)

Adapted from VCAA 2014 Section B Q7

Question 17 (3 MARKS)

The following diagram outlines various events that occur in cells when DNA is activated.

Label the structures and stages shown in the diagram.

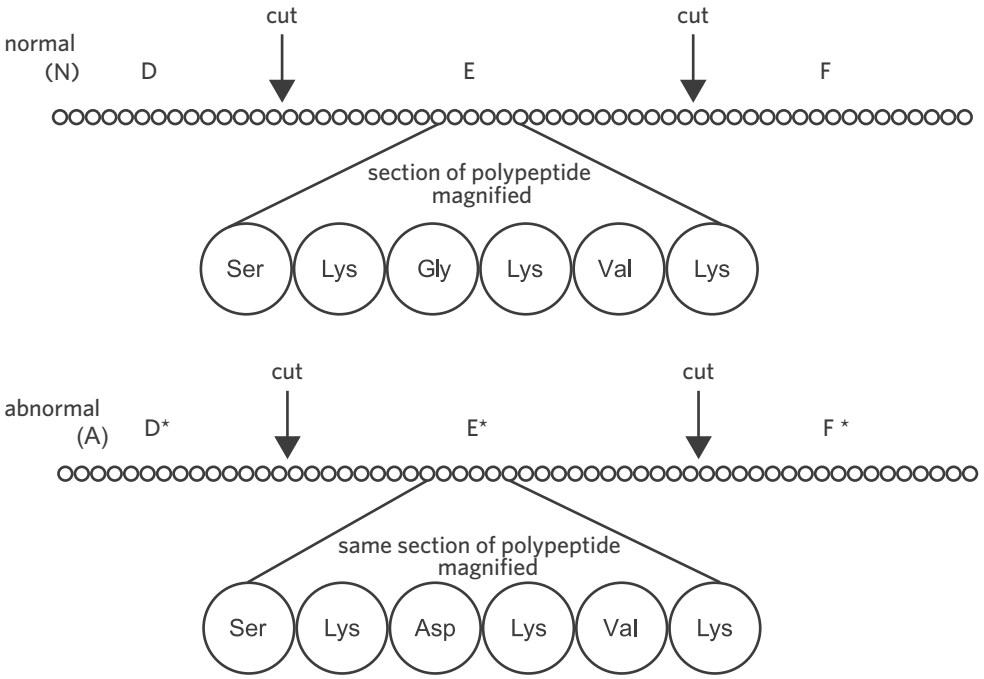


Adapted from VCAA 2013 Section B Q6

Key science skills and ethical understanding

Question 18 (9 MARKS)

Scientists have investigated how the nucleus accumulates the essential proteins that it needs to function. They found a possible reason while studying a certain normal polypeptide sequence, and an abnormal version. The abnormal polypeptide produced was not able to cross the nuclear membrane in the same way the normal form did. The amino acids in the normal (N) and abnormal (A) polypeptides are shown. A sequence of six amino acids from the middle is magnified.



An experiment was carried out where both polypeptides were cut into three smaller chains – D, E, and F (D*, E*, and F*) – as shown. Each polypeptide was put into a cell and then its accumulation in the nucleus was measured. The table shows the results of the experiment.

Polypeptide	Accumulates in nucleus
N	yes
A	no
D	no
E	yes
F	no
D*	no
E*	no
F*	no

a Identify the independent and dependent variables of this experiment. (2 MARKS)

b Which polypeptides were easily able to cross the nuclear envelope? Justify your response. (2 MARKS)

c Polypeptide E accumulated in the nucleus whereas polypeptide E* did not. Suggest a reason why polypeptide E* did not accumulate in the nucleus. (1 MARK)

d How could the scientists improve the precision of their experiment? (2 MARKS)

e Medical research often requires large amounts of funding. However, ethical dilemmas can often arise when deciding which research projects deserve the most funding. With reference to one bioethical concept, suggest one criterion that should be considered when determining which research projects deserve the most funding. (2 MARKS)

Adapted from VCAA 2014 Section B Q2

2E GENE REGULATION

Teachers are a crucial component of every classroom. We all know that when teachers are away and replaced by substitute teachers, there is no chance of any work getting completed. Or if the teacher doesn't show up after 15 minutes, there is almost no chance of finding the students for the rest of the day as they've all disbanded and gone for an early lunch. But just like teachers, is there something inside our cells that keeps everyone in check? What makes sure that our cells don't just stop working after 15 minutes?

Image: alphaspirit.it/Shutterstock.com

Lesson 2E

In this lesson you will learn how regulatory genes initiate or inhibit transcription, specifically how the *trp* operon is switched on and off as a simple model of gene regulation.

◀ *Prerequisite knowledge*

Lesson 2C
Genes are composed of many different regions, including a promoter region, coding regions known as exons, a termination region, and an operator region.

Lesson 2D
Gene regulation is the modulation of gene expression, which primarily involves the production of proteins via transcription, RNA processing, and translation.

Future applications ▶

Lesson 2F
The amount of protein exported by a cell is dependent on the amount of protein produced. Gene regulation is the mechanism which ultimately determines the amount of protein produced within a cell.

Chapter 3
The mechanism of how a repressor can be deactivated by another molecule through a change in its functional 3D shape is also applicable to enzymes. Enzymes can be inhibited by other molecules through a 3D conformational change.

Study design dot point

- the basic elements of gene regulation: prokaryotic *trp* operon as a simplified example of a regulatory process

Key knowledge units

Gene regulation	3.1.4.1
How the *trp* operon works	3.1.4.2

Gene regulation 3.1.4.1

OVERVIEW

Regulatory genes code for proteins that influence the expression of structural genes, preventing the over or under expression of a particular protein and allowing the cell to adapt to its needs.

THEORY DETAILS

The human body uses a significant amount of energy each day – with every heartbeat, every breath, and even every thought, energy must be expended. Therefore, in order to maintain adequate energy levels, we need to be smart about how our limited energy supply is used and distributed. But apart from staying completely still or clearing our minds of their thoughts, how can our cells conserve energy? **Gene regulation**!

gene regulation the control of gene expression, typically achieved by switching transcription on or off

Gene regulation involves the process of either inhibiting or activating **gene expression**. In doing so, organisms can prevent the unnecessary production of gene products such as proteins when they are not required, thereby conserving energy. There are two types of genes involved in gene regulation – **structural genes** and **regulatory genes** (Table 1). The important link between these two types of genes is that regulatory genes are responsible for controlling the expression of other genes, such as structural genes.

gene expression the process of reading the information stored within a gene to create a functional product, typically a protein

structural gene a segment of DNA that doesn't code for regulatory proteins, but instead codes for proteins that play a role in the structure or function of a cell or organism

regulatory gene a segment of DNA responsible for producing proteins that control the expression of other genes

repressor protein a protein coded for by a regulatory gene that prevents gene expression by binding to its operator

activator protein a protein coded for by a regulatory gene that increases gene expression

promoter the sequence of DNA to which RNA polymerase binds

operator a short region of DNA that interacts with repressor proteins to alter the transcription of an operon

operon a cluster of linked genes that all share a common promoter and operator and are transcribed at the same time

Table 1 Structural and regulatory genes

Gene	Description
Structural gene	Structural genes are responsible for producing proteins that are involved in the structure or function of a cell. For example, they may code for enzymes, transport proteins, receptors, or peptide hormones. These genes are often found downstream (towards the 3′ end of the coding strand) of the regulatory gene that controls them.
Regulatory gene	Regulatory genes are responsible for the production of regulatory proteins such as **repressor proteins**, which inhibit or decrease the expression of structural genes. **Activator proteins**, on the other hand, can initiate or increase the expression of structural genes. Regulatory proteins can turn gene expression off or on, as well as increase or decrease the rate of gene expression by promoting or hindering transcription. They can also control the types of post-transcriptional modifications that occur, such as the way in which a gene's pre-mRNA is spliced.

From lesson 2C, you should remember that genes are composed of many different components such as a **promoter**, an **operator**, initiation and termination sequences, introns and exons, and the influence each of these has on protein production. In certain organisms, such as prokaryotes, multiple structural genes that share a common purpose can often be arranged into groups so that their expression is efficiently controlled by a single promoter and operator in what is known as an **operon** (Figure 1).

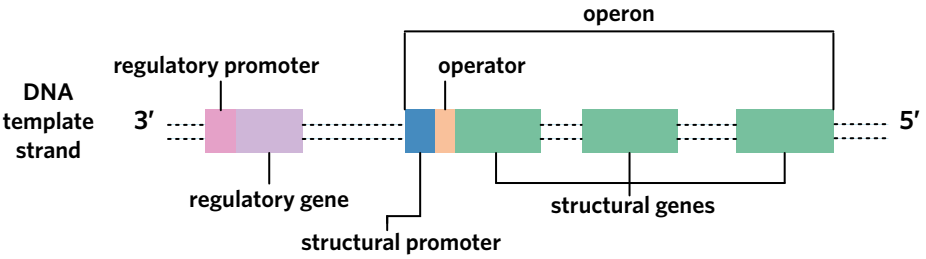


Figure 1 The basic structure of an operon with three structural genes

Because operators are always located downstream of the gene's promoter region, they can be used as a binding site for repressor or activator proteins, which are produced by regulatory genes, and control gene expression. For example, consider the following scenarios:

- operator region is bound with a repressor protein – RNA polymerase cannot move downstream from the promoter region, inhibiting transcription of the gene.
- operator region is not bound with a repressor protein – RNA polymerase is free to move downstream from the promoter region, allowing for the transcription of the gene as usual.

Apart from helping organisms conserve energy, gene regulation also ensures that cells produce the appropriate proteins. For example, even though every somatic cell within the human body is genetically identical and contains all the same genes, not all cells express the same genes. For example, skin cells express and produce different proteins to heart cells.

How the *trp* operon works 3.1.4.2

OVERVIEW

The *trp* operon contains a series of genes that are involved in the production of the amino acid tryptophan, which can subsequently be used in protein production.

THEORY DETAILS

In certain species of bacteria such as *Escherichia coli* (*E. coli*), the *trp* operon regulates the expression of structural genes which code for proteins that are involved in the production of the amino acid tryptophan. The *trp* operon is composed of a series of structural genes (*trpE*, *trpD*, *trpC*, *trpB*, and *trpA*) which are controlled by a common promoter and operator. The entire *trp* operon is controlled by a regulatory gene located upstream (Figure 2).

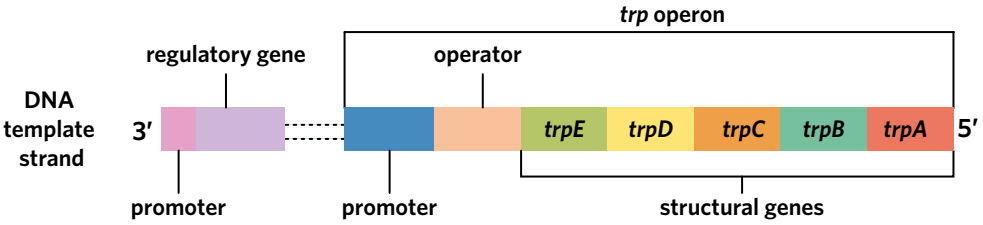


Figure 2 The *trp* operon involves a series of structural genes, a promoter, and an operator.

The amino acid tryptophan can be used as a building block for the formation of large and complex proteins. However, because energy is a finite resource and should be conserved, the expression of the structural genes coding for proteins involved in the production of the amino acid tryptophan should only be expressed when required. Therefore, the expression of the *trp* operon and the *trp* structural genes it contains depends on the levels of tryptophan present within the cell:

- high levels of tryptophan – transcription of the *trp* structural genes is repressed in order to prevent unnecessary production of tryptophan.
- low levels of tryptophan – transcription of the *trp* structural genes is activated in order to increase the amount of tryptophan available.

To regulate the expression of the structural genes, the regulatory gene for the *trp* operon is constantly expressed, producing a repressor protein. When high levels of tryptophan are present, tryptophan binds to the repressor protein which induces a **conformational change** in the repressor protein. This allows the repressor protein to bind to the operator region (Figure 3). In this way, the repressor protein can prevent transcription of the structural genes by blocking the path of RNA polymerase, inhibiting unnecessary production of tryptophan.

conformational change a change in the three-dimensional shape of macromolecules such as proteins

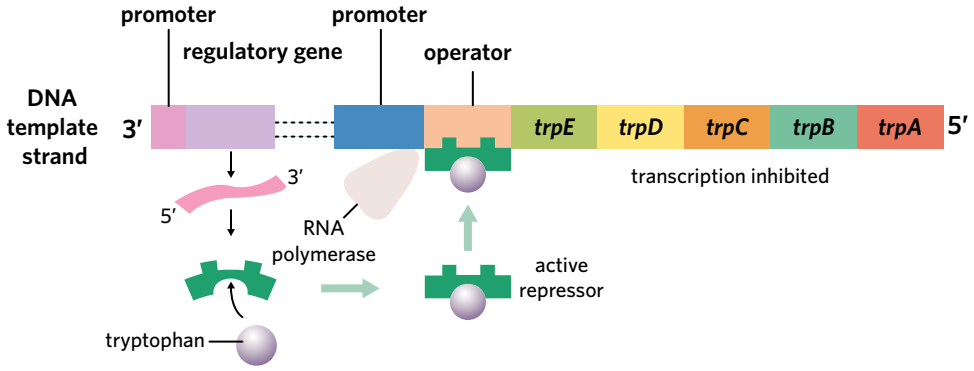


Figure 3 When tryptophan levels are high, the repressor protein binds to the operator and prevents transcription of the *trp* operon.

Conversely, when tryptophan levels are low, there is an insufficient quantity of tryptophan molecules available to bind consistently to the repressor protein (Figure 4). This causes the repressor protein to detach from the operator region, allowing RNA polymerase to transcribe the *trp* structural genes so that the level of tryptophan can increase. However, as tryptophan accumulates in the cell, it will once again bind to the repressor protein, slowly inhibiting transcription of the *trp* structural genes. Together, these mechanisms keep the amount of tryptophan available at a relatively constant level to ensure that energy and resources are expended appropriately.

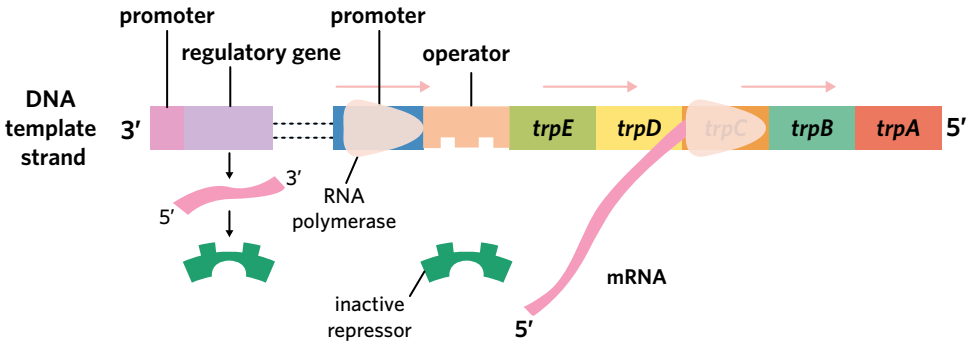


Figure 4 When tryptophan levels are low, the repressor protein detaches from the operator, allowing transcription of the *trp* operon to occur.

Theory summary

Gene regulation involves the regulation of gene expression in order to prevent the overproduction or underproduction of proteins. Genes can be classified into two types – structural and regulatory genes – with regulatory genes controlling the expression of structural genes. The *trp* operon provides a simple model of prokaryotic gene regulation, involving a repressor molecule that is either bound or unbound to the operator region, preventing or allowing transcription to occur depending on the levels of the amino acid tryptophan (Table 2).

Table 2 Summary of *trp* operon

Tryptophan levels	Regulatory gene	Repressor protein	Structural genes
high	transcribed	bound to operator	not transcribed
low	transcribed	unbound to operator	transcribed

Just like our structural genes represent students within a classroom, our regulatory genes represent the teachers that control the classroom. In doing so, regulatory genes are responsible for controlling the expression of structural genes. Without regulatory genes, our cells would not be able to control the expression of genes, leading to the production of unnecessary proteins and the inability to maintain stable energy levels.

Image: jittawit21/Shutterstock.com

2E QUESTIONS

Theory review questions

Question 1

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- repressor proteins
- structural proteins
- RNA polymerase
- DNA polymerase
- regulatory genes
- structural genes
- promoter
- operator

_____ are responsible for the production of proteins that are involved in the structure and functioning of an organism. Conversely, _____ are responsible for producing regulatory proteins such as _____ which control the expression of other genes. These proteins bind to the _____ region of operons, preventing _____ from transcribing the genes.

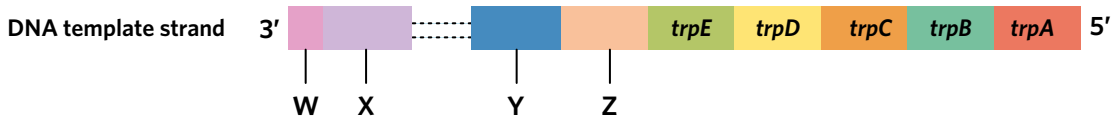
Question 2

Which one of the following statements is false?

A Regulatory genes can code for proteins that regulate the expression of structural genes.
B Operons consist of a series of genes controlled by a common promoter and operator.
C Organisms can conserve energy by preventing the transcription of genes.
D Regulatory genes do not contain a promoter region.

Question 3

Label the parts of the following diagram.



Question 4

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- transcribed
- preventing
- translated
- allowing
- high
- low

When tryptophan levels are _____, the repressor protein detaches from the operator region, _____ *trp* structural genes to be _____. Conversely, when tryptophan levels are _____, tryptophan binds to the repressor protein, allowing it to bind to the operator region, thereby _____ transcription of the *trp* structural genes.

SAC skills questions

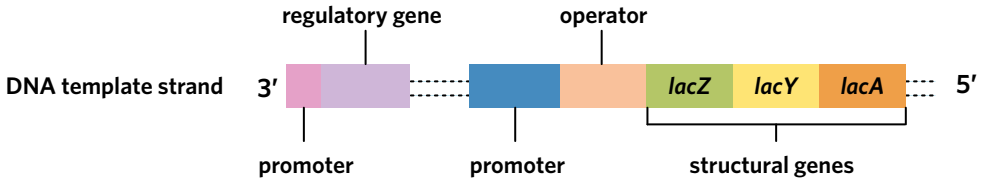
Case study analysis

Use the following information to answer Questions 5–8.

Escherichia coli is a species of bacteria that uses glucose as its primary source of energy. However, when glucose is scarce, *E. coli* must use alternate pathways to generate energy. One of these pathways involves the *lac* operon, which is a series of genes that share a common promoter and operator involved in the production of enzymes responsible for the digestion of lactose into glucose and galactose. By digesting lactose into glucose and galactose, glucose can be used in cellular respiration to produce energy. However, the activation of the *lac* operon is tightly regulated so that *E. coli* can conserve energy. Transcription of *lac* operon genes is only required when glucose levels are low and lactose levels are high.

When lactose levels are high, some lactose is converted into allolactose, which involves a slight change in its chemical structure. Allolactose binds to and induces a 3D conformational change in the *lac* repressor protein, causing it to detach from the DNA strand. This allows RNA polymerase to bind to the promoter region, initiating transcription of the *lac* operon and the three structural proteins encoded by the structural genes are produced.

Conversely, when lactose levels are low, the repressor protein binds to the DNA strand, thereby preventing the transcription of the *lac* operon. This allows for bacteria, such as *E. coli*, which have the *lac* operon, to conserve energy and only produce the enzymes required for the digestion of lactose when required. The following diagram depicts the *lac* operon.



Question 5

The *lac* operon is expressed when

A lactose levels are high and glucose levels are high.
B lactose levels are high and glucose levels are low.
C lactose levels are low and glucose levels are high.
D lactose levels are low and glucose levels are low.

Question 6

Allolactose can

A bind to the repressor protein, causing it to detach from the DNA strand.
B bind to the promoter region, causing transcription of the *lac* operon.
C inhibit RNA polymerase, causing transcription of the *lac* operon.
D be directly used as an energy source in *E. coli*.

Question 7

The *lac* repressor protein is produced by a

A regulatory gene and binds to the promoter region of the *lac* operon.
B structural gene and binds to the promoter region of the *lac* operon.
C regulatory gene and binds to the operator region of the *lac* operon.
D structural gene and binds to the operator region of the *lac* operon.

Question 8

When lactose levels are low and glucose levels are high

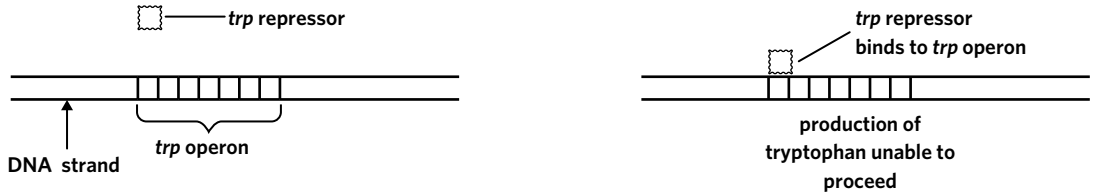
A the repressor protein inhibits the translation of the *lac* operon.
B RNA polymerase is actively transcribing the *lac* operon.
C glucose is being used as the primary source of energy.
D the production of the repressor protein is inhibited.

Exam-style questions

Within lesson

Use the following information to answer Questions 9 and 10.

Tryptophan is an amino acid that is produced by many bacteria. Genes that code for enzymes that produce tryptophan are found on bacterial DNA and together are called the *trp* operon. When the binding of a *trp* repressor protein occurs, the process of tryptophan production is inhibited. This process is illustrated in the following diagram.



Question 9 (1 MARK)

The *trp* repressor will bind to

A the promoter region.
B the operator region.
C pre-mRNA.
D tRNA.

Adapted from VCAA 2017 Northern Hemisphere Exam Section A Q27

Question 10 (1 MARK)

Which one of the following statements is correct concerning the *trp* operon?

A The *trp* repressor molecule is encoded by a structural gene.
B The *trp* operon is most active when *trp* concentration is high.
C Low concentrations of tryptophan increase the likelihood of *trp* repressor releasing from the DNA.
D High concentrations of RNA polymerase increase the likelihood of *trp* repressor releasing from the DNA.

Adapted from VCAA 2017 Northern Hemisphere Exam Section A Q28

Multiple lessons

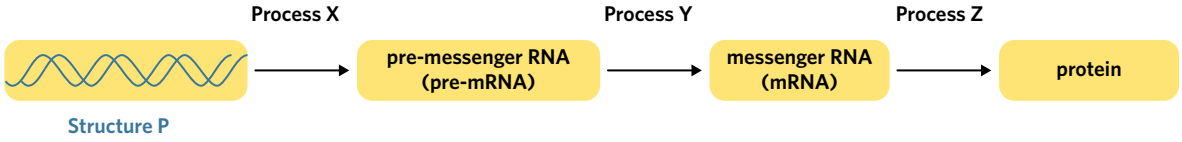
Question 11 (1 MARK)

Which one of the following statements regarding the structure of a gene in a eukaryotic cell is correct?

A Only prokaryotes exhibit gene regulation.
B An intron is transcribed but not translated.
C Structural genes encode for repressor proteins.
D A regulatory gene is always found downstream of the gene it controls.

Question 12 (9 MARKS)

The following diagram outlines the processes involved in the production of proteins within a cell.

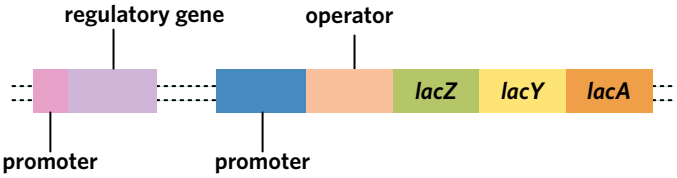


a Identify and describe the purpose of Process X. (2 MARKS)
b Identify and describe the purpose of Process Z. (2 MARKS)
c Outline the key events that occur during Process Y. (3 MARKS)
d Provided that Structure P is composed of two different types of genes, describe the roles of each type of gene. (2 MARKS)

Question 13 (8 MARKS)

In certain species of bacteria such as *E. coli*, glucose is the primary source of energy. However, glucose is not always available, forcing bacteria to produce energy via other pathways. For example, the *lac* operon contains genes for enzymes that allow bacteria to break down lactose into glucose and galactose, thereby providing another source of energy.

However, when a *lac* repressor protein is bound to the *lac* operon, the production of these enzymes is inhibited. The following diagram shows the order of the genes found in the *lac* operon. The dots represent sequences of DNA nucleotides between genes.



a Name a structural gene found in the *lac* operon. (1 MARK)

b Outline the role of the repressor protein in the *lac* operon. (2 MARKS)

c Describe the role of the promoter regions in the *lac* operon. (1 MARK)

d Suggest whether the *lac* operon would be active in the absence of lactose. Justify your response. (2 MARKS)

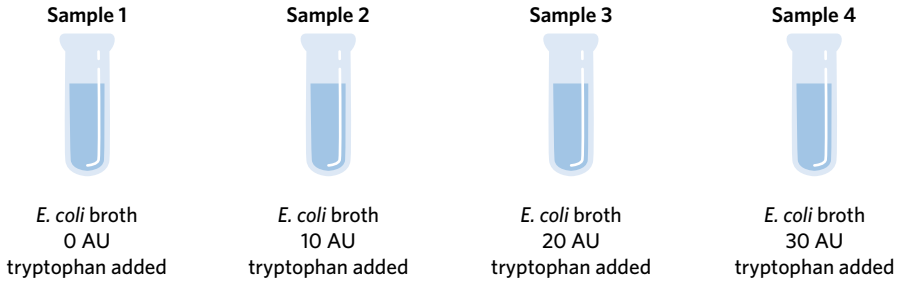
e Suggest why the controlled regulation of the *lac* operon is crucial for the survival of *E. coli*. (2 MARKS)

Adapted from VCAA 2017 Section A Q2

Key science skills and ethical understanding

Question 14 (9 MARKS)

Sinead and Gillian designed an experiment to test the effect of tryptophan concentration on the production of enzymes involved in tryptophan synthesis, which are encoded for by *trp* structural genes found in the *trp* operon. The experimental setup is shown in the following diagram. AU represents arbitrary units.



a Outline what an experimental control is and explain its purpose. Identify the sample which acts as an experimental control in this experiment. (3 MARKS)

b Suggest one safety consideration that should be applied in this experiment. (1 MARK)

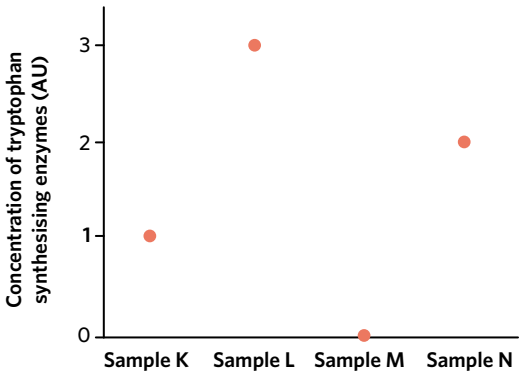
c State a possible hypothesis Sinead and Gillian were testing. (1 MARK)

d Sinead and Gillian forgot to label each test tube in their experiment.

i Identify the type of error that has occurred. (1 MARK)

ii In order to cover up their error, Sinead and Gillian decided to falsify their results. Identify the bioethical concept which has been violated by their actions. (1 MARK)

iii Considering the following graph, match each test tube to its expected corresponding sample. Using your knowledge of the *trp* operon, briefly explain your response. (2 MARKS)



2F THE PROTEIN SECRETORY PATHWAY

When was the last time you sent a letter? You might find it hard to believe, but just a few decades ago, before the rise of phones, laptops, and computers, to communicate with other people, you had to send them a letter. You had to physically write a letter before packaging it into an envelope and placing it within a post box. Afterwards, it would be whirled away by the post office who would sort the letters, eventually delivering it to your target letterbox. Just like the post office, within your cells there is a complex postal network involved in the transportation of substances around cells. Who's responsible for sorting and delivering the letters in your cells?

Image: Nils Versemann/Shutterstock.com

Lesson 2F

In this lesson you will explore how proteins are exported from cells through the process of exocytosis and the various organelles involved.

◀ *Prerequisite knowledge* — *Future applications* ▶

Year 11

There are many different organelles involved in the protein secretory pathway such as the rough endoplasmic reticulum, the Golgi apparatus, mitochondria, and secretory vesicles.

Lesson 2D

Proteins are products of gene expression, which involves the processes of transcription and translation. Following the translation of proteins at ribosomes, they can then be exported from the cell for use elsewhere.

Chapter 3

Enzymes are proteins responsible for increasing the rate of reactions. They can often be exported out of cells for use elsewhere in the body.

Lesson 6A

In order to export proteins from a cell, energy is required. Energy, in the form of ATP, can be produced in large quantities by the mitochondria for use within cells through the process of aerobic cellular respiration.

Study design dot point

- the role of rough endoplasmic reticulum, Golgi apparatus, and associated vesicles in the export of proteins from a cell via the protein secretory pathway

Key knowledge unit	
The protein secretory pathway	3.1.7.1

The protein secretory pathway 3.1.7.1

OVERVIEW

The protein secretory pathway involves various different organelles that produce, fold, modify, and package proteins, eventually exporting them from the cell via the process of exocytosis.

THEORY DETAILS

Many of the proteins produced by cells do not actually remain in the cell that produced them. Instead, they are often exported out of the cell so that they can be used elsewhere in the body. For example, while peptide hormones such as insulin are produced by cells in the pancreas, they are released into the bloodstream so that they can travel around the body and reach target cells found in the liver.

In VCE Biology, you need to understand how a protein made at a **ribosome** can travel through various organelles, and how it is eventually secreted via a process known as **exocytosis** into the extracellular environment.

ribosome an organelle made of rRNA and protein that is the site of protein synthesis. Can be free in the cytosol or attached to the rough endoplasmic reticulum

exocytosis a type of bulk transport that moves large substances out of a cell

Exocytosis

Exocytosis is the process by which the contents of a **vesicle** are released from a cell. It is a form of **bulk transport**, which allows for the movement of large substances such as proteins out of cells. Importantly, bulk transport is also a form of **active transport**, and therefore, the process of exocytosis requires the input of energy. The stages involved in exocytosis include:

1 A vesicle containing **secretory products** is transported to the **plasma membrane**.

2 The membrane of the vesicle fuses with the plasma membrane.

3 The secretory products are released from the cell into the extracellular environment.

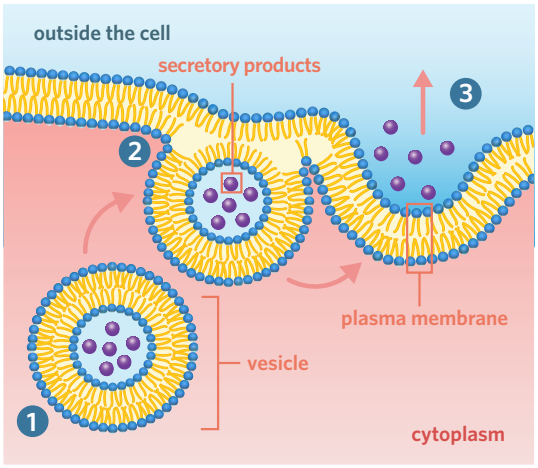


Figure 1 The process of exocytosis

Exocytosis is possible due to the fluid nature of the plasma membrane, which allows it to fuse with the vesicle. The fluid nature of the plasma membrane refers to its ability to be mobile and flexible – that is, the plasma membrane is not a static structure incapable of moving. Additionally, aside from the export of proteins, the process of exocytosis can also be used in the export of waste products to ensure that toxins do not build up within the intracellular environment.

The protein secretory pathway

While there are many different organelles involved in the protein secretory pathway, the primary organelles involved include the ribosomes, the **rough endoplasmic reticulum**, the **Golgi apparatus**, and transport and secretory vesicles. The roles of these organelles are described in Table 1 and the protein secretory pathway is depicted in Figure 2.

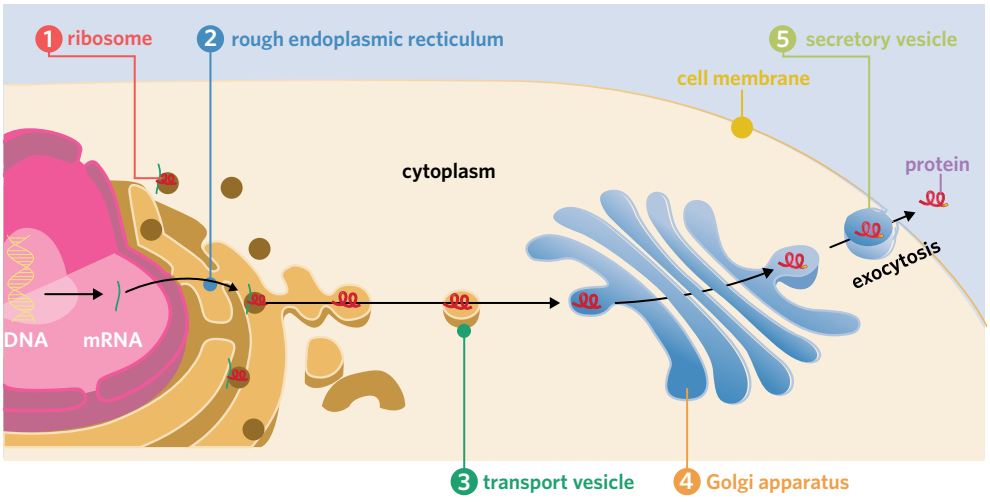


Figure 2 The protein secretory pathway

vesicle a small fluid-filled organelle enclosed in a phospholipid membrane that transports substances around the cell

bulk transport a type of active transport that uses vesicles to move large molecules or groups of molecules into or out of the cell

active transport the movement of molecules across a semipermeable membrane requiring an energy input

secretory products the substances inside a vesicle that are being transported out of the cell

plasma membrane the phospholipid bilayer with embedded proteins which separates the intracellular environment from the extracellular environment

Examiners' tip

The other form of bulk transport is called endocytosis, and is simply the opposite of exocytosis. Instead of exporting large molecules, endocytosis involves the engulfment of molecules by extensions of the plasma membrane, importing them into the cell.

rough endoplasmic reticulum (RER) a membranous organelle shaped like a series of connected, flattened cylinders that folds and transports proteins via its attached ribosomes

Golgi apparatus an organelle made of flattened sacs of membrane involved in modifying, sorting, and packaging proteins. Also known as the **Golgi body** or **Golgi complex**

Table 1 The function of key organelles in the protein secretory pathway

Organelle	Function	Description
1 Ribosome	Synthesises proteins	The ribosomes are the sites of protein synthesis. They assemble polypeptide chains from amino acids by translating mRNA.
2 Rough endoplasmic reticulum	Folds and transports proteins	If a protein is destined to be secreted, the ribosome synthesising it is usually attached to the rough endoplasmic reticulum rather than being free in the cytosol. The environment inside the rough endoplasmic reticulum allows for the correct folding of the newly formed polypeptide chain before being passed to the Golgi apparatus.
3 Transport vesicle	Transports proteins	A transport vesicle containing the protein buds off the rough endoplasmic reticulum and travels to the Golgi apparatus. The vesicle fuses with the Golgi membrane and releases the protein into its lumen.
4 Golgi apparatus	Modifies and packages proteins	Proteins can have chemical groups (e.g. sugar molecules) added or removed at the Golgi apparatus, where they are often packaged into secretory vesicles for export or released directly into the cytosol for use by the cell.
5 Secretory vesicle	Transports proteins	Secretory vesicles containing proteins for export bud off the Golgi apparatus and travel through the cytoplasm, fusing with the plasma membrane. This releases the proteins contained from within, into the extracellular environment through the process of exocytosis.

It's important to note that other organelles can also be involved in the protein secretory pathway, but to a lesser extent. For example, **mitochondria** are the site of ATP synthesis and provide the energy required to move vesicles around the cell and modify the proteins produced. The plasma membrane is another organelle involved, fusing with vesicles and facilitating the release of proteins from the cell. Additionally, the nucleus stores DNA, which contains the instructions for mRNA and therefore downstream protein synthesis.

mitochondrion (pl. mitochondria) a double-membrane-bound organelle that is the site of the second and third stages of aerobic cellular respiration

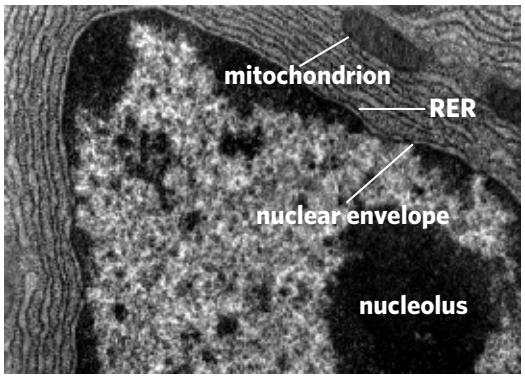


Figure 3 An electron micrograph depicting the rough endoplasmic reticulum, a mitochondrion, and the nucleus

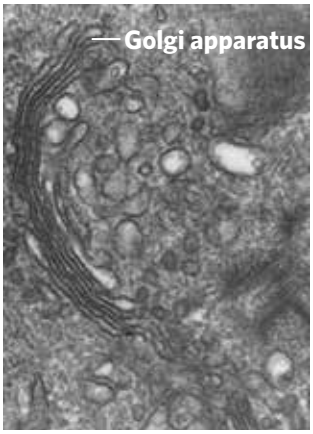


Figure 4 An electron micrograph depicting the Golgi apparatus

Theory summary

Proteins are produced at ribosomes, folded in the rough endoplasmic reticulum, transported via transport vesicles to the Golgi apparatus, where they are modified and packaged into secretory vesicles, and then subsequently exported from the cell via the process of exocytosis. Exocytosis is a form of bulk transport, involving the fusion of a secretory vesicle with the plasma membrane, releasing its contents into the extracellular environment.

The mastermind behind the cellular post office within your cells is the Golgi apparatus. After receiving proteins synthesised by the ribosomes, which are transported there from the rough endoplasmic reticulum via transport vesicles, it modifies the proteins and packages them into secretory vesicles for export. From there, the proteins can then be transported to their final destination. However, the only difference between the Golgi apparatus and your local post office is that the Golgi apparatus will never lose a single protein, while you might not be able to say the same for your local post office.

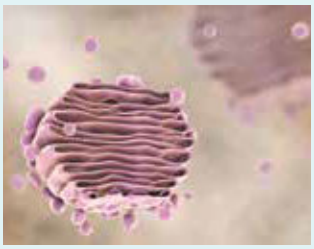


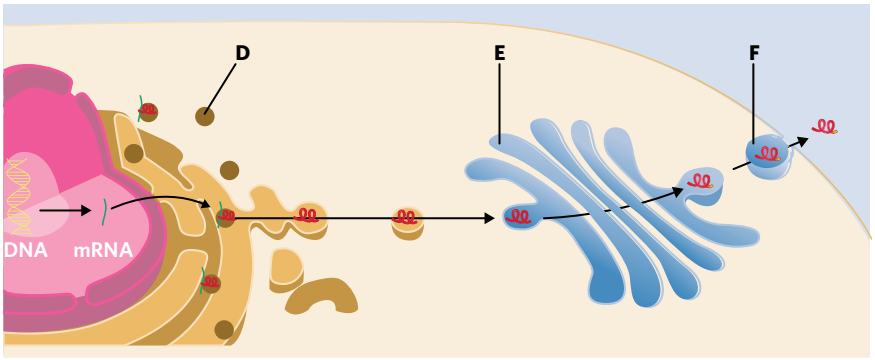
Image: Kateryna Kon/Shutterstock.com

2F QUESTIONS

Theory review questions

Question 1

Label the parts of the following diagram.



Question 2

Match the organelle to its description.

Organelle	Description
• ribosome	**I** _____ can have ribosomes attached to it, facilitating the folding of proteins
• mitochondrion	**II** _____ a fluid-filled sac containing substances such as proteins for export
• Golgi apparatus	**III** _____ the site of ATP production via aerobic cellular respiration
• secretory vesicle	**IV** _____ involved in the modification and packaging of proteins
• endoplasmic reticulum	**V** _____ the site of protein synthesis

Question 3

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- into
- out of
- energy
- glucose
- intracellular
- extracellular
- secretory vesicle

Exocytosis is a form of bulk transport, involving the movement of large substances such as proteins _____ a cell. Because it is also a form of active transport, the direct input of _____ is required. The process of exocytosis involves the fusion of a _____ to the plasma membrane, releasing its contents into the _____ environment.

Question 4

Which one of the following processes correctly depicts the process of exocytosis?

A Process J
B Process K
C Process L

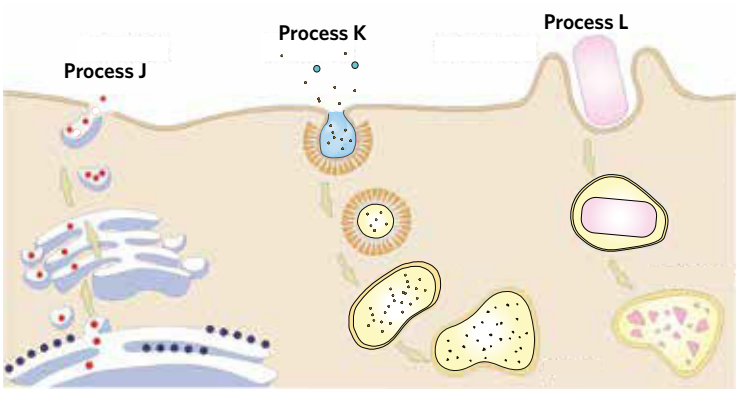


Image: Soleil Nordic/Shutterstock.com

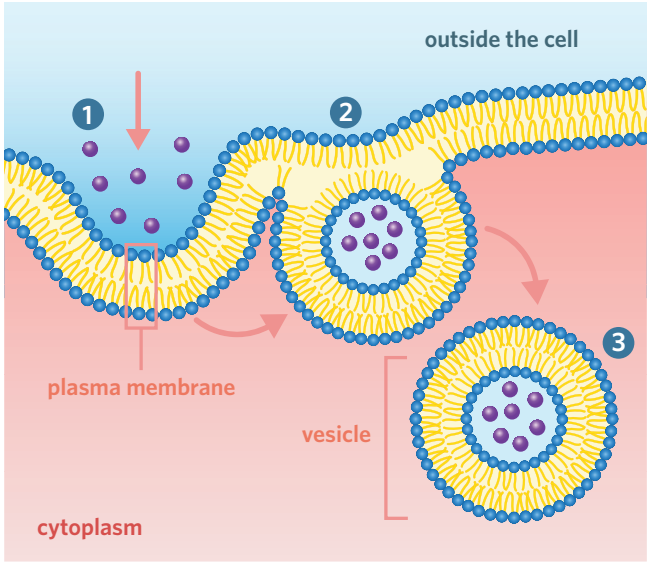
SAC skills questions

Case study analysis

Use the following information to answer Questions 5–8.

Endocytosis involves the bulk transport of large molecules or groups of molecules into cells. It is crucial to the functioning of cells, because many of the molecules that cells need to survive are too large to travel across the plasma membrane through alternative methods such as protein channels. Once inside the cell, these substances can be used for metabolic processes or structural elements of the cell. Endocytosis can also be an effective defence mechanism. Certain immune cells can engulf invading microorganisms or toxins and destroy them through the fusion of the vesicle with a lysosome. The stages involved in endocytosis include:

1. The folding of the plasma membrane inwards to form a cavity that fills with extracellular fluid and the target molecules.
2. The entrapment of the target molecules through the continued folding of the plasma membrane back on itself until the two ends of the membrane meet and fuse, trapping the target molecules inside the vesicle.
3. The budding of the vesicle off the plasma membrane and into the cell, where it can be transported to the appropriate cellular location.



There are also two subcategories of endocytosis – phagocytosis and pinocytosis. While phagocytosis involves the engulfment of solid material, pinocytosis involves the process of engulfing molecules that are dissolved in the extracellular fluid.

Question 5

Endocytosis involves the

A movement of substances into a cell.

B movement of substances out of a cell.

Question 6

The molecules entering a cell via endocytosis are

A enclosed within a vesicle.

B not enclosed within a vesicle.

Question 7

Phagocytosis involves the movement of

A solid material out of a cell.

B solid material into a cell.

C liquids out of a cell.

D liquids into a cell.

Question 8

Proteins found in the extracellular environment would have been produced at

A the smooth endoplasmic reticulum.
B the nucleus.
C a ribosome.
D a vesicle.

Exam-style questions

Within lesson

Question 9 (1 MARK)

All specialised cells that secrete protein molecules

A have minimal ribosomes.
B contain numerous vacuoles.
C do not have a plasma membrane.
D have an extensive rough endoplasmic reticulum.

Question 10 (1 MARK)

Which one of the following statements is false?

A Protein export involves vesicular transport of proteins out of the cell.
B Protein export involves the fusion of vesicles with the plasma membrane.
C Protein export involves specialised vesicles transporting specific proteins.
D Protein export involves sorting and modification of proteins at the Golgi apparatus.

Question 11 (1 MARK)

Gastrin is a peptide hormone that is released from cells in the stomach, duodenum, and pancreas. It aids digestion by stimulating the secretion of gastric acid by cells that line the stomach. One likely pathway for the production of gastrin in stomach cells could be

A nucleus → ribosome → rough endoplasmic reticulum → vesicle → Golgi apparatus
B nucleus → ribosome → Golgi apparatus → vesicle → rough endoplasmic reticulum
C nucleus → vesicle → rough endoplasmic reticulum → Golgi apparatus → ribosome
D nucleus → Golgi apparatus → rough endoplasmic reticulum → vesicle

Use the following information to answer Questions 12 and 13.

The following diagram shows the structure of certain organelles within a cell.

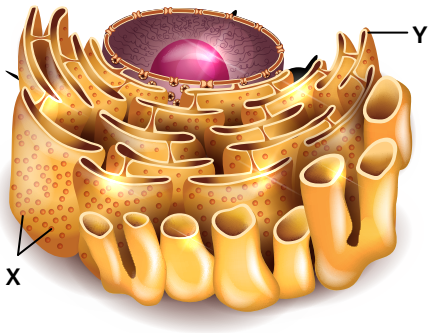


Image: Tefi/Shutterstock.com

Question 12 (1 MARK)

Organelle Y

A folds and dispatches protein.

B is involved in the production of lipids.

C contains numerous vesicles for protein transport.

D synthesises most of the ATP molecules required for active transport.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q1

Question 13 (1 MARK)

Organelle X

A is only made of protein.

B contains many phospholipids.

C is the site of protein modification.

D can also exist in the cytosol, unattached to other organelles.

Question 14 (1 MARK)

B lymphocytes are a type of immune cell that produce large amounts of antibodies. Antibodies are proteins that bind to and deactivate foreign substances like bacteria or toxins. B lymphocytes

A undertake large amounts of endocytosis.

B have extensive networks of endoplasmic reticulum.

C must use facilitated diffusion to transport antibodies out of the cell.

D do not have a Golgi apparatus, as they are specialised to make one type of protein.

Adapted from VCAA 2015 Section B Q5

Question 15 (6 MARKS)

In animal cells, tight junctions are multi-protein complexes that mediate cell-to-cell adhesion and regulate transport through the extracellular matrix. Proteins that form these complexes are made within the cell.

a Identify the cellular organelle where the primary structure of these proteins is made. (1 MARK)

b Outline the secretory pathway of these proteins. (3 MARKS)

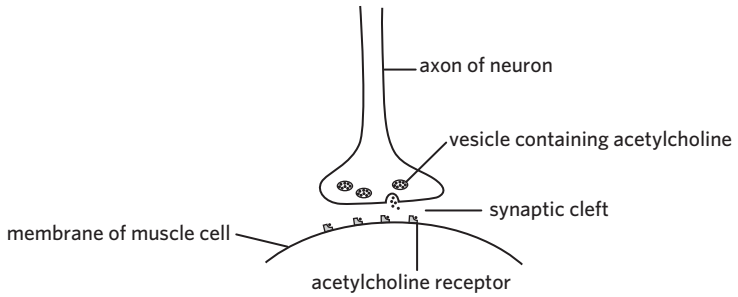
c Draw a labelled diagram to illustrate the exocytosis of these complex proteins from a cell. (2 MARKS)

Adapted from VCAA 2016 Section A Q7

Multiple lessons

Question 16 (5 MARKS)

Neurotransmitters are signalling molecules used to communicate between neurons and certain tissues of the body. One type of neurotransmitter is called acetylcholine, which can be released from the ends of neurons via the process of exocytosis. From there, it can diffuse across the synaptic cleft and bind to receptors on another neuron or tissue type. The receptors that interact with acetylcholine are proteins embedded within the plasma membrane. The following diagram shows the release of acetylcholine at the junction between a neuron and a muscle cell.



a Outline the events that would occur at a ribosome in the synthesis of an acetylcholine receptor. (3 MARKS)

b Describe the role of mRNA in the synthesis of the acetylcholine receptor. (1 MARK)

c Identify the organelle where the acetylcholine receptor would be packaged into secretory vesicles. (1 MARK)

Adapted from VCAA 2017 Section A Q21

Key science skills and ethical understanding

Question 17 (8 MARKS)

Alzheimer's disease is a progressive brain disorder that causes problems with memory, thinking, and behaviour. It is characterised by the build-up of a protein called beta-amyloid around neurons as individuals grow older. A group of scientists hypothesised that increased endocytosis, which involves the uptake of substances via a vesicle, by neurons of amyloid precursor protein (APP) may contribute to the accumulation of beta-amyloid proteins. The following diagram shows the process of endocytosis.

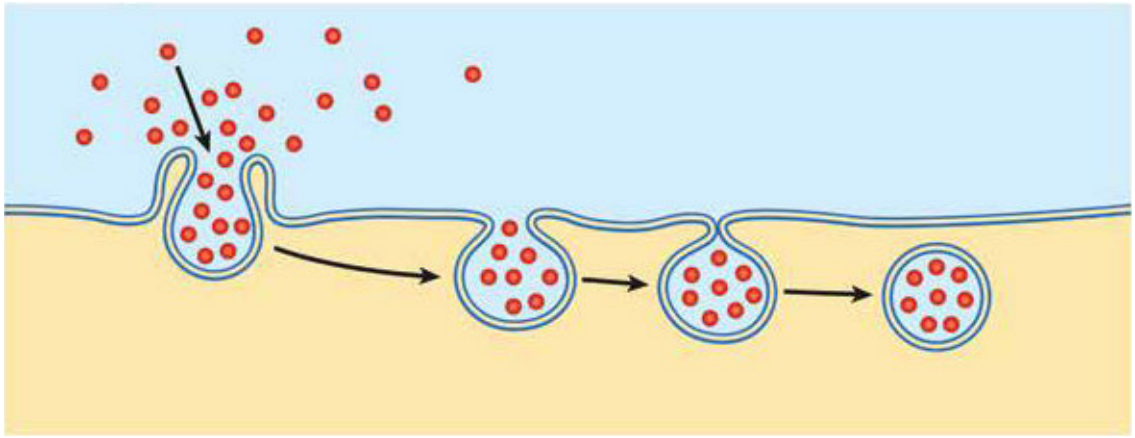


Image: Aldona Griskeviciene/Shutterstock.com

The scientists cultured neurons from mice and aged them *in vitro*. Mouse neurons undergo three developmental stages: they develop axons and dendrites after a week, reach peak maturation at 21 days, and exhibit aging at 28 days. The researchers compared 21-day-old neurons with 28-day-old ones for differences in APP endocytosis and beta-amyloid levels.

a Identify the independent and dependent variables of this experiment. (2 MARKS)

b The scientists found that aged neurons had 50% more beta-amyloid proteins, double the amount of APP endocytosis, and larger vesicles.

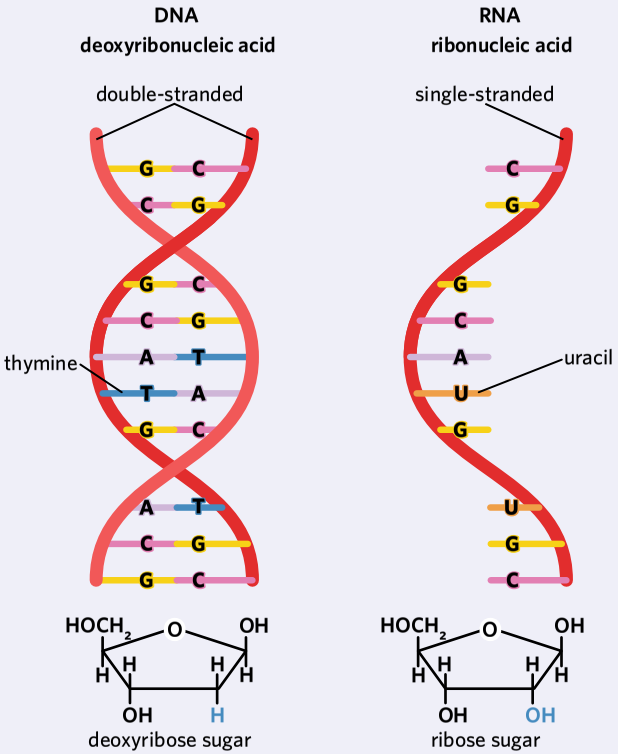
- **i** Do these results support or disprove the scientist's hypothesis? Justify your response. (1 MARK)
- **ii** Explain why the size of vesicles may be significant. (1 MARK)
- **iii** Identify and explain one limitation of these results. (2 MARKS)

c Describe the relevance of the bioethical concept of non-maleficence to the experimental scenario. (2 MARKS)

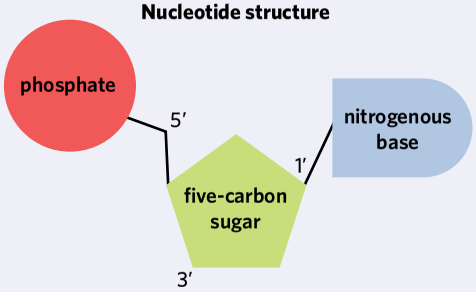
CHAPTER 2 SUMMARY

Nucleic acids

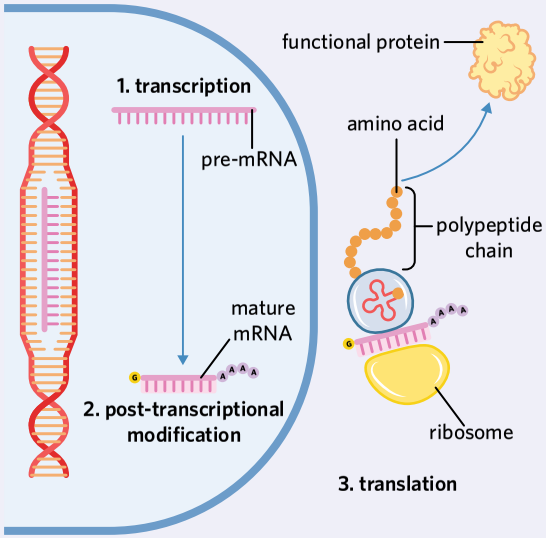
- DNA stores genetic information
- mRNA transports genetic information to the ribosomes
- tRNA carries specific amino acids to ribosomes for translation
- rRNA forms ribosomes that synthesise proteins



Nucleotide structure

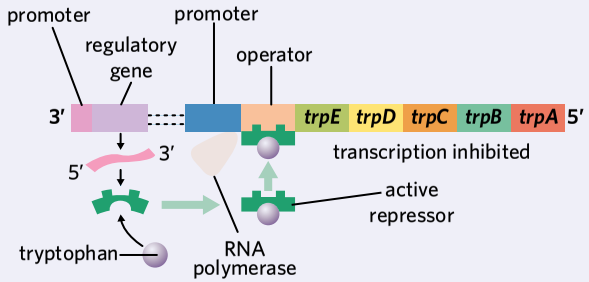


Protein synthesis



Gene regulation – *trp* operon

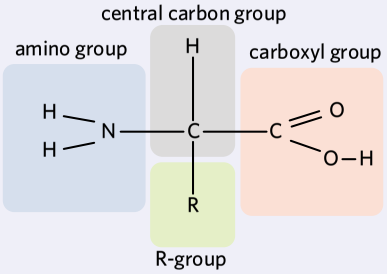
When tryptophan levels are high, tryptophan binds to the repressor which allows the repressor to bind to the operator to inhibit transcription of *trp* structural genes, thereby conserving energy. Conversely, when tryptophan levels are low, the repressor is released from the operator, allowing transcription to occur.



Proteins

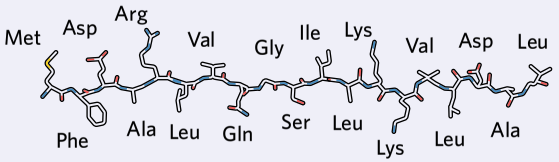
- Proteins have a diverse range of functions (e.g. structural, enzyme, motor, transport, signalling, storage).

Amino acid structure

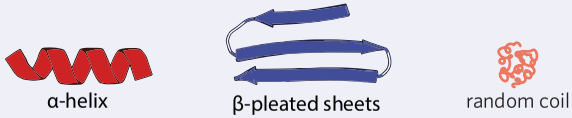


Protein structure

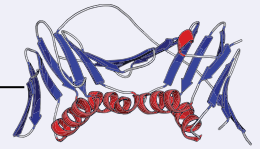
Primary structure involves the sequence of amino acids.



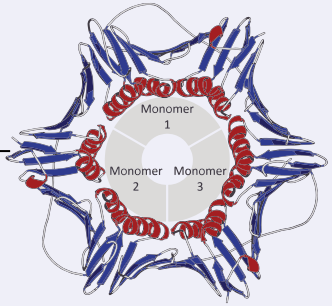
Secondary structure involves folding into alpha-helices, beta-pleated sheets, and random coils.



Tertiary structure involves the functional 3D structure of a protein.



Quaternary structure involves two or more polypeptide chains joining together.



CHAPTER 2 SAC PRACTICE

SAC skills covered in this section:

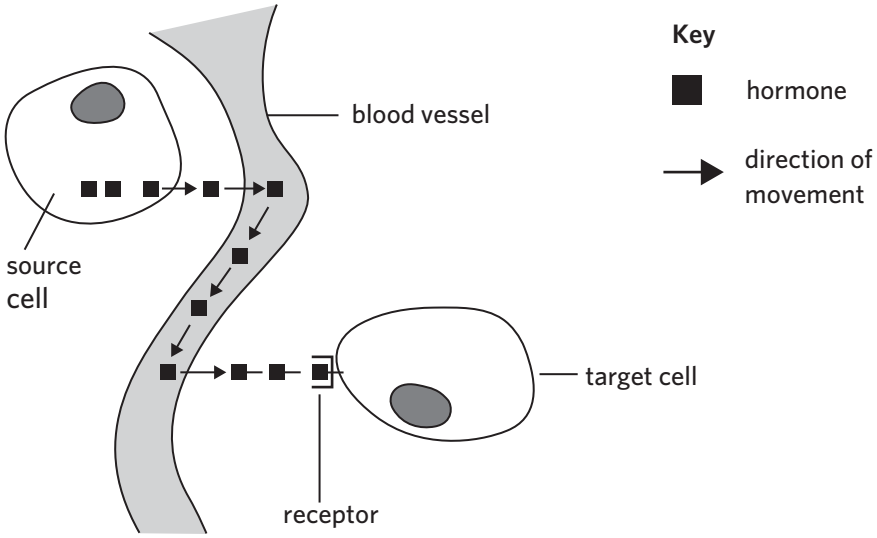
✓ Case study analysis ✓ Data analysis ✓ Bioethical deep dive

INSULIN (24 MARKS)

Hormones

There are many different mechanisms that exist within the body to help maintain a stable internal environment so that the body functions correctly. Factors which must be kept relatively constant include blood glucose levels, pH, temperature, the concentration of ions such as sodium and potassium, and many more. One of the mechanisms that the body can use to maintain this stable internal environment includes the use of hormones, which are cell signalling molecules.

Hormones are commonly composed of protein and can be used to transmit signals from one part of the body to another. For example, hormones are often released by cells into the bloodstream, where they travel to distant target cells. On those distant target cells are receptors, which are also typically composed of protein, with a complementary shape to the hormone. When the hormone binds to the receptor, specific cellular pathways are activated. The following diagram illustrates the binding of a hormone to its receptor.



1 Describe the function of a hormone. (1 MARK)

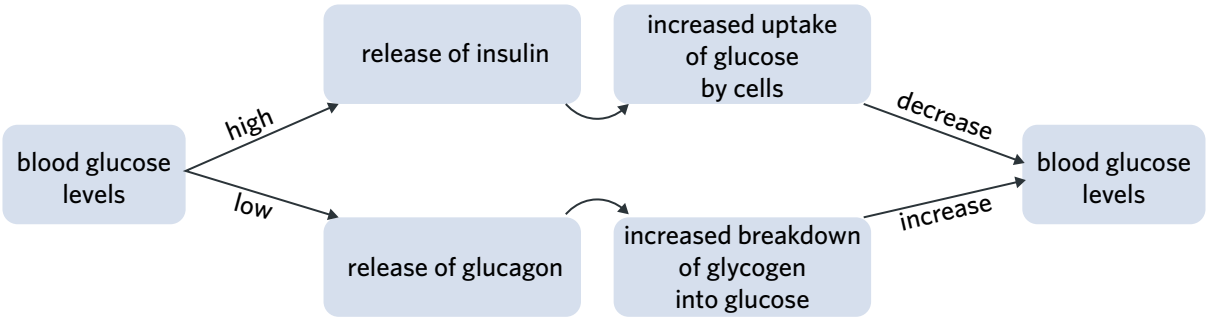
2 Identify two factors that should be kept relatively constant within the body. (2 MARKS)

3 Explain the significance of shape in the role of hormones and their receptors. (1 MARK)

Blood glucose levels

Blood glucose levels are one of the many factors that must be kept at a relatively constant level within the body. Typically, normal blood glucose levels range from 4.0–7.8 mmol/L, with the average level being around 5.0 mmol/L. When blood glucose levels deviate from their normal value, the body releases certain hormones in order to reduce or increase the amount of glucose within the blood. For example, if blood glucose levels rise above the normal value, then hormones will be released to reduce blood glucose levels. Conversely, if blood glucose levels fall below the normal value, then hormones will be released to increase blood glucose levels. The two hormones which regulate this process are known as:

- insulin, which reduces blood glucose levels by increasing the uptake of glucose by cells
- glucagon, which increases blood glucose levels by increasing the breakdown of glycogen into glucose.



Both insulin and glucagon are hormones composed of protein and, when required, are exported from the cell that produced them. Insulin is produced by beta cells in the pancreas, whereas glucagon is produced by alpha cells in the pancreas. Both hormones play a crucial role in regulating blood glucose levels, and ensure that they remain relatively constant within the body.

4 Name the monomer that makes up insulin. (1 MARK)

5 There are many stages involved in the production of insulin. Outline the process that occurs in the cytosol which directly leads to the production of insulin. (3 MARKS)

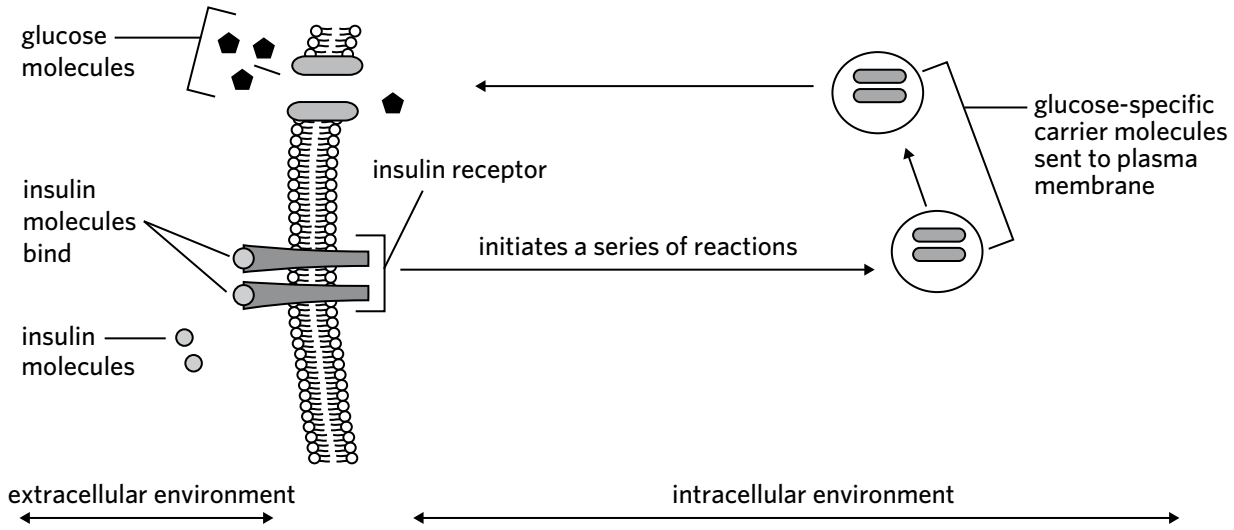
6 Outline the pathway insulin would take to be exported out of a cell after its production. (3 MARKS)

7 Describe the post-transcriptional modifications that would occur to a strand of pre-mRNA coding for insulin. (3 MARKS)

8 Describe the difference between structural and regulatory genes. (1 MARK)

Insulin

When insulin binds to receptors located on the cell surface, a series of events are initiated that lead to the insertion of glucose channels within the plasma membrane. These glucose channels are capable of transporting glucose from the extracellular environment into the cell, thereby increasing cellular uptake of glucose and decreasing blood glucose levels. The following diagram depicts the binding of insulin to its receptor, which increases the number of glucose channels.



9 Describe the events that would occur if blood glucose levels increased. (2 MARKS)

10 Suggest a reason other than diabetes why blood glucose levels may rise. (1 MARK)

11 Insulin is composed of two polypeptide chains, an alpha chain and a beta chain. Identify the level of protein structure that insulin displays. Justify your response. (2 MARKS)

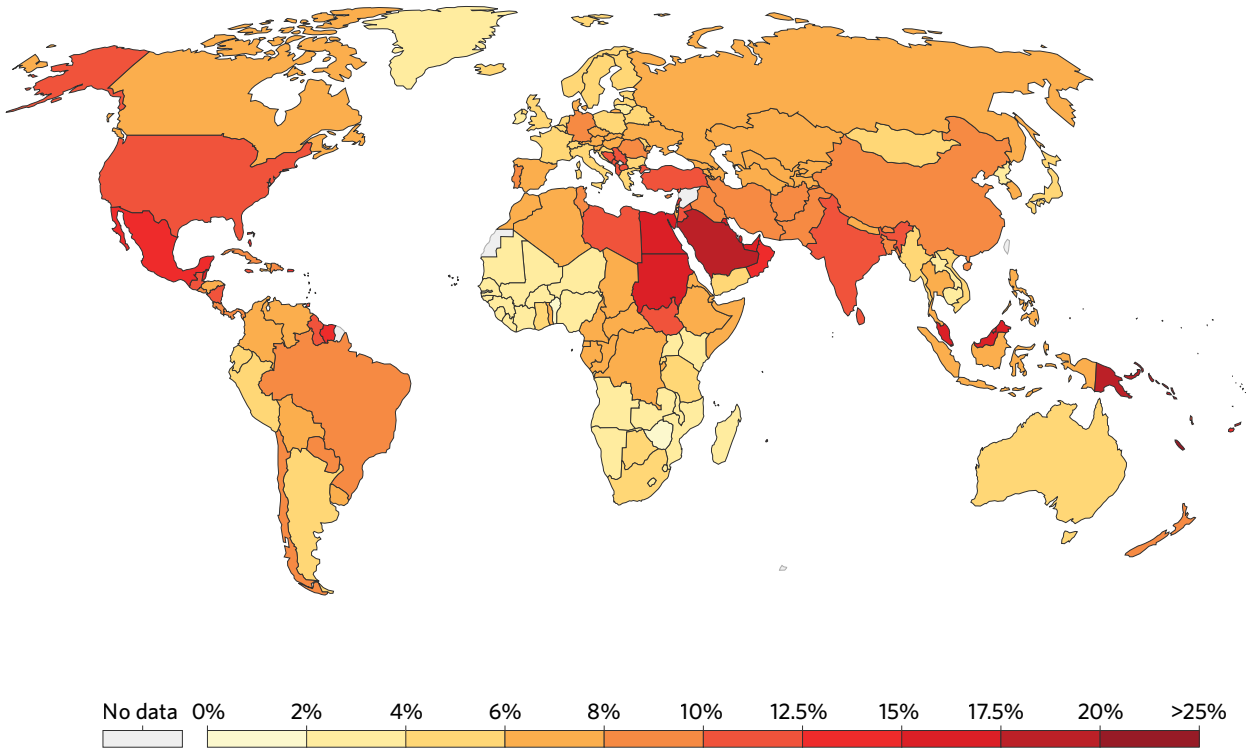
12 Bacteria, humans, pigs, and cows all produce insulin through the same pathway of protein synthesis. Explain why this is possible. (1 MARK)

Diabetes

Malfunctions can arise in the regulation of blood glucose levels. When this occurs, individuals can develop diabetes, which can be categorised into:

- type 1 diabetes, which occurs when individuals do not produce enough insulin
- type 2 diabetes, which occurs when insulin receptors become resistant to insulin, potentially due to changes in shape, and can no longer adequately bind to it.

The percentage of individuals between 20–79 who have been diagnosed with either type 1 or type 2 diabetes



Source: International Diabetes Federation, Diabetes Atlas

Due to their inability to effectively control blood glucose levels, diabetics often require daily injections of insulin to help manage their blood glucose levels. While insulin used to be harvested from other animals, it is now commonly made artificially. However, in many countries, there are significant barriers in terms of the availability and accessibility of insulin. For example, in America, it was reported that in 2018, the average price of insulin per unit was nearly $100. In Australia, however, the average price per unit of insulin in 2018 was reported to be under $10. This significant difference in price has largely been attributed to the power that large pharmaceutical companies hold, where they effectively hold a monopoly over the production and distribution of insulin.

13 Based on the image, what is the percentage of individuals diagnosed with either type 1 or type 2 diabetes in Australia? (1 MARK)

14 With reference to the bioethical concept of justice, suggest whether pharmaceutical companies should decrease their pricing of insulin. (2 MARKS)

CHAPTER 2 EXAM PRACTICE

60 min

Section A (16 MARKS)

Question 1 (1 MARK)

The genetic code specifies 20 different amino acids that can form proteins. Which one of the following explains the functional diversity of proteins?

A 20 amino acids allow for a large number of different combinations within a polypeptide.

B The processes of transcription and translation determine a protein's function.

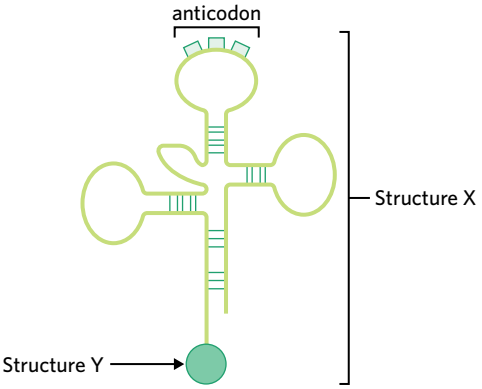
C Nucleic acids are synthesised frequently within a cell.

D Proteins cannot be inhibited or broken down.

Adapted from VCAA 2017 Sample Exam Section A Q1

Use the following information to answer Questions 2 and 3.

The following diagram depicts a molecule crucial to the process of protein synthesis.



Question 2 (1 MARK)

Structure X is known as

A DNA.

B tRNA.

C rRNA.

D mRNA.

Question 3 (1 MARK)

Structure Y is a molecular monomer of

A DNA.

B RNA.

C lipids.

D proteins.

Adapted from VCAA 2012 Exam 1 Section A Q4

Question 4 (1 MARK)

The proteome is

A the set of proteins undergoing the transcription process in an organism.

B all proteins, carbohydrates, lipids, and nucleic acids in an organism.

C the entire set of proteins expressed by an organism.

D the most common protein in an organism.

Adapted from VCAA 2011 Exam 1 Section A Q1

Question 5 (1 MARK)

A particular DNA double helix is 100 nucleotide pairs long and contains 25 adenine bases. The number of uracil bases in this DNA double helix would be

A 0.
B 25.
C 75.
D 100.

Adapted from VCAA 2012 Exam 1 Section A Q5

Question 6 (1 MARK)

Which one of the following events does not occur when tryptophan levels are high in a cell?

A Tryptophan binds to the *trp* repressor.
B Enzymes that produce tryptophan are produced.
C The transcription factor is inhibited from binding to the promoter.
D A repressor protein is bound to the operator region of the *trp* operon.

Question 7 (1 MARK)

Which of the following statements about gene regulation is false?

A Repressor proteins bind to the operator region.
B Structural genes code for proteins that are not involved in gene regulation.
C Regulatory genes control the expression of structural genes using transcription factors.
D Transcription factors are proteins that control gene expression at the transcription and translation stages.

Adapted from VCAA 2016 Section A Q32

Question 8 (1 MARK)

Different cells within an organism have different proteins. In some cases, different proteins can be coded for by the same gene. One gene can code for several proteins because

A of alternative splicing.
B there are 20 amino acids.
C of the specificity of the genetic code.
D genes can alter their sequence during transcription.

Adapted from VCAA 2017 Section B Q1c

Question 9 (1 MARK)

Proteins are not part of the structure of

A the plasma membrane.
B messenger RNA.
C haemoglobin.
D antibodies.

Adapted from VCAA 2012 Exam 1 Section A Q8

Question 10 (1 MARK)

Which one of the following statements about the structure of a eukaryotic gene is false?

A Introns undergo the transcription process.
B Exons contain the protein-coding sequence.
C A promoter region is found upstream of a gene.
D Alternative splicing results in different sets of introns being translated.

Adapted from VCAA 2017 Sample Exam Section A Q6

Use the following information to answer Questions 11 and 12.

The following diagram shows one of the major biomacromolecules in living things.

Question 11 (1 MARK)

The monomers comprising the macromolecule vary in their

A sugar-phosphate backbones.

B nitrogen-containing bases.

C phosphate groups.

D sugar groups.

Adapted from VCAA 2016 Section A Q3

Question 12 (1 MARK)

A portion of the coding strand of a macromolecule has the sequence -TACGTGCTTGAT-. The mRNA strand produced from this coding strand during transcription would be

A -TACGTGCTTGAT-

B -ATGCACGAACTA-

C -AUGCACGAACUA-

D -UACGUGCUUGAU-

Adapted from VCAA 2016 Section A Q4

Question 13 (1 MARK)

Bacteria can have a gene from another species inserted (e.g. a human gene), and they can be cultured to produce a given protein in large quantities. It is possible to introduce a human gene into bacteria because the DNA code is

A universal.

B redundant.

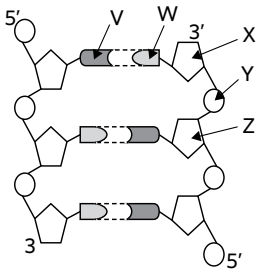
C degenerate.

D non-overlapping.

Adapted from VCAA 2013 Section A Q36

Use the following information to answer Questions 14 and 15.

The following diagram represents part of a DNA molecule.



Question 14 (1 MARK)

A hydrogen bond is formed between sub-units

A X and Y.

B Y and Z.

C V and W.

D X and Y and Y and Z.

Adapted from VCAA 2015 Section A Q3

Question 15 (1 MARK)

Which one of the following statements is false?

A Sub-unit V could be adenine.

B Sub-unit Y is a deoxyribose sugar.

C Sub-unit X is the same in every DNA nucleotide.

D This diagram displays the deoxyribose sugar-phosphate backbone.

Question 16 (1 MARK)

The following codon table can be used to determine the sequence of amino acids coded for by a nucleotide sequence.

1st position (5′ end)	2nd position				3rd position (3′ end)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	**U**
	Phe	Ser	Tyr	Cys	**C**
	Leu	Ser	STOP	STOP	**A**
	Leu	Ser	STOP	Trp	**G**
C	Leu	Pro	His	Arg	**U**
	Leu	Pro	His	Arg	**C**
	Leu	Pro	Gln	Arg	**A**
	Leu	Pro	Gln	Arg	**G**
A	Ile	Thr	Asn	Ser	**U**
	Ile	Thr	Asn	Ser	**C**
	Ile	Thr	Lys	Arg	**A**
	Met	Thr	Lys	Arg	**G**
B	Val	Ala	Asp	Gly	**U**
	Val	Ala	Asp	Gly	**C**
	Val	Ala	Glu	Gly	**A**
	Val	Ala	Glu	Gly	**G**

The following nucleotide sequence is found on the template strand at a particular site in the genome.

GCT TTA CGG TTA TA**T** ACC

Due to a DNA change, the bolded nucleotide in this sequence was changed from a T to a G. What would be the result of this DNA change?

A The peptide chain would be shortened.

B The fifth amino acid would change from tyr to cys.

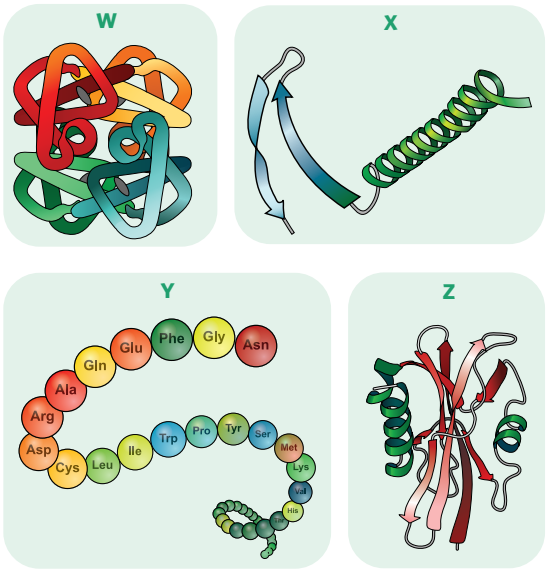
C The sixth amino acid would change from cys to STOP.

D There would be no change in the amino acid sequence.

Section B (24 MARKS)

Question 17 (6 MARKS)

The following diagrams represent examples of the four levels of protein structure. Note that the diagrams are not to scale.



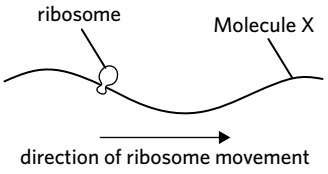
a Identify the level of protein structure represented in the diagrams W, X, Y, and Z. (2 MARKS)

b Describe the functional significance of Z. (1 MARK)

c Outline the process that occurs at a ribosome which leads to the production of Y. (3 MARKS)

Question 18 (6 MARKS)

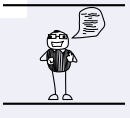
Diphtheria is a serious bacterial infection caused by the bacterium *Corynebacterium diphtheriae*, with symptoms including difficulty breathing, heart failure, paralysis and in severe cases, death. It achieves this through the production of toxins which kill healthy tissues within the respiratory tract, causing the buildup of dead cells in what is known as a pseudomembrane, eventually blocking the airways. One way diphtheria toxin works is by stopping the movement of ribosomes along a certain molecule. This molecule has been labelled as Molecule X in the following diagram.



a Identify Molecule X and describe its function. (2 MARKS)

b Outline the process involved in the production of Molecule X. (3 MARKS)

c State the cellular process which diphtheria toxin is thought to interfere with. (1 MARK)



Question 19 (6 MARKS)

Human insulin is a macromolecule composed of two polypeptide chains. The chains are connected by disulphide bonds.

a Identify the monomers that make up insulin. (1 MARK)

b Describe the structure of the monomers of insulin, and explain how they differ from the monomers of DNA. (2 MARKS)

c Insulin found in other animals differs from human insulin. The following table compares the differences seen in the primary structure of human, cow, pig, and sheep insulin.

	Amino acid position number within	
	Alpha chain	**Beta chain**
	- 8 - 9 - 10 -	**- 30 -**
human	- thr - ser- ile -	thr
cow	- ala - ser - val -	ala
pig	- thr - ser - ile -	ala
sheep	- ala - gly - val -	ala

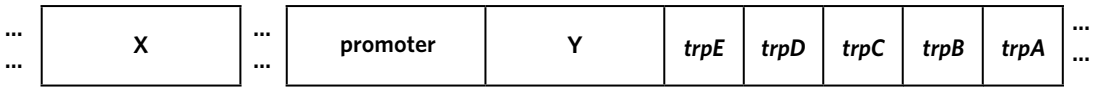
i Humans with diabetes take insulin injections to help control their blood glucose levels. If no human insulin is available, it is possible to use similar insulin from another animal. According to the table, explain which animal's insulin structure is the least similar to human insulin. (1 MARK)

ii Do cows, pigs, and sheep have an identical sequence of nucleotides at amino acid position 30 in the beta chain? Justify your response. (2 MARKS)

Adapted from VCAA 2012 Exam 1 Section B Q3

Question 20 (6 MARKS)

The *trp* operon was originally identified in *Escherichia coli*. The *trp* operon has five structural genes: *trpE*, *trpD*, *trpC*, *trpB*, and *trpA*. These genes code for proteins which help a cell produce the amino acid tryptophan. The diagram shows the order of the genes found in the *trp* operon. The dotted lines represent the DNA nucleotides between the genes.



a What is an operon? (1 MARK)

b Identify regions X and Y. (2 MARKS)

c Identify the region to which RNA polymerase binds to. (1 MARK)

d Explain how the *trp* operon functions when tryptophan levels are low. (2 MARKS)

Adapted from VCAA 2017 Section A Q2

CHAPTER

3

Enzymes

3A Introducing enzymes

3B Factors that affect enzymes

Key knowledge

- proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways
- the general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors
- the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration

Image: Design_Cells/Shutterstock.com

3A INTRODUCING ENZYMES

It sounds un-Brie-lievable, but some individuals simply Camembert eating dairy products. People with lactose intolerance struggle to digest the sugar lactose that is found in milk and other dairy products. As a result, they experience a range of not Gouda digestive issues if they eat dairy products. Lactose intolerance is pretty Edam common, with some estimates stating that roughly 65% of the global population have a reduced ability to digest lactose. So if you're someone who finds it necessary to have an emergency bathroom visit after sculling a glass of choccy milk, don't be ashamed – you're one of the normal ones, it's those cow-suckling freaks that are the weird ones! But, is there anything we can fondue about lactose intolerance? Or will these poor people be forever blue about the fact that they miss out on some of life's grate-est pleasures?

Just like a chocolate milkshake, only... warm.
Image: ifong/Shutterstock.com

Lesson 3A

In this lesson you will learn about enzymes, which are catalysts that speed up chemical reactions in many important biochemical pathways.

◀ *Prerequisite knowledge*

Years 7–10
The rate of a chemical reaction is dependent on a number of factors, one of which includes the presence or absence of enzymes.

Year 11
Many different body systems are dependent on enzymes to function, such as the digestive system which relies on the presence of enzymes to digest food.

Lesson 2D
RNA polymerase is the enzyme responsible for transcribing DNA.

Future applications ▶

Lesson 3B
There are many factors that affect the activity of enzymes such as temperature, pH, and enzyme inhibitors.

Lesson 4A
DNA can be manipulated by scientists with the use of specific enzymes capable of cutting, joining, and amplifying DNA.

Chapters 5 and 6
The complex chemical reactions of photosynthesis and cellular respiration are catalysed by a variety of different enzymes.

Study design dot point

- proteins as a diverse group of molecules that collectively make an organism's proteome, including enzymes as catalysts in biochemical pathways

Key knowledge unit

Enzymes	3.1.6.2

Enzymes 3.1.6.2

OVERVIEW

Enzymes speed up biochemical reactions by lowering the activation energy required to initiate a given reaction.

THEORY DETAILS

It is estimated that every second in your body, there are 37 thousand billion billion chemical reactions occurring. These chemical reactions include DNA replication, cell communication, cellular respiration, and the breakdown of nasty toxins in the liver. However, most of these reactions can't just happen on their own. All reactions require energy to initiate the process. This is where **enzymes** come in.

enzyme an organic molecule, typically a protein, that catalyses (speeds up) specific reactions

What are enzymes?

Enzymes are molecules that are organic (carbon-based) **catalysts**. This means that they speed up, or **catalyse**, chemical reactions that would normally take much longer to occur. Enzymes bind to a molecule called a **substrate**, which is the name given to the **reactant** undergoing an enzyme facilitated reaction. Upon binding, the substrate undergoes a chemical reaction and forms a **product**(s), which then leaves the enzyme. Importantly, the enzyme remains unchanged from the reaction and is now free to catalyse further reactions.

The enzyme apha-glucosidase is shown in detail in Figure 1a. Note the alpha-helices and beta-pleated sheets which form the three-dimensional structure of the enzyme. As you can see, the accurate representation of enzymes is relatively complex and difficult to understand. Therefore, simplified two-dimensional diagrams of enzymes and substrates will instead be used throughout this lesson (Figure 1b).

catalyst a substance capable of increasing the rate of a reaction without being used up

catalyse to increase the rate of a reaction

substrate the reactant of a reaction catalysed by an enzyme

reactant a molecule that undergoes a transformation into a product. When enzymes are involved, the reactant is called a substrate

product the transformed molecule created in a reaction

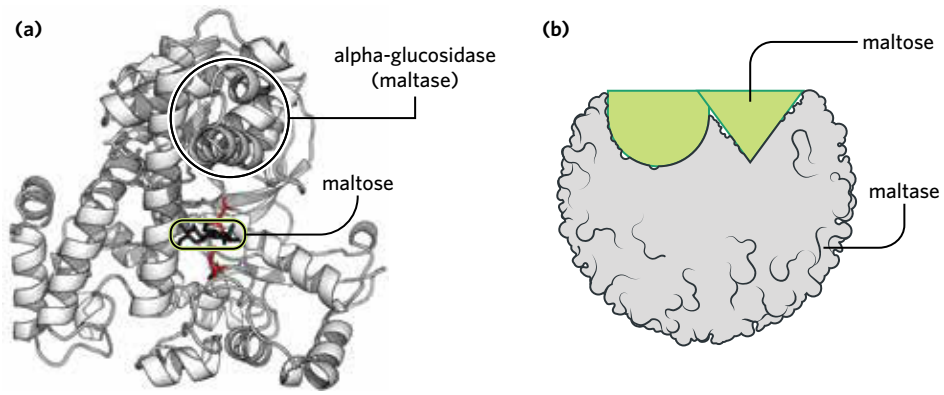


Image: StudioMolekuul/Shutterstock.com

Figure 1 **(a)**Three-dimensional representation of the enzyme alpha-glucosidase, also known as maltase (white), bound to the substrate maltose (black) at the active site (red) and **(b)** a simplified representation of the enzyme.

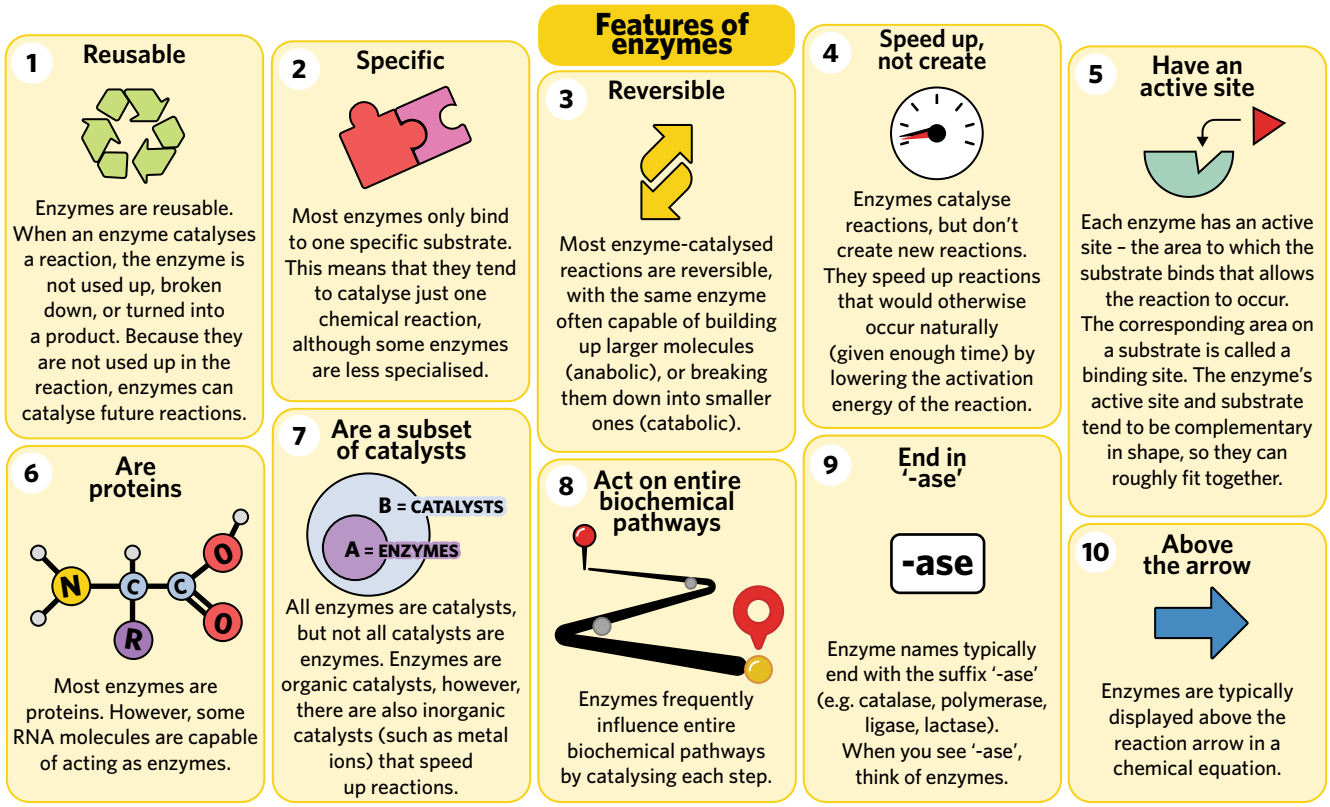


Figure 2 The key features of enzymes you should know.

What do enzyme-catalysed reactions look like?

As mentioned in Figure 2, each enzyme has an **active site**. The active site is a pocket-like area of the enzyme's tertiary structure where the substrate binds to. Due to the compatibility of the complex three-dimensional structures, we say that an enzyme's active site and substrate are complementary in shape.

active site the part of an enzyme where the substrate binds

When a substrate binds to an enzyme's active site, together they form an **enzyme-substrate complex**. Upon binding, the active site undergoes a **conformational change** to accommodate the substrate, and the substrate undergoes a small change in turn. Think of the change as a handshake or a hug – both parties adjust to allow for a stronger connection. Many chemical bonds (e.g. hydrogen bonds, hydrophobic interactions) hold the substrate and active site together in the enzyme-substrate complex.

enzyme-substrate complex the structure formed when an enzyme and substrate are bound together

conformational change a change in the three-dimensional shape of macromolecules such as proteins

You can see in Figure 3 that the substrate (green) binds to the enzyme (grey) at the active site which is complementary in shape to the substrate. The reaction then occurs and the products (blue and red) leave the enzyme so that it can continue to catalyse future reactions.

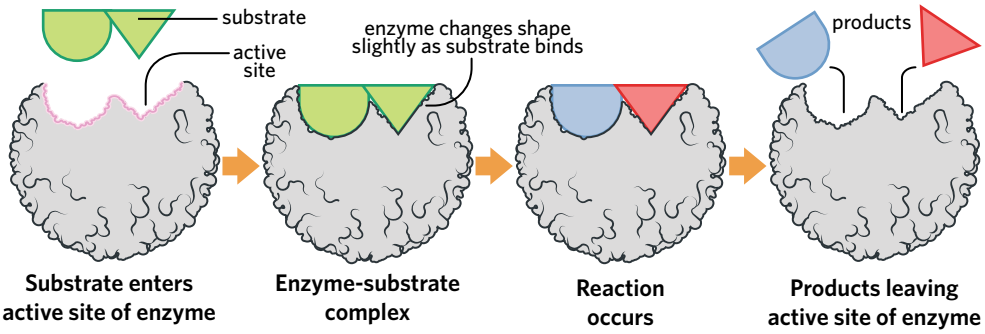


Figure 3 An enzyme undergoes a conformational change to catalyse a reaction.

Theory in context

LOCK AND KEY VS INDUCED FIT

Because enzymatic activity is happening on such a minute scale, it is often difficult to understand exactly what is going on. Biologists use models to visualise what they believe is happening. It was once thought that a substrate fits into an active site perfectly, like how a key fits into a lock. This was known as the lock and key model of the enzyme-substrate complex. It is now believed to be incredibly rare for an enzyme and its substrate to fit together perfectly. Usually, there is a slight adjustment upon binding to better fit one another. This model is known as the induced fit model, which states that an enzyme undergoes a conformational change to become complementary in shape to the substrate.

Memory device

Imagine you are tasked with screwing 100 metal screws into a piece of wood during your woodwork class. Instinctively, you should immediately pick up a screwdriver to help you complete your task. Just like a screwdriver makes screwing screws much easier and faster, enzymes make chemical reactions occur faster. The screwdriver (enzyme), is specific to certain types of screws (substrate), and the tip of the screwdriver (active site) is where contact is made. After acting on a screw, the screwdriver (or enzyme) is unchanged and free to act on many more screws.

Enzyme-catalysed reactions are incredibly similar to the chemical reactions and equations you may have already come across (Figure 4). The only difference is that the reactant is called the substrate. Because the enzyme is considered neither a reactant nor a product, it is written above the arrow in an equation.

Figure 4 The enzyme-catalysed breakdown of hydrogen peroxide

Theory in action

Check out scientific investigation 3.1 to put this into action!

Activation energy

Every chemical reaction requires an input of energy to start. This initial requirement is the **activation energy**. The activation energy is defined as the minimum amount of energy required to energise atoms or molecules to a state where they can undergo a chemical transformation. Think of it as the hurdle that reactants need to get over to start a chemical reaction.

activation energy the energy required to initiate a reaction

All reactions have an activation energy, regardless of whether they are anabolic or catabolic. An anabolic reaction is when two or more smaller molecules combine to form a larger one (i.e. building things up), whereas a catabolic reaction is a larger molecule turning into two or more smaller molecules (i.e. breaking things down).

Enzymes function to lower the activation energy of chemical reactions by bringing reactants closer to the state they need to be in order to react. In other words, enzymes significantly reduce the size of the hurdle. This allows reactions to proceed at a much quicker rate. For example, the enzyme carbonic anhydrase can catalyse the reaction of carbon dioxide and water into carbonic acid 10 million times quicker than the uncatalysed reaction. The activation energy of a reaction is typically displayed in graphical form (Figure 5).

Lesson link

Many immune defences rely on the presence of enzymes, which can aid in the degradation of invading microorganisms – see ***chapter 7***.

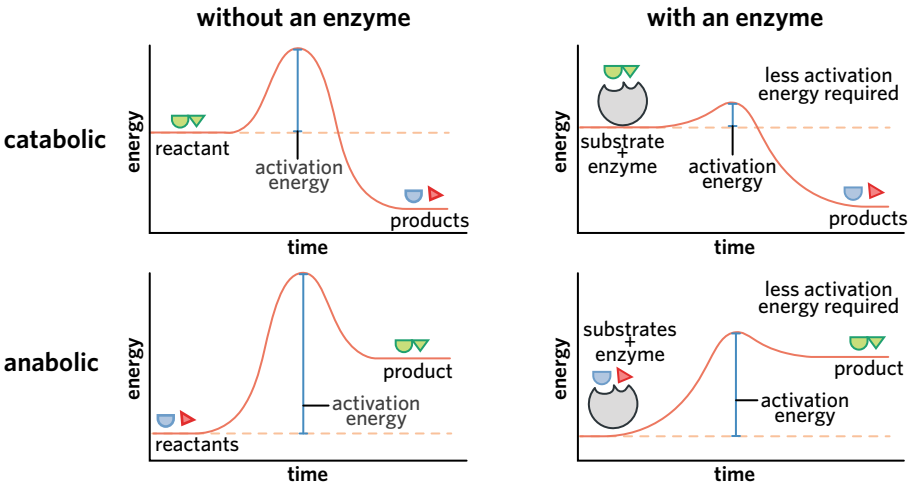


Figure 5 The activation energy required in a reaction without and with an enzyme

Enzymes and biochemical pathways

Enzymes are capable of catalysing reactions continuously, and frequently 'team up' to work in chains of reactions. In a **biochemical pathway**, one enzyme will function to catalyse a substrate into a product, which will then become the substrate of a second enzyme. Recall that enzymes are specific to their substrate, so enzymes must function in pathways to reach the desired outcome.

In Figure 6, the first three steps and enzymes of the biochemical pathway of glycolysis are shown. From the diagram, we can see that hexokinase is the enzyme responsible for catalysing the reaction of glucose into glucose 6-phosphate. The phospho-hexose isomerase enzyme then catalyses the conversion of glucose 6-phosphate into fructose 6-phosphate and so on. While you don't need to know the specifics of glycolysis, just appreciate how vital enzymes are and how complicated biochemical pathways can be!

biochemical pathway a series of enzyme-catalysed biochemical reactions in which the product of one reaction becomes the substrate of the next reaction. Also known as a **metabolic pathway**

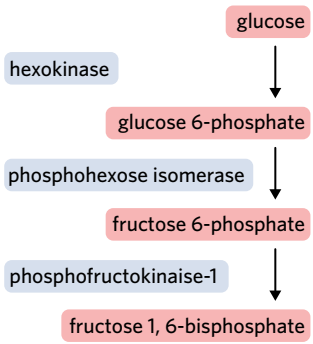


Figure 6 The first three of ten enzymatic reactions in the biochemical pathway of glycolysis. Note: this is not assessible material.

> ***Lesson link***
>
> While you may not be assessed directly about them, enzymes are a critical part of a number of different areas of the VCE Biology study design. Two biochemical processes heavily reliant on the use of enzymes are photosynthesis and cellular respiration, which are explored in ***chapter 5*** and ***chapter 6***.

Theory summary

Enzymes are organic catalysts that lower the activation energy of reactions. They are specific, are not used up in reactions, can sometimes work in both directions of a reaction, and can catalyst each step of entire metabolic pathways.

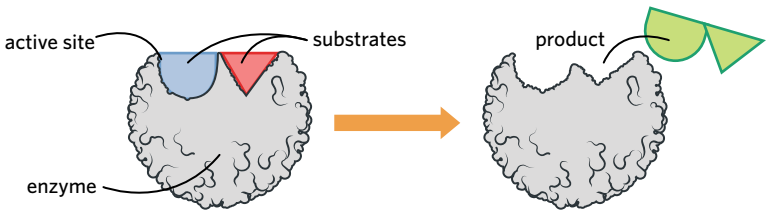


Figure 7 An enzyme catalysing a reaction

Lactose intolerance is a result of an enzyme deficiency. Normally when a person consumes lactose, an enzyme in the small intestine called lactase breaks down lactose into glucose and galactose. Nearly everyone has lactase in their digestive system when they are young in order to digest their mother's milk, but as you age, your lactase levels typically decrease, often resulting in an intolerance. Without lactase, lactose is instead broken down by probiotic bacteria within the intestines. A nasty side-effect of this bacteria-led breakdown is the production of large amounts of gas – which can manifest in the symptoms associated with lactose intolerance such as bloating, cramps, and diarrhoea. If you are in desperate need, some oral enzyme supplements are available as short-term aids for those lactose intolerant individuals that are screaming for ice-cream.

Image: KomootP/Shutterstock.com

3A QUESTIONS

Theory review questions

Question 1

Enzymes are

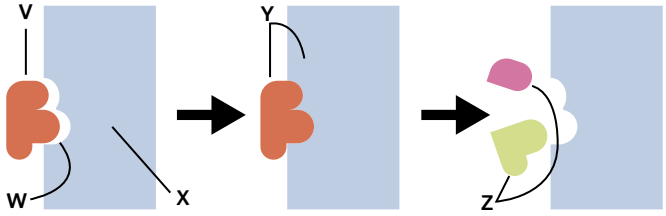
A proteins that raise the activation energy of reactions.

B organic catalysts that often influence entire biochemical pathways.

Question 2

Label the parts of the diagram from the list of terms.

- enzyme
- products
- substrate
- active site
- enzyme-substrate complex



Question 3

Which are key features of enzymes? (*Select all that apply*)

I reusable

II non-specific

III are mostly proteins

IV contain an active site

V shown below the arrow

VI most reactions are reversible

Question 4

Fill in the blanks in the following sentences.

Enzymes are catalysts that have an _____ where a substrate binds. They function to _____ the activation energy of reactions in order to _____ the reaction rate. Following a reaction, an enzyme _____ go on to catalyse further reactions.

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–9.

Amino acids are the building blocks of proteins and are essential for growth and development. However, the accumulation of certain amino acids within the body can be highly toxic. Phenylketonuria (PKU) is an inherited disorder that causes a deficiency in the enzyme phenylalanine hydroxylase (PAH), resulting in reduced levels or the complete absence of PAH. Because the role of PAH is to catalyse the conversion of the amino acid phenylalanine into another amino acid, tyrosine, those suffering from PKU accumulate high levels of phenylalanine in their bodies. Unfortunately, the buildup of phenylalanine can have devastating effects, resulting in the death of neurons within the brain.

Without treatment, most individuals with PKU develop severe intellectual disabilities. Fortunately, PKU is detectable during routine newborn screening, which is carried out in the first few days of a baby's life. A form of PKU has also been discovered in mice and testing on these model organisms can provide us with a better understanding of PKU and its treatments in humans. Currently, treatment for individuals diagnosed with PKU consists of a carefully controlled diet. The diet restricts the intake of foods containing phenylalanine and involves ongoing monitoring of their phenylalanine levels to ensure they don't reach toxic levels.

Question 5

Phenylalanine hydroxylase is

A a condition where individuals experience a buildup of amino acids leading to the death of neurons.

B an enzyme responsible for the conversion of one type of amino acid into another.

Question 6

Intellectual disabilities in individuals with PKU are caused by

A a deficiency in PAH leading to excess tyrosine buildup resulting in brain damage.

B a lack of phenylalanine breakdown leading to toxic levels and brain damage.

Question 7

Newborns screened to have PKU are put on a strict diet in order to

A limit the amount of phenylalanine intake to prevent the buildup of phenylalanine due to the presence of a PAH deficiency.

B increase the levels of tyrosine intake to encourage the PAH enzyme to function in reverse by catalysing the conversion of tyrosine to phenylalanine.

Question 8

The PAH-catalysed reaction can be represented by

A PKU $\xrightarrow{\text{PAH}}$ phenylalanine

B phenylalanine $\xrightarrow{\text{PAH}}$ tyrosine

C PAH $\xrightarrow{\text{phenylalanine}}$ tyrosine

D tyrosine $\xrightarrow{\text{PKU}}$ phenylalanine

Question 9

The ethical concept of integrity should be applied in PKU studies on mice by

A ensuring that physical harm to the mice is minimised in each step of the study.

B publishing all results, positive and negative, of the study to the scientific community.

Exam-style questions

Within lesson

Question 10 (1 MARK)

The activation energy in a biological reaction is

A completely removed in the presence of an enzyme.

B the energy required to finish the reaction.

C lowered in the presence of an enzyme.

D involved in catabolic reactions only.

Adapted from VCAA 2008 Exam 1 Section A Q18

Use the following information to answer Questions 11–13.

In an experiment, two students added 5 mL of a glucose solution, 5 mL of a maltose solution, 1 mL of a maltase solution, and 100 mL of water to a beaker. After an hour, the amount of glucose in the beaker increased, the amount of maltose decreased, the amount of water also decreased, and the amount of maltase remained unchanged.

Question 11 (1 MARK)

The product in this scenario is

A maltose.

B maltase.

C glucose.

D water.

Question 12 (1 MARK)

The enzyme in this scenario is

A maltose.
B maltase.
C glucose.
D water.

Question 13 (1 MARK)

Which of the following displays the equation of the reaction?

A maltose $\xrightarrow{\text{maltase}}$ glucose
B maltase + water $\xrightarrow{\text{maltose}}$ glucose
C maltose $\xrightarrow{\text{glucose}}$ maltase
D maltose + water $\xrightarrow{\text{maltase}}$ glucose

Question 14 (1 MARK)

The enzyme lactase digests lactose.

lactose $\xrightarrow{\text{lactase}}$ glucose + galactose

Two test tubes were set up in an experiment. Test tube one contained 5 mL of lactose syrup and 0.5 mL of lactase extracted from humans. Test tube two contained 5.5 mL of lactose syrup and no lactase.

After 10 minutes, the amount of glucose produced in test tube one when compared to test tube two would be

A equal as both reactions occur at the same rate.
B lower as test tube two contained more lactose syrup to breakdown.
C higher as the presence of lactase speeds up the production of glucose.
D higher as the greater amount of substrate present allows for faster glucose production.

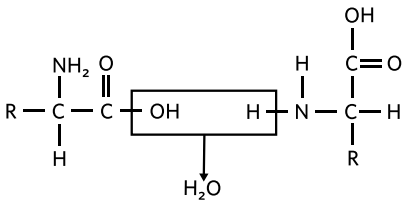
Adapted from VCAA 2009 Exam 1 Section A Q7

Multiple lessons

Question 15 (6 MARKS)

Enzymes are proteins that are formed by adjacent amino acids being joined together.

The following diagram represents this joining of amino acids.



a Name the type of bond formed when two amino acids join together. (1 MARK)

Adapted from VCAA 2018 Section A Q3

b Proteins have four levels of basic structure.

i Which level of structure is represented in the diagram? (1 MARK)

ii Describe the differences between the secondary and tertiary levels of structure in a functional enzyme. (2 MARKS)

iii Suggest why not all proteins possess a quaternary structure. (2 MARKS)

Question 16 (5 MARKS)

Amylase is critical for catalysing the breakdown of foods in saliva. Salivary amylase is produced when the gene *AMY1* is transcribed and translated. The diagram shows the relative positions of the three introns and four exons in an unknown molecule called Molecule Z which leads to the production of amylase.

'Molecule Z'

A cell initiates the process of producing Molecule Z by having RNA polymerase attach to the promoter region of the *AMY1* gene.

a What type of molecule is Molecule Z? (1 MARK)

b After Molecule Z undergoes post-transcriptional modifications, it is transported to the ribosomes for translation. Describe the steps that occur to produce amylase from Molecule Z. (3 MARKS)

c Explain how Molecule Z could give rise to proteins with different functions in different cells of the body. (1 MARK)

Adapted from VCAA 2017 Section B Q1c

Key science skills and ethical understanding

Question 17 (5 MARKS)

Many living cells produce hydrogen peroxide as a by-product of metabolic reactions. However, hydrogen peroxide is poisonous for these cells and is immediately decomposed into water and oxygen by an enzyme called catalase.

The reaction is represented by the following equation:

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

a What is the product(s) in this chemical reaction? (1 MARK)

Adapted from VCAA 2007 Exam 1 Section B Q3

b Explain the biological significance of the 3D shapes of catalase and hydrogen peroxide. (2 MARKS)

c In an experiment, three flasks were set up each containing 10 mL of hydrogen peroxide. In Flask 1, 0.5 mL of catalase solution was added, in Flask 2, no enzyme solution was added, and in Flask 3, 0.5 mL of maltase solution was added. The results of oxygen build up in the three flasks over time are displayed in the table.

	Oxygen volume (mL)		
Time (minutes)	**Flask 1**	**Flask 2**	**Flask 3**
1	0.8	0.0	0.1
2	1.4	0.1	0.1
3	1.8	0.1	0.2
4	2.4	0.2	0.2
5	3.0	0.3	0.2

Explain the role of Flasks 2 and 3 in the experiment. (1 MARK)

d Two Year 12 students completed this experiment in class and obtained very different results to the table shown. They knew oxygen volume should increase substantially in Flask 1 and very little in Flasks 2 and 3 from their knowledge of the theory, so they decided to forge their results in case they got marked down. Identify which bioethical concept the students are ignoring. (1 MARK)

3B FACTORS THAT AFFECT ENZYMES

How to fry the perfect egg:

1. *Gather your ingredients*
2. *First, gently crack the egg into the preheated pan...*
3. *Oh no! It's puffing up like crazy!*
4. *The egg is far too solid and chewy*

What went so wrong with the egg?

Image: LightField Studios/Shutterstock.com

Lesson 3B

In this lesson you will learn about the different factors that influence enzymatic activity.

◀ *Prerequisite knowledge* — *Future applications* ▶

Years 7–10
Reaction rates are often dependent on the enzymes that catalyse biochemical pathways and the factors that influence them.

Year 11
Enzymes in your digestive system are influenced by changes in temperature or pH.

Lesson 5C
Factors that affect enzyme activity are pivotal in influencing the rate of photosynthesis.

Lesson 6C
Cellular respiration relies upon enzymes. Factors that affect these enzymes alter the rate of cellular respiration.

Chapter 7
Pathogenic enzymes can be affected by the internal environment of an organism, reducing the pathogen's ability to cause disease.

Study design dot points

- the general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors
- the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration

Key knowledge units

Temperature	3.2.3.1
pH	3.2.3.2
Concentration	3.2.3.3
Competitive inhibition	3.2.3.4
Non-competitive inhibition	3.2.3.5
Coenzymes	3.2.2.1

Temperature 3.2.3.1

OVERVIEW

The activity of an enzyme is affected by temperature. When it gets too hot, an enzyme can denature and stop functioning.

THEORY DETAILS

Optimal temperatures

Chemical reactions inside our bodies speed up as our internal body temperature increases. This is because when the temperature increases, molecules have greater kinetic energy and collide with one another more frequently. This is also true for enzyme-catalysed reactions – when the temperature increases, enzymes and substrates move faster and collide with each other more frequently, allowing reactions to occur faster.

However, this is only true to a certain point and does not mean that an enzyme's activity will increase indefinitely with temperature. Each enzyme has its own specific **optimal** temperature at which its activity is greatest, meaning that the enzyme and substrate collide and bind most frequently. The enzymes found within the human body have an optimal temperature range of 36–38 °C, meaning they function most effectively at the human body temperature of 37 °C. Enzymes differ greatly, however some enzymes found in bacteria that live in hot springs have optimal temperatures above 70 °C!

optimal the point at which for a given condition (e.g. temperature), the maximum function of an enzyme occurs. Also known as **optimum**

What happens when it's too hot?

As we learned in lesson 3A, enzymes are mostly proteins, and proteins can be **denatured** under certain conditions. If the temperature goes beyond its optimal, an enzyme is at risk of denaturing, where the bonds that create its tertiary and quaternary structures are broken down. Denaturation causes a **conformational change** in the active site of an enzyme, causing the substrate to no longer fit (Figure 1). It's important to note that this change is irreversible, meaning that a denatured enzyme cannot regain its function even if the temperature decreases.

denature the disruption of a molecule's structure by an external factor such as heat

conformational change a change in the three-dimensional shape of macromolecules such as proteins

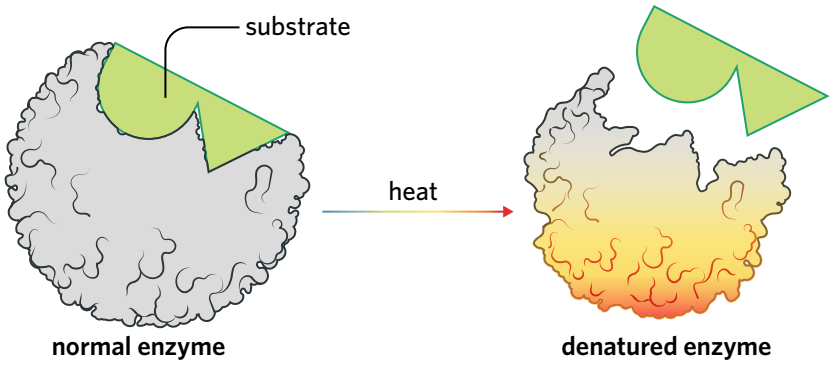


Figure 1 Higher temperatures can denature enzymes and irreversibly damage their active site.

What happens when it's too cold?

Conversely, what happens if the temperature decreases below the optimal temperature of the enzyme? An enzyme's activity decreases below the optimal temperature, as the enzyme and substrate molecules move slower and collide less frequently. When it becomes too cold, enzymes experience little to no activity and can freeze. However, unlike with denaturation at high temperatures, enzymes can regain functionality when reheated as significant denaturation does not occur at low temperatures.

Tolerance ranges

Beyond the optimal temperature, the narrow range at which enzyme function is best, we can also use the term tolerance range to describe the wider range of a given condition (like temperature) that an enzyme can function under. Outside of its tolerance range, an enzyme is inactive. For example, an enzyme might have an optimal temperature of 58–60 °C but could have a tolerance range of 30–70 °C. In this case, the enzyme will function variably within this range but might freeze if the temperature drops below 30 °C, or denature if the temperature goes above 70 °C.

Memory device

The effect of temperature on enzymes can be illustrated by raw meat. Raw meat can be frozen, and then thawed to return back to raw meat. However, you can't reverse the process of cooking meat, just like you can't reverse denaturation.

The relationship between enzymes and temperature can be displayed graphically (Figure 2). In summary:

- enzymes have a tolerance range and will catalyse reactions differently depending on what temperature they are exposed to. Inside this tolerance range, we know that:
  - the enzyme works best at its optimal temperature (point X).
  - as temperature increases towards the optimal, the kinetic energy increases and more enzyme-substrate complexes form.
  - as temperature decreases from the optimal, the kinetic energy decreases and fewer enzyme-substrate complexes form.
- as the temperature decreases towards the lower limit of the tolerance range, enzyme activity slows until freezing occurs, causing loss of function. This freezing is reversible as it does not cause an irreversible conformational change (point Y).
- as the temperature increases towards the upper limit of the tolerance range, enzyme activity drops steeply until denaturation causes a complete loss of function. This denaturation is irreversible as it causes an irreversible conformational change (point Z).

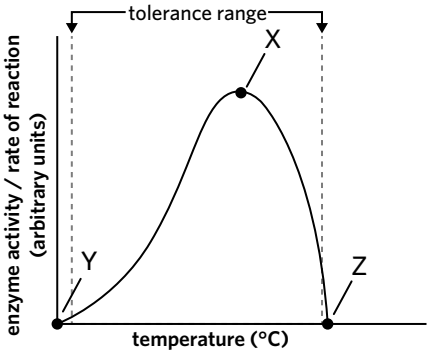
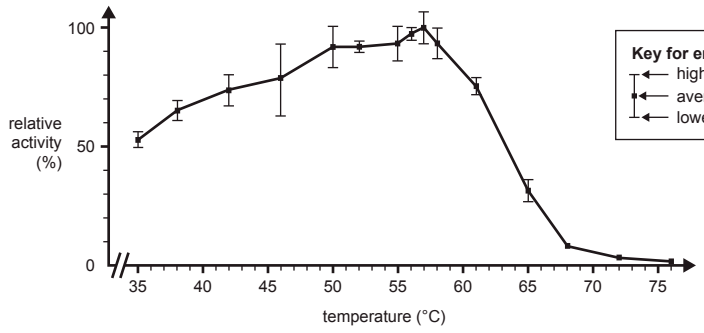


Figure 2 The general relationship between enzyme activity and temperature

Examiners' tip

The VCAA frequently tests students' understanding of enzyme function using graph-based questions like that shown in Figure 2. For instance, let's look at Question 13 in Section A of the 2020 VCE Biology Exam.

Relative activity of Celluclast ® at a range of temperatures



Source: J Herlet et al , 'A new method to evaluate temperature vs pH activity profiles for biotechnological relevant enzymes', *Biotechnology for Biofuels*, 10, 234 (2017), <https://doi org/10 1186/s13068-017-0923-9>

Question 13

It is reasonable to conclude that

A. Celluclast® is inactive at 61 C.

B. Celluclast® is denatured at 35 C.

C. the optimum pH for Celluclast® is pH 5.

D. the optimum temperature for Celluclast® is around 57 C.

The answer is D, as we can see that the greatest enzyme activity is around 57 °C. Although the graph's overall shape is slightly different to that of Figure 2, the situation remains the same. You need to be able to (1) understand the terms used in this section and (2) interpret the general shape of these graphs and make inferences about temperature ranges and the optimal temperature.

Lesson link

The VCAA often tests students' understanding of enzyme function and temperature/pH in relation to photosynthesis and cellular respiration. Both of these are covered in ***lesson 5C*** and ***lesson 6C*** respectively.

pH 3.2.3.2

OVERVIEW

The activity of an enzyme is affected by pH. When the pH becomes too acidic or basic for an enzyme, it can denature.

THEORY DETAILS

The pH scale is used to measure the acidity or alkalinity of a solution (Figure 4). Acids have low pH values (<7) and alkaline (basic) solutions have high pH values (>7). Just like with temperature, enzymes have their optimal pHs at which they function best. Unlike temperature, however, the denaturation of an enzyme occurs if it is exposed to an environment that is either above or below the optimal pH. That is, both overly acidic or basic environments can denature a given enzyme.

The pH ranges of different enzymes vary greatly depending on where the enzymes are located (e.g. pepsin is the main digestive enzyme in the human stomach and has an optimal pH around 1.5–2, whilst pancreatic lipase has an optimal pH of 8). Because denaturation occurs at both extremes, plotting enzyme activity (or reaction rate) against pH results in a symmetrical, bell-shaped curve (Figure 3).

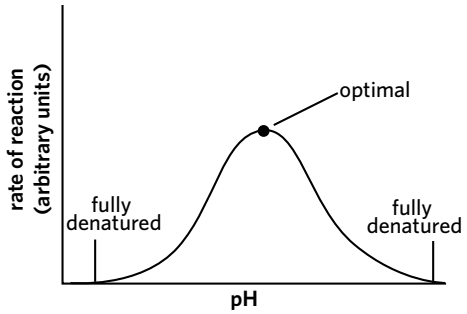


Figure 3 The relationship between enzyme activity and pH

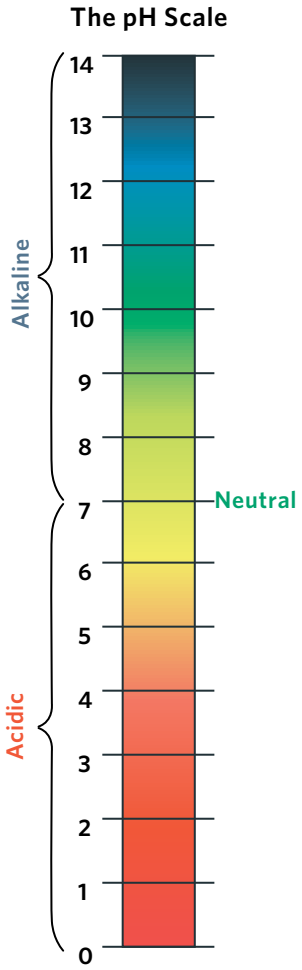


Figure 4 The pH scale

Concentration 3.2.3.3

OVERVIEW

The concentrations of both substrate and enzyme molecules influence the overall reaction rate.

THEORY DETAILS

Substrate concentration

If the enzyme concentration remains constant while the substrate concentration increases, then the reaction rate will increase. This is because there are more reactants available to undergo the reaction (Figure 5). Think of it like a dinner party – if you only put a small handful of chips out in a single bowl, the eating rate will be very slow, but if you have heaps and heaps of chippies in different bowls then your guests will gobble them down at a much faster rate.

Theory in action

Check out scientific investigation 3.2 to put this into action!

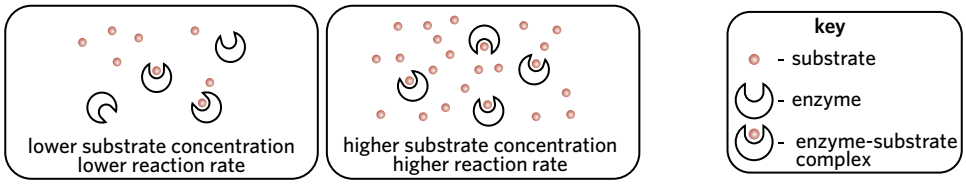
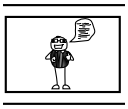


Figure 5 Increasing substrate concentration increases the reaction rate due to more frequent collisions with enzymes.

However, a point will be reached where there are so many substrate molecules that continuously occupy all active sites, meaning that the enzymes are saturated with substrate. This is called the **saturation point**. At this point, even if the amount of substrate increases, the reaction rate can no longer increase. Back to our dinner party analogy, this is like saying 'well we only have a certain amount of guests, each with only one mouth. At some point, there is such a thing as too many chips, and even if you brought out another packet we wouldn't be able to eat what's here any faster.'

saturation point the point at which a substance (e.g. an enzyme) cannot receive more of another substance (e.g. a substrate)



Consider the graph shown in Figure 6 displaying the relationship between the substrate concentration and the rate of reaction. After the saturation point is reached, the rate of reaction remains constant, resulting in a plateau. It is important to note, however, that at the saturation point and beyond, the reaction is still occurring very quickly – the rate of reaction, however, is no longer increasing.

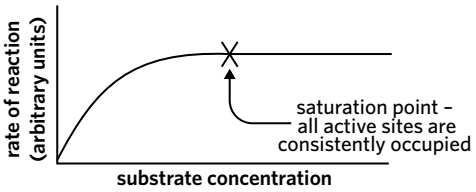


Figure 6 The rate of reaction increases as substrate concentration increases until saturation occurs.

Before the graph plateaus, we can say that the substrate concentration is a **limiting factor** in the reaction. A limiting factor is a factor that is preventing an increase in reaction rate. To put it another way, were we to have more of the limiting factor (i.e. substrate), then our reaction rate would increase. Thinking of our dinner party analogy – if we have more chips then our guests will eat more. When the graph's plateau starts, however, we can say that the substrate concentration is no longer the limiting factor in the reaction. This means that another factor such as temperature, pH, or enzyme concentration is the limiting factor preventing the reaction rate from increasing.

limiting factor a factor that prevents the rate of reaction from increasing

Enzyme concentration

Enzyme concentration can also influence the reaction rate. If the enzyme concentration is high, then the reaction rate will be high (Figure 7). This is due to the large number of active sites available for the substrate to bind to. Think of this like the inverse of our dinner party example from earlier – in this case it is like saying, 'we have so many chippies (substrate), if only we had more guests (enzymes) to help us eat them all'.

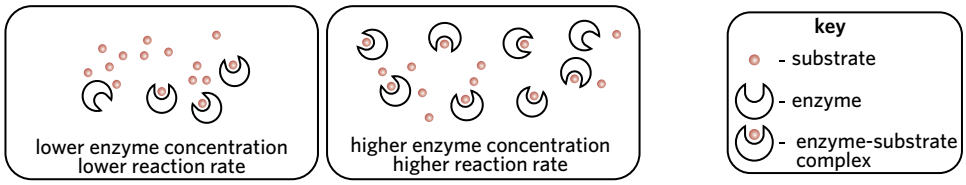


Figure 7 Increasing enzyme concentration increases the reaction rate due to more frequent collisions with substrates.

The relationship between enzyme concentration and reaction rate can be displayed graphically (Figure 8). In summary:

- If the enzyme concentration rises (while the substrate concentration is kept constant), then the reaction rate will increase. Again, there are more mouths to eat the chippies.
- This is true until enzymes are in excess, at which point the reaction rate will plateau regardless of any continued increase in enzyme concentration. Adding more mouths to the party won't help if there aren't more chippies to eat.

It is theoretically possible that with an ever-increasing enzyme concentration, a point will be reached where all the substrates are used up and the rate of reaction decreases rather than continuing to plateau – all the chippies will be gone. This is more theoretical than practical, however, as in biological systems, there is typically far more substrate than enzyme, so an increase in the enzyme concentration usually always increases the reaction rate. When representing the relationship between enzyme concentration and reaction rate, the VCAA uses the graph shape represented in Figure 8.

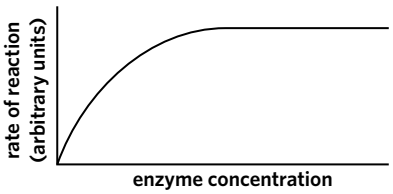
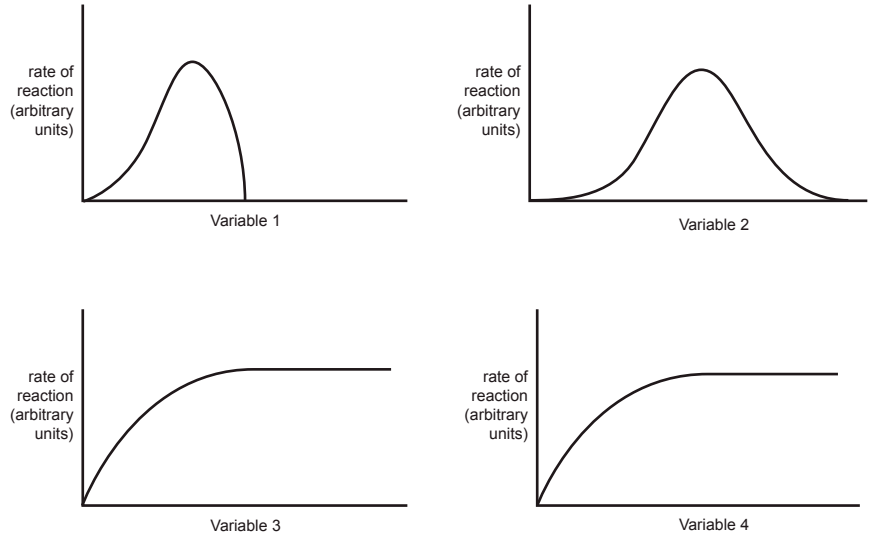


Figure 8 The relationship between enzyme activity and enzyme concentration

Examiners' tip

Let's take a look at another graph-based enzyme question from a previous exam. This time, consider Question 7 in Section A of the 2018 VCE Biology Exam:

Four students performed a series of experiments to investigate the effect of four different variables on the rate of an enzyme-catalysed reaction In each experiment the students changed one of the following variables: substrate concentration, pH, temperature and enzyme concentration After recording their data, the students displayed their results in a series of graphs, as shown below Each graph is a line of best fit for their data



The students did not label the horizontal axis on any of their graphs The next day, the students could not agree on which variable should be labelled on the horizontal axis of each graph The students made the following suggestions as to what each variable could be

Student	Variable 1	Variable 2	Variable 3	Variable 4
Marcus	substrate concentration	temperature	pH	enzyme concentration
Billy	temperature	substrate concentration	enzyme concentration	pH
Voula	enzyme concentration	temperature	substrate concentration	pH
Sheena	temperature	pH	enzyme concentration	substrate concentration

Out of these four students, Sheena has correctly identified all four variables. This is a stellar summary of the knowledge you should take from this lesson, as the VCAA requires you to be able to instantly recognise and differentiate the factors that affect enzyme functioning when represented graphically. To test yourself, ask whether you can (1) identify the factor in question from the shape of its graph, and (2) explain the trend of the graph in relation to reaction rate.

Competitive inhibition 3.2.3.4

OVERVIEW

Enzymes can be hindered by molecules known as competitive inhibitors that impede enzymes by blocking their active site.

THEORY DETAILS

Enzyme inhibitors are molecules that bind to an enzyme and prevent it from performing its function. When an inhibitor is bound to an enzyme, the enzyme can either no longer catalyse its specific reaction, or its functioning is greatly reduced.

enzyme inhibitor a molecule that binds to and prevents an enzyme from functioning

Competitive inhibition

Competitive inhibition occurs when an inhibitor molecule binds to an enzyme's active site. This binding directly occupies and blocks the active site, meaning the substrate is now unable to bind with the enzyme and no reaction will occur (Figure 9b). To block an active site, a competitive inhibitor has to have a shape that is complementary to the active site in some way, and therefore must share similarities in shape to the substrate. Unlike the substrate, however, when an inhibitor binds to an active site, it does not trigger a reaction. This form of inhibition is said to be 'competitive' because both the substrate and inhibitor are attempting to bind to the active site – they are competing for the active site.

competitive inhibition the hindrance of an enzyme by blocking the active site and preventing the substrate from binding

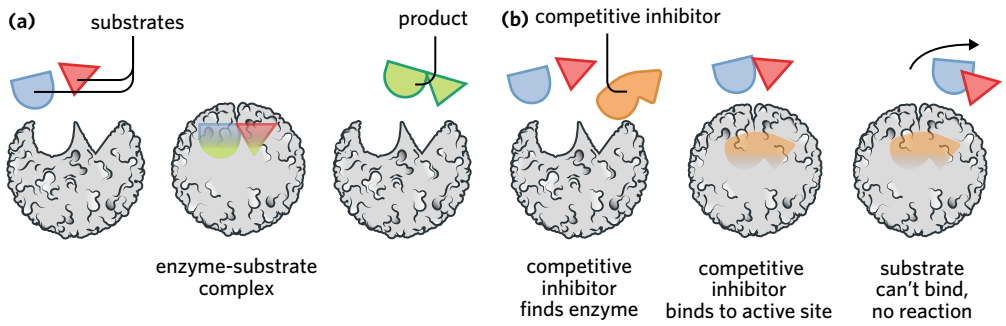


Figure 9 **(a)** Normal enzyme functioning vs **(b)** a competitively inhibited enzyme that can no longer catalyse substrate as its active site is blocked.

Non-competitive inhibition 3.2.3.5

OVERVIEW

Non-competitive inhibitors interfere with enzymes by binding to a site other than the active site and inducing a conformational change.

THEORY DETAILS

Non-competitive inhibition (also known as allosteric inhibition) occurs when an inhibitor binds to an enzyme at a site other than the active site (an **allosteric site**). This binding causes a conformational change in the active site of the enzyme. The conformational change in the active site's structure prevents the substrate from binding to it, preventing the reaction from occurring (Figure 10).

non-competitive inhibition the hindrance of an enzyme by binding to an allosteric site and changing the shape of the active site to prevent the substrate from binding

allosteric site a region on an enzyme that is not the active site

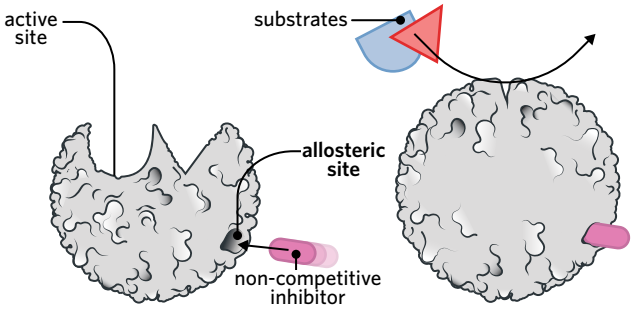


Figure 10 Non-competitive inhibition of an enzyme. The inhibitor binds to a site that is not the active site, causing a change in the active site's structure which halts enzyme functionality.

Inhibition of biochemical pathways

Enzyme inhibitors play an important role in regulating a range of **biochemical pathways** in the body. One way this is done is by regulating how much of a certain product is created, depending on the body's needs. Take Figure 11 for example, which shows a biochemical pathway where the product of one enzyme-catalysed reaction becomes the substrate of the next reaction in a series of connected reactions. In other words, the product of the reaction catalysed by Enzyme 1 is the substrate needed for the reaction catalysed by Enzyme 2. However, in this scenario, the product of Enzyme 3 in the pathway non-competitively inhibits Enzyme 1, altering its active site and halting its function. When Enzyme 1 is inhibited, all subsequent reactions are also affected, as less of Substrate 2 is available for the second reaction in our pathway.

biochemical pathway a series of enzyme-catalysed biochemical reactions in which the product of one reaction becomes the substrate of the next reaction. Also known as a **metabolic pathway**

Why is this important? Well, thanks to this, the products of this pathway will not be overproduced. In Figure 11, for example, the cell will need a specific amount of Substrate 3 to perform whatever function that molecule is responsible for. Sometimes though, the cell has enough Substrate 3, meaning that production needs to slow down. That's where inhibition comes in. The product of Enzyme 3 inhibits the function of Enzyme 1, leading to a reduction in Substrate 2 and a lower reaction rate for Enzyme 2. We call this pathway a self-regulating pathway, as the amount of product is regulated by one enzyme in the pathway (in this case, Enzyme 3). Of course, in this scenario, inhibition of Enzyme 1 could also lead to a buildup of Substrate 1, meaning that the pathway will need to recommence to convert it to the product.

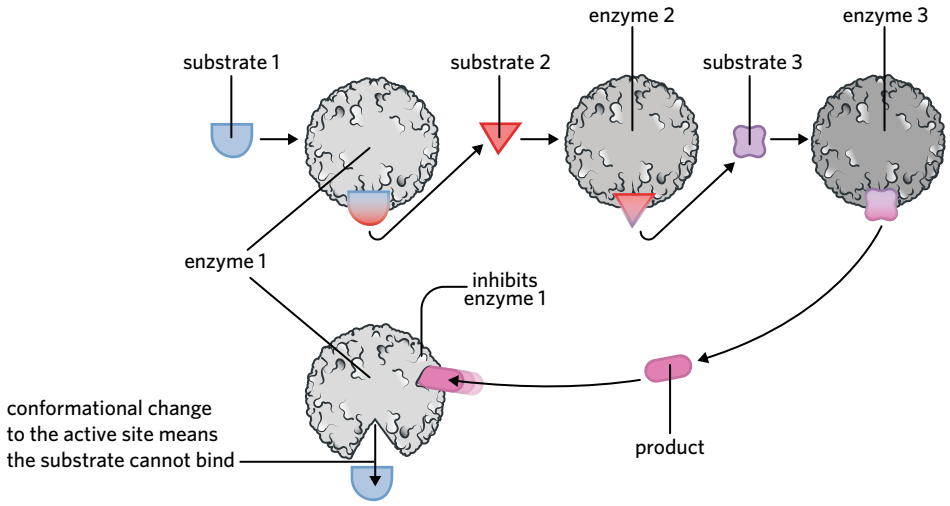


Figure 11 Enzyme inhibitors can disrupt entire biochemical pathways. In this pathway, the product of Enzyme 3 non-competitively inhibits Enzyme 1, effectively regulating the pathway.

Coenzymes 3.2.2.1

OVERVIEW

Coenzymes assist enzymes in catalysing reactions. The cycling of coenzymes is integral to many biochemical processes.

THEORY DETAILS

Some enzymes require assistance from a **cofactor** to catalyse their reactions. A cofactor is a molecule that assists enzyme functioning. **Coenzymes** are a subset of cofactors that are organic, non-protein molecules, with their role being to assist enzymes in catalysing reactions.

cofactor any organic or inorganic molecule, such as a coenzyme or metal ion, that assists enzyme function

coenzyme a non-protein organic cofactor that assists enzyme function. They release energy and are recycled during a reaction

In coenzyme-assisted enzymatic reactions, the enzyme remains unchanged as always. The structure of the coenzyme, however, is changed (Figure 12). During the reaction, the coenzyme binds to the active site, donates energy or molecules, and then cannot be immediately reused. After the reaction, the coenzyme leaves the enzyme and is recycled by accepting more energy, so it can then go on to assist in more reactions. This is referred to as the cycling of coenzymes and is integral to certain biochemical processes.

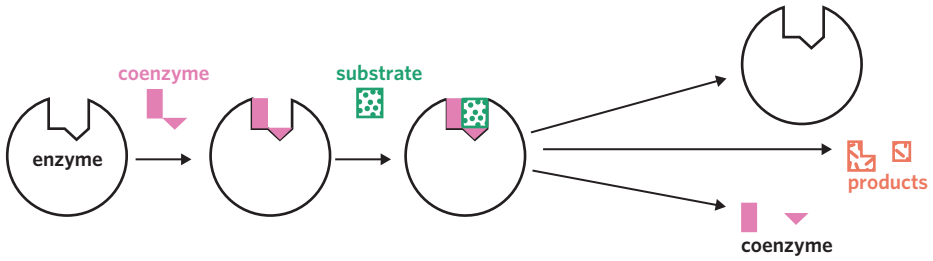


Figure 12 Coenzymes assist enzymes. After a coenzyme binds, the substrate can bind to the active site. Following the reaction the coenzyme is unloaded and must be recycled.

Lesson link

The coenzymes ATP and NADPH are critical for photosynthesis (***lesson 5A***), and the coenzymes ATP, NADH, $FADH_2$ and acetyl-CoA are integral to cellular respiration (***lesson 6A***).

ATP and ADP

The rockstar of all coenzymes is adenosine triphosphate (**ATP**) and its partner molecule adenosine diphosphate (**ADP**) (Figure 13). ATP is the main energy transfer unit of the cell, as cells frequently rely on this coenzyme to donate energy to catalyse reactions (Figure 14). Upon releasing energy for a reaction, ATP loses a phosphate group and becomes ADP (triphosphate = three phosphates, diphosphate = two phosphates). The ADP molecule then goes on to have a phosphate group re-added, so it can become ATP and go on to catalyse more ATP-assisted reactions. The conversion of ADP to ATP is a phosphorylation (adding phosphate) reaction, whilst ATP turns into ADP via a dephosphorylation (removing phosphate) reaction. This back and forth process is coenzyme cycling, and we can refer to ATP as loaded and ADP as unloaded. Coenzymes are used so frequently that the same ATP molecule can be cycled to ADP and back over 1 000 times every day.

ATP adenosine triphosphate, a high energy molecule that, when broken down, provides energy for cellular processes

ADP adenosine diphosphate, the unloaded form of ATP

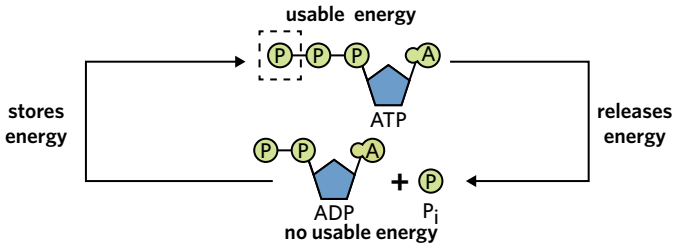


Figure 13 The cycling of ATP loaded with energy and ADP unloaded with energy

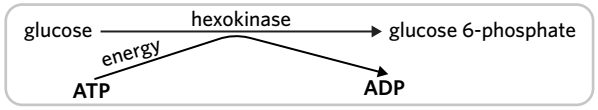


Figure 14 The coenzyme ATP assists the enzyme hexokinase in catalysing glucose into glucose 6-phosphate. Note that the function of a coenzyme can be represented using a curved arrow as shown.

Theory summary

There are many factors that affect enzyme functioning:

1 Enzymes have optimal temperatures and pHs at which they function best. However, enzymes can be denatured in extreme conditions.

2 Keeping everything else constant, an increase in substrate or enzyme concentration will increase reaction rate up to a certain point.

3 Competitive inhibitors block an enzyme's active site, whereas non-competitive inhibitors bind elsewhere and alter the structure of the active site.

4 Unlike inhibitors, coenzymes assist enzyme functioning and must be recycled after undergoing a reaction.

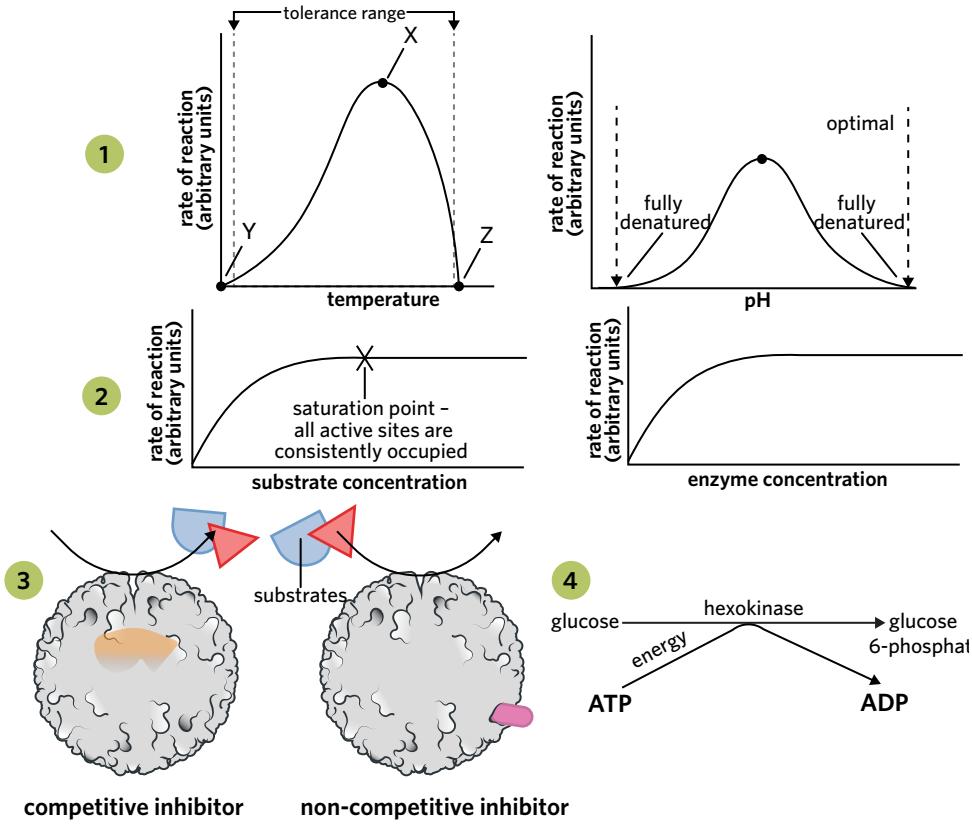


Figure 15 Summary of the factors affecting enzyme function

You had the pan way too hot! The extreme heat caused rapid denaturation of the enzymes and other proteins within the egg, leading to quick coagulation and a chewy, rubbery, failure of an egg. Cooking the egg at a slow rate under a lower temperature is best, as it allows for more gradual denaturation and coagulation which can give you the soft and tender egg you so desire.

3B QUESTIONS

Theory review questions

Question 1

Enzymes can be denatured by

A high and low temperatures and pH values.

B high and low pH values but only high temperatures.

Question 2

Which of the following are true of non-competitive inhibition? (*Select all that apply*)

I A reaction is triggered by the inhibitor.

II The active site of an enzyme is blocked.

III The enzyme's function is reduced or eliminated.

IV The active site undergoes a change in structure.

V The substrate can no longer bind to the enzyme.

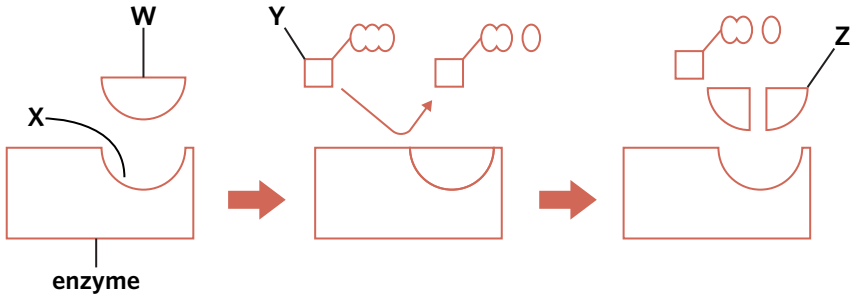
VI Binding occurs at a site other than the active site.

VII High levels of non-competitive inhibition can cause denaturation.

Question 3

Label the parts of the enzymatic reaction from the list of terms.

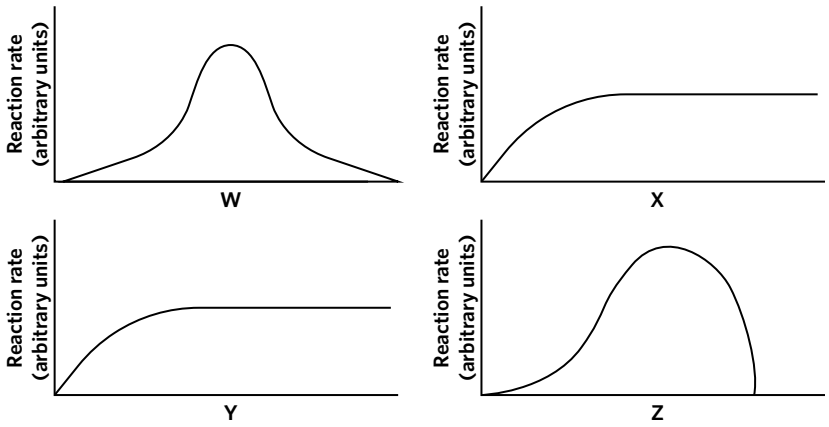
- coenzyme
- active site
- substrate
- product



Question 4

Label the parts of the graph axes from the list of terms.

- pH
- temperature
- enzyme concentration
- substrate concentration



Question 5

Order the steps to correctly describe a coenzyme-assisted enzymatic reaction.

I The reaction occurs.

II An enzyme is present.

III The substrate can now bind to the active site.

IV The enzyme is now free to catalyse further reactions.

V Substrate arrives at the enzyme but cannot be catalysed yet.

VI A loaded coenzyme arrives at the enzyme to bind and donate energy.

VII The products are released from the active site and the unloaded coenzyme leaves to be recycled.

SAC skills questions

Case study analysis

Use the following information to answer Questions 6–9.

Cytochromes P450 (CYPs) are a large group of enzymes found in all kingdoms of life. CYPs are characterised by the presence of an attached haem group that is a cofactor responsible for assisting enzymatic activity. Haem is a substance that helps bind oxygen within the bloodstream. In humans, CYPs are found in most tissues in the body and play important roles in hormone synthesis, cholesterol synthesis, vitamin metabolism, and in metabolising potentially toxic compounds such as drugs.

In fact, CYPs are the major enzymes responsible for drug metabolism in the body, accounting for approximately 75% of total drug metabolism. Certain medications and naturally occurring compounds, however, can function as inhibitors to CYP enzymes. In the presence of such inhibiting compounds, individuals can be susceptible to harmful side-effects of any drugs or medications taken.

Certain compounds known to inhibit CYPs are found within grapefruit juice. When ingested, the inhibition of CYPs reduces the potential to metabolise certain medications. If these specific medications are administered with grapefruit juice in the body, it can cause dangerous buildups of the drug, which can lead to drug overdoses and even death. When prescribed with medication, it is critical that you read all labels and listen to professional advice to know if you should avoid certain foods such as grapefruit and grapefruit juice. Because of the risk, avoiding grapefruit entirely while on any prescription medication is usually advised.

Question 6

The function of CYPs in the body is to

A inhibit potentially dangerous drug compounds by blocking their active site.

B lower the activation energy of a variety of reactions responsible for outcomes such as cholesterol synthesis.

Question 7

Compounds within grapefruit juice can be dangerous as they inhibit

A enzymes responsible for processing certain medications in the body.

B approximately 75% of enzymes in the body.

Question 8

If a given compound in grapefruit juice competitively inhibited one CYP, then it would have to be

A of similar structure to the type of drug that the CYP catalyses.

B observed occupying many different locations on the CYP molecule.

Question 9

Some health professionals may advise for the total avoidance of grapefruit when taking prescription medication even when the likelihood of negative effects of the specific medication is extremely low to minimal. What bioethical principle is guiding this decision?

A Beneficence – as benefits are maximised by deepening people's knowledge of CYPs.

B Non-maleficence – as potential harm is minimised as much as possible even if the likelihood of harm is extremely small.

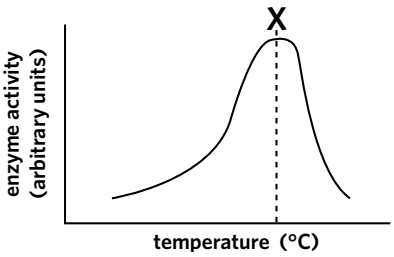
C Integrity – as the advice to not ingest grapefruit will continue to expand the understanding of how the grapefruit compounds and CYPs react in the body.

Exam-style questions

Within lesson

Question 10 (1 MARK)

Scientists have found microorganisms living in hot springs of boiling water. The enzyme activity of these microorganisms was investigated over a range of temperatures. The results obtained were plotted and are shown in the following graph.



Which of the following is correct?

A Point X is the denaturation point.

B The temperature at point X is likely to be 37 °C.

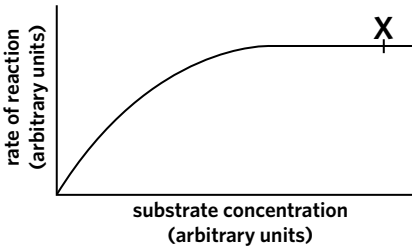
C Below point X the enzyme would likely denature.

D Point X is the optimal temperature of the enzyme.

Adapted from VCAA 2004 Exam 1 Section A Q7

Question 11 (1 MARK)

The following graph illustrates the effect of different substrate concentrations on the reaction rate.



In this series of experiments, the amount of enzyme, the pH, and the temperature remain constant.

At point X

A all active sites are consistently occupied.

B the substrate is the limiting reactant.

C the rate of reaction is decreasing.

D no reactions are occurring.

Adapted from VCAA 2003 Exam 1 Section A Q17

Question 12 (1 MARK)

Bacteria such as *Thermus aquaticus* live in hot springs where temperatures are around 90 °C. What can be said about the temperature tolerance range of enzymes found in *T. aquaticus*?

A The enzymes' tolerance range likely centres around 90 °C.

B The enzymes' tolerance range is limited to a narrow range.

C 90 °C is outside the enzymes' tolerance range, however, they can still operate.

D The enzymes must be capable of operating over a wide range of temperatures.

Adapted from VCAA 2006 Exam 1 Section A Q19

Question 13 (1 MARK)

Which of the following is false when considering this reaction?

$$ADP + P_i \longrightarrow ATP$$

A This reaction is reversible.

B The product of this reaction stores energy.

C The product of this reaction contains three phosphate subunits.

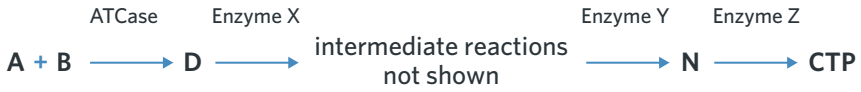
D This reaction is catalysed by a coenzyme to produce adenosine triphosphate.

Adapted from VCAA 2011 Exam 1 Section A Q20

Use the following information to answer Questions 14 and 15.

CTP is a substance used by cells to make RNA. The cell initially synthesises CTP using a metabolic pathway starting with the amino acid aspartate (A) and another complex molecule (B).

The pathway for making CTP is represented. The enzyme involved in the first step of the pathway is called ATCase.



Question 14 (1 MARK)

Inhibiting the action of Enzyme X would

A result in a buildup of molecule D.

B cause Enzyme Z to function faster.

C decrease the concentration of ATCase.

D increase the concentration of CTP produced.

Adapted from VCAA 2014 Section B Q1

Question 15 (1 MARK)

An inhibitor that competitively inhibits Enzyme X would be

A complementary to ATCase.

B similar in structure to Enzyme X.

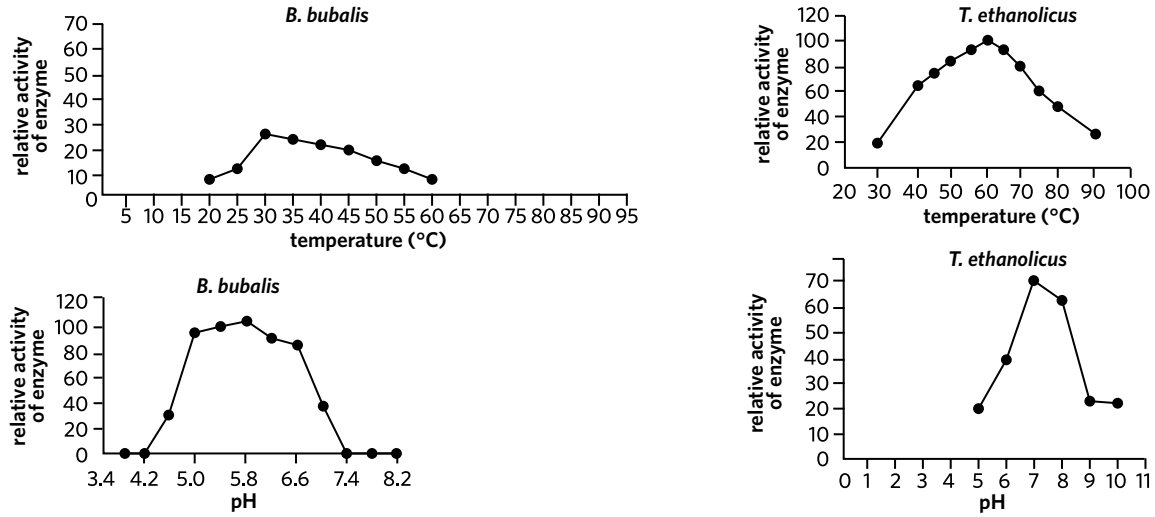
C similar in structure to molecule D.

D complementary to the substrate of Enzyme Z.

Adapted from VCAA 2014 Section B Q1

Question 16 (1 MARK)

The enzyme lactate dehydrogenase is found in a wide variety of organisms. It catalyses the conversion of both pyruvate to lactate, and lactate to pyruvate. The bacterium *Thermoanaerobacter ethanolicus* lives in geothermal (hot) springs. The river buffalo (*Bubalus bubalis*) is a domestic animal common in Pakistan. Scientists studying the enzyme lactate dehydrogenase from these two organisms produced the following graphs (adapted from Nadeem et al. (2011) (left) and Zhou and Shao (2010) (right)).



From the graphs, which of the following conclusions is false?

A The optimal pH of the bacterial lactate dehydrogenase is 7.0.

B Above 60 °C the buffalo form of the enzyme would likely denature.

C Below 40 °C the bacterial form of the enzyme would likely denature.

D The form of enzyme found in the buffalo operates over a narrower pH range than the bacterial form.

Adapted from VCAA 2017 Sample Exam Section A Q9

Question 17 (1 MARK)

Laundry powder is sometimes advertised as containing powerful enzymes that break down dirt. These enzymes are called extremozymes. They come from some species of bacteria and archaea. The following table gives the optimal functioning of enzymes from some of these species.

Species	Enzyme	Optimal temperature (°C)	Optimal pH
Psychrobacter sp.	J	10–30	7.0–9.0
Pseudomonas sp.	K	40	10.0
Methanococcus sp.	L	120	5.0–8.0
Cystofilobasidium sp.	M	40–42	5.0

Given this information and your knowledge of enzyme function, which of the following conclusions could be made?

A Enzyme K would likely denature in extremely acidic environments.

B Enzyme L has the widest optimal temperature range.

C Enzyme M functions well in a basic environment.

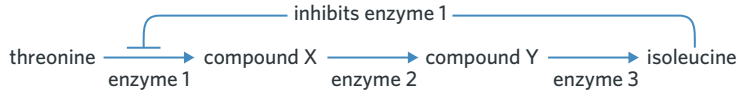
D Enzyme J is likely found in the human body.

Adapted from VCAA 2010 Exam 1 Section A Q23

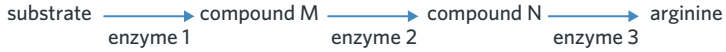
Question 18 (1 MARK)

In the production of isoleucine from threonine in bacteria (Biochemical Pathway 1 [BP 1]), the end product acts as a competitive inhibitor of the first enzyme in the pathway. In the production of arginine (Biochemical Pathway 2 [BP 2]), the end product has no influence on other enzymes in the pathway.

Biochemical Pathway 1 (BP 1)



Biochemical Pathway 2 (BP 2)



It is reasonable to conclude that in

A BP 1, an increase in isoleucine results in an increase in enzyme 2.

B BP 2, an increase in arginine results in an increase in substrate.

C BP 1, isoleucine regulates the production of compound X.

D BP 2, arginine regulates the production of compound M.

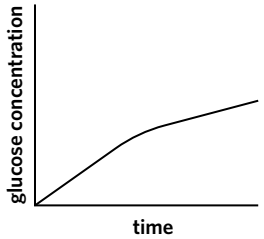
Adapted from VCAA 2006 Exam 1 Section A Q25

Question 19 (1 MARK)

The enzyme maltase catalyses the breakdown of maltose into glucose. Maltase was added to a tube containing a solution of maltose in water and incubated at 37 °C. The amount of glucose produced was monitored over a period of time. Some maltose remained at the end.

The graph showing the change in glucose concentration in the tube is

A



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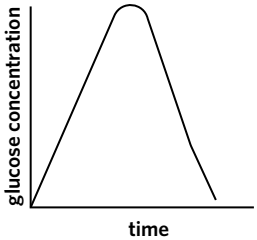

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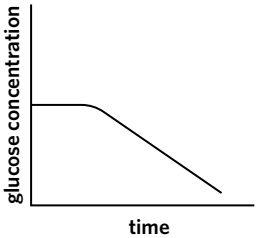

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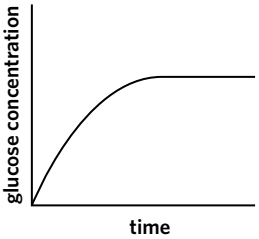


Adapted from VCAA 2008 Exam 1 Section A Q5

Multiple lessons

Question 20 (1 MARK)

Sucrose (cane sugar) is a disaccharide used by plants as a transport molecule. Sucrose is formed in the reaction shown.

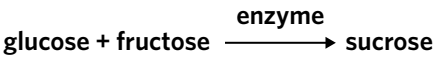
With reference to this process, which of the following statements is false?

A Fructose acts as a coenzyme to produce sucrose from glucose.

B The enzyme lowers the activation energy of the reaction.

C The production of sucrose is an energy-storing reaction.

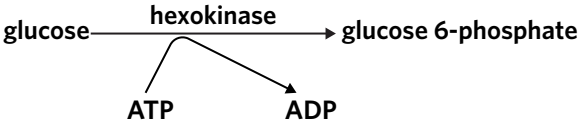
D An enzyme can be used to form sucrose.



Adapted from VCAA 2008 Exam 1 Section A Q18

Question 21 (3 MARKS)

Examine the following reaction.



a Identify the substrate(s) and enzyme in the reaction. (1 MARK)

b How would the structure of hexokinase likely compare to the structure of glucose? (1 MARK)

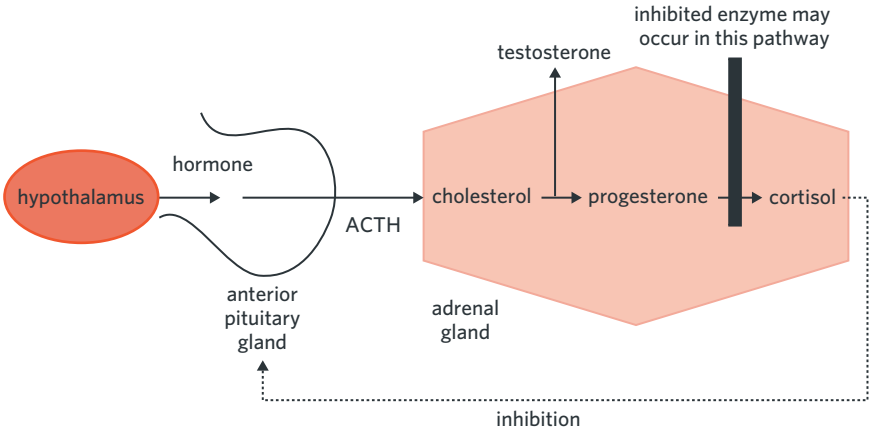
c Briefly describe the role ATP plays in the reaction. (1 MARK)

Key science skills and ethical understanding

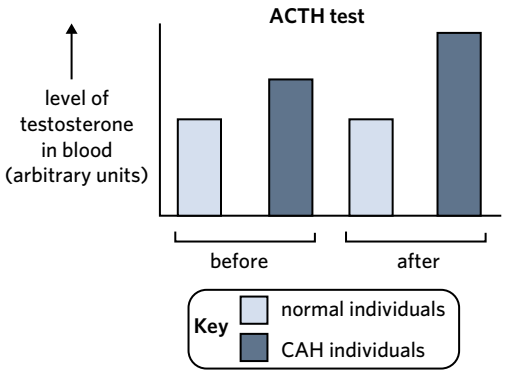
Question 22 (6 MARKS)

Cholesterol can be converted to progesterone and testosterone in a biochemical pathway. Progesterone is then converted to cortisol due to the action of an enzyme known as 21-hydroxylase. If 21-hydroxylase is inhibited, the synthesis of cortisol is reduced and an excess of testosterone is produced. This excess of testosterone results in a disorder called congenital adrenal hyperplasia (CAH).

ACTH is the hormone that stimulates this pathway to begin. In normal individuals, the production of cortisol helps to regulate the ACTH levels via inhibition. To diagnose if an individual has CAH disorder, an ACTH stimulation test is performed. Blood is measured for starting levels of testosterone. ACTH is then injected and another blood sample is taken and analysed after 60 minutes.



The graph shows the changes in testosterone levels in an ACTH stimulation test.



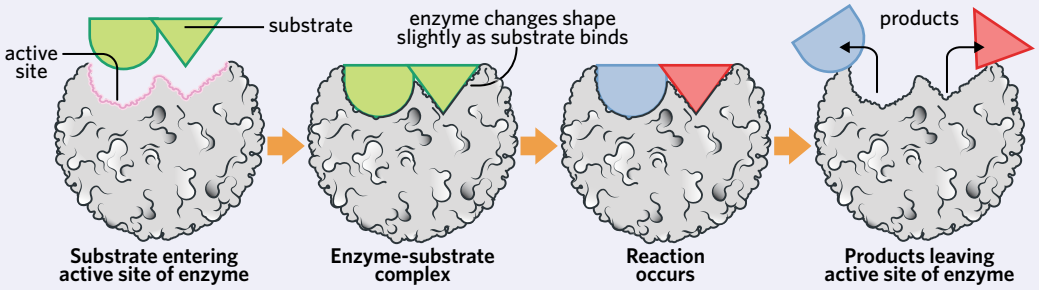
a Describe what is seen in the graph and compare the results of the two groups of individuals. (2 MARKS)

b In relation to the pathway, explain these trends in the graph. (2 MARKS)

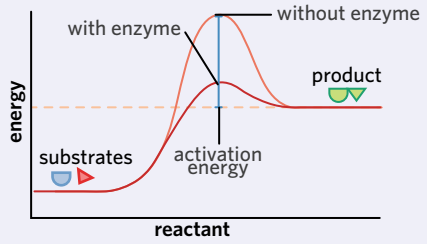
c The inhibitor of 21-hydroxylase shares extreme similarities in structure to the structure of progesterone. Identify the type of enzyme inhibition likely occurring here. Justify your response. (2 MARKS)

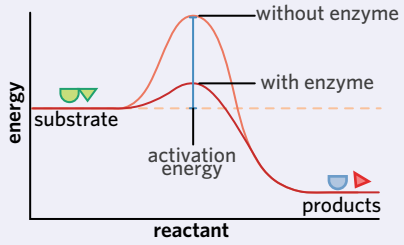
Adapted from VCAA 2010 Exam 1 Section A Q24

CHAPTER 3 SUMMARY



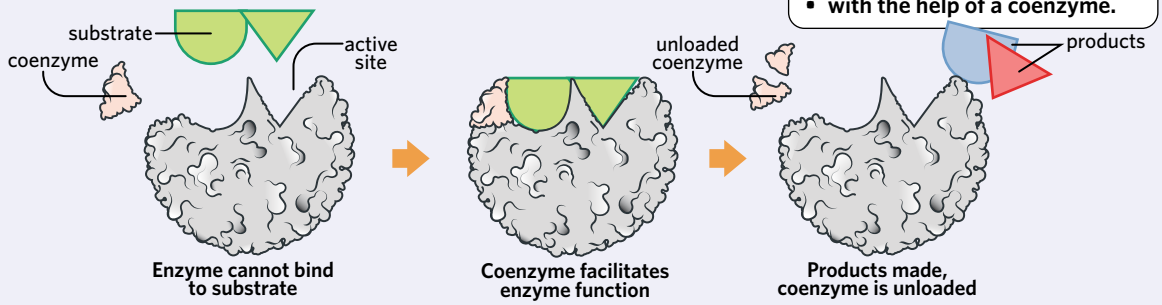
Enzymes catalyse biochemical reactions by binding to a substrate and lowering the activation energy of a reaction. Binding occurs at the enzyme's active site.



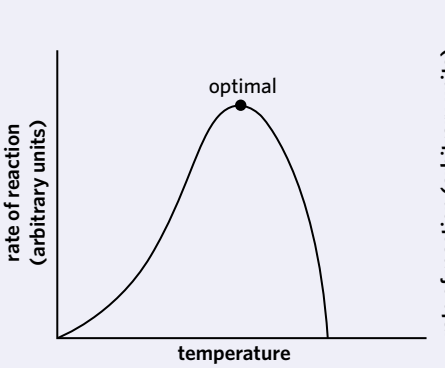


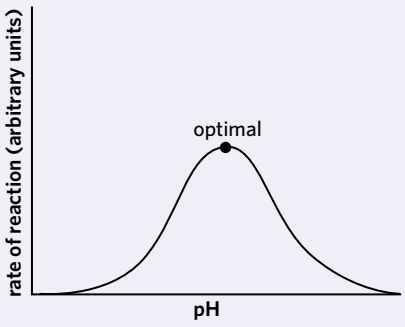
Enzymes are:
- reusable
- specific to their substrate
- mostly proteins.

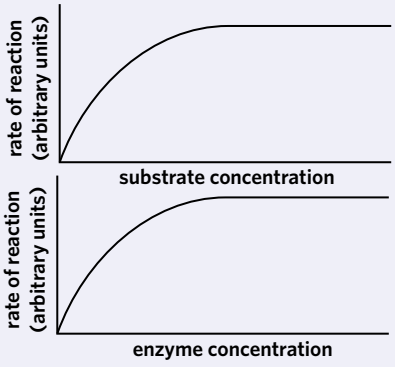
They often function:
- throughout entire biochemical pathways
- in both directions of a reaction
- with the help of a coenzyme.



Some enzymes require a coenzyme to function

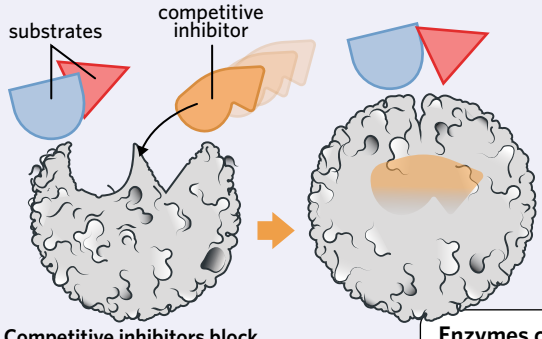


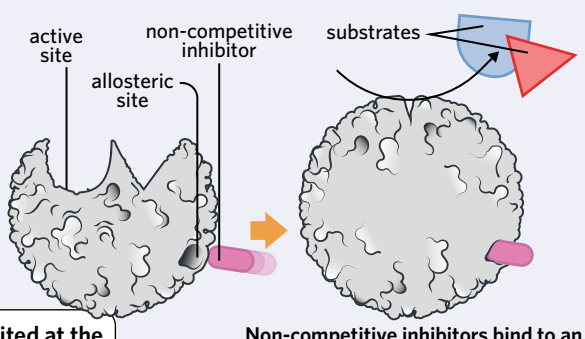




Enzyme activity is the highest at the optimal temperature, but denatures above this. Outside of the optimal pH value, enzymes begin to denature.

Increasing substrate or enzyme concentration increases the rate of reaction, until a maximum is reached.





Enzymes can be inhibited at the active site or an allosteric site.

CHAPTER 3 SAC PRACTICE

SAC skills covered in this section:

✓ Case study analysis ✓ Data analysis ✓ Scientific methodology comparison

ENZYMES IN THE HUMAN BODY (21 MARKS)

A diverse range of enzymes are found within the human body. Each type of enzyme is typically responsible for catalysing a particular reaction. As biochemical reactions require an input of energy to occur, enzymes are crucial for lowering the activation energy of nearly every reaction in the body. A few key activities that enzymes facilitate include digestion, respiration, DNA replication, the breakdown of toxins, immunity, growth, and reproduction. Some common enzymes within the human body are listed in the table.

Enzyme	Substrate	Location	Optimal pH	Optimal temperature (°C)
Catalase	Hydrogen peroxide	Liver	7.0	37
Amylase	Starch	Pancreas	6.7–7.0	32–37
Pepsin	Proteins	Stomach	1.0–2.0	37–42
Lipase	Lipids	Stomach & pancreas	3.0–6.0 & 8.0–9.0	37
Sucrase	Sucrose	Small intestine	6.2	37

1 Briefly describe the importance of enzymes by referring to what would happen in the body without enzymes. (2 MARKS)

2 Differentiate between a reactant and a substrate. (1 MARK)

3 What is meant by the term 'enzyme-substrate complex'? (1 MARK)

4 Two types of lipase are listed in the table gastric lipase (found in the stomach) and pancreatic lipase (pancreas). Which type of lipase is able to function in alkaline (basic) environments? (1 MARK)

5 Suggest what would happen to the functioning of gastric (stomach) pepsin if it were introduced into a neutral pH environment. (1 MARK)

6 Hypothermia is a condition that occurs when the human body temperature drops below 35 °C, with severe and life-threatening impacts arising as body temperature falls below 32 °C. If an individual is suffering from severe hypothermia, would any of these enzymes denature? Why/why not? (2 MARKS)

Aside from pH and temperature, the presence of coenzymes or enzyme inhibitors, as well as substrate and enzyme concentrations, can also influence the rate of enzymatic reactions. Enzyme inhibitors can be classified as either competitive or non-competitive. In the presence of an inhibitor, the functioning of an enzyme is lowered. In this way, inhibitors can control biochemical pathways by regulating enzymatic function. Because of this, pharmaceutical companies and medical professionals frequently look to synthesise drugs that can act as enzyme inhibitors in order to target human enzymes that are reacting too much (e.g. enzymes in certain cancer cells) or enzymes within foreign bodies that are doing harm (e.g. enzymes in bacteria responsible for metabolism).

7 Briefly describe what happens to a coenzyme molecule after it assists an enzymatic reaction. (1 MARK)

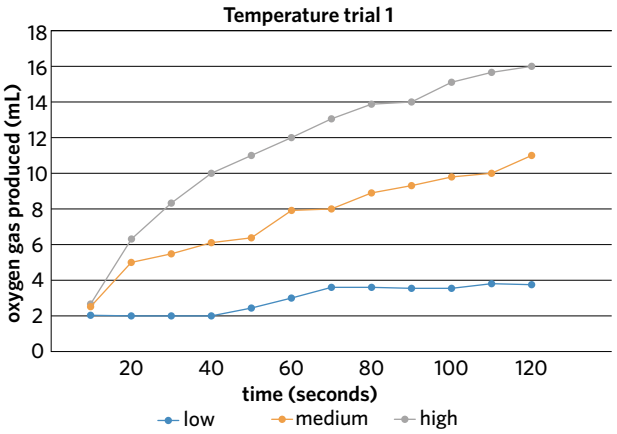
8 State what would happen to a reaction rate if the substrate concentration continually increased whilst the enzyme concentration remained the same. (1 MARK)

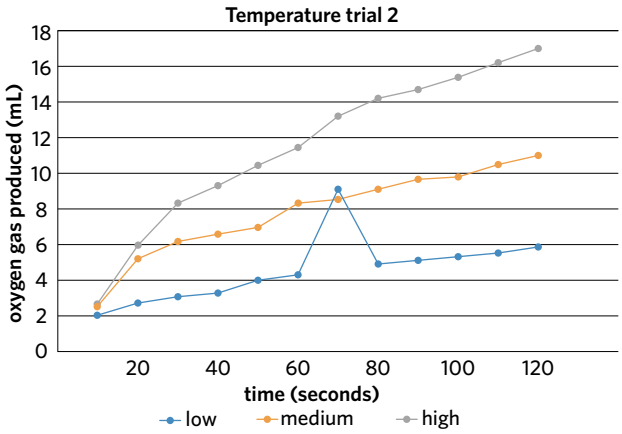
9 A pharmaceutical company wants to synthesise a drug that is a competitive inhibitor of a well-studied enzyme within the human body. For it to be effective, explain how the structure of the drug would relate to the structure of the target enzyme, as well as how it would relate to the structure of the enzyme's substrate. (2 MARKS)

Two students, Bridget and Jarrod, were tasked with designing and conducting their own experiment that investigated the relationship between the activity of a human enzyme, Enzyme Q, and a certain variable. Naturally, they could not agree on one factor so they each ran their own separate experiment. Bridget explored the effects of temperature on Enzyme Q's activity, whereas Jarrod investigated the effects of varying pH values on Enzyme Q's activity. The enzyme is known to break down its substrate into two product molecules – one of which is oxygen. So, by using sealed containers and an oxygen meter, the change of oxygen concentration, and therefore reaction rate, could be measured under varying temperature or pH values. The following is a summary of the methods and results of the two experiments.

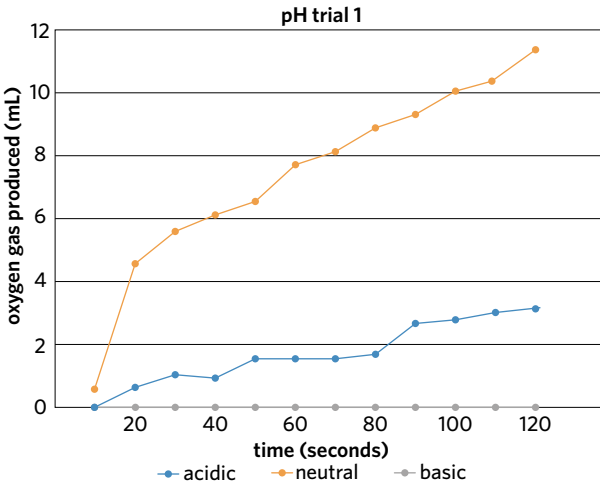
Bridget's method	Jarrod's method
1 Set up a sealed container and oxygen meter within a water bath with a medium (37 °C) temperature and a neutral pH value	1 Set up a sealed container and oxygen meter within a water bath with a medium (37 °C) temperature and a neutral pH value
2 Place 1 mL of Enzyme Q solution and 20 mL of substrate solution and some distilled water into the sealed container	2 Place 1 mL of Enzyme Q solution and 20 mL of substrate solution and some distilled water into the sealed container
3 Record levels of oxygen in the container at 10-second intervals for two minutes	3 Record levels of oxygen in the container at 10-second intervals for two minutes
4 Repeat steps 1–3 again, this time with the bath set to 33 °C, and then again at 41 °C	4 Repeat steps 1–3 again, this time add 5 drops of a strong acidic (pH of 2) solution to the container, and then again, this time using 5 drops of a strong alkaline (pH of 12) solution
5 Repeat the whole method again (two trials in total) to address replication	5 Repeat the whole method two more times (three trials in total) to address replication

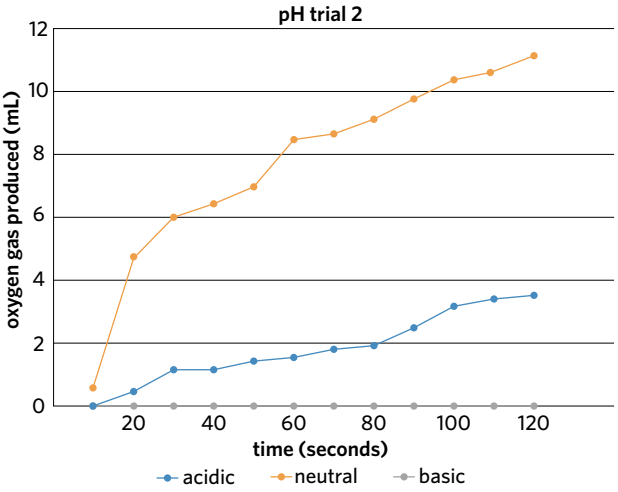
Bridget's results

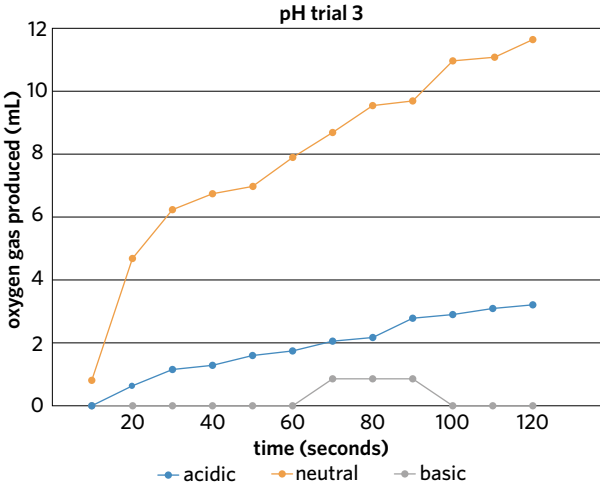




Jarrod's results







10 Describe the results seen in Bridget's graphs. (2 MARKS)

11 Describe the results seen in Jarrod's graphs. (2 MARKS)

12 In one of Bridget's trials, the meter malfunctioned during one reading. Identify this type of error and describe how this could affect the results. (1 MARK)

13 Jarrod made a personal error by misreading several of the values for one of his trials. Identify which trial this was and describe how this could affect the results. (2 MARKS)

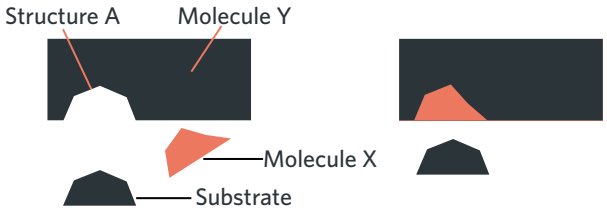
14 Given that both students investigated the same enzyme, summarise what can be concluded about Enzyme Q. In your response, identify which temperature and pH value tested in this experiment are closest to the enzyme's optimal. (2 MARKS)

CHAPTER 3 EXAM PRACTICE

Section A (11 MARKS)

Question 1 (1 MARK)

The diagram represents a generalised biochemical process.



Which one of the following statements is correct?

A Molecule X is an enzyme.

B Structure A is a coenzyme.

C This is an example of competitive inhibition.

D Molecule X binds allosterically to the active site.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q8

Question 2 (1 MARK)

An experiment was conducted to investigate enzyme activity. A small amount of amylase solution was added to a solution of starch dissolved in water at 35 °C. It was observed that maltose was produced.

In this reaction, the enzyme is

A water.

B starch.

C maltose.

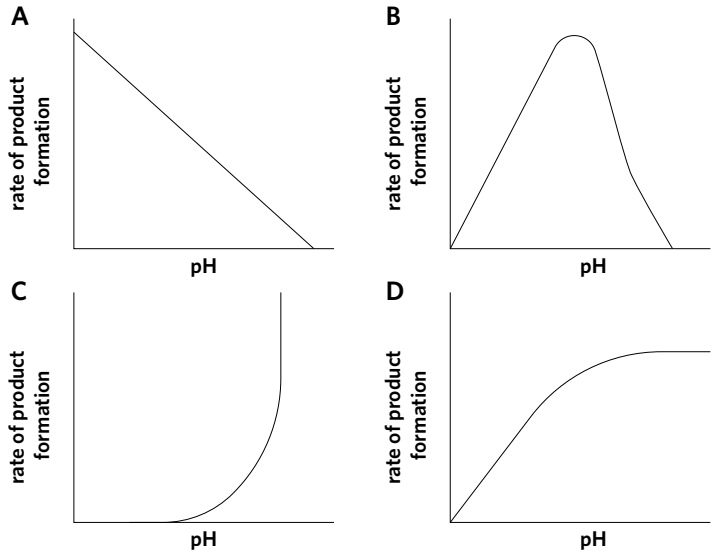
D amylase.

Adapted from VCAA 2015 Section A Q6

Question 3 (1 MARK)

Consider an enzyme-facilitated reaction in which the concentration of the enzyme is kept constant.

Which of the following graphs shows the effect of increasing the pH of the solution on the rate of product formation?



Adapted from VCAA 2017 Sample Exam Section A Q10

Question 4 (1 MARK)

The activity of an enzyme does not

A increase with temperature indefinitely, even though more molecular collisions typically occur as temperature increases.

B decrease with pH values outside an enzyme's optimal range.

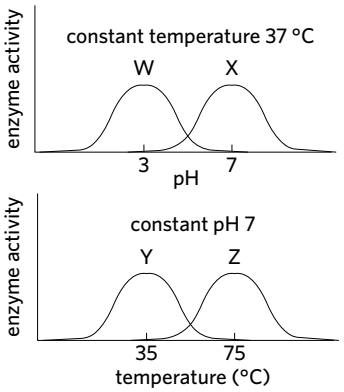
C increase when temperature is within its optimum range.

D decrease in the presence of an inhibitor.

Question 5 (1 MARK)

The following graphs show the way four enzymes, W, X, Y, and Z, change their activity under different pH or temperature situations.

Which of the following statements about the activity of the four enzymes is correct?



A The optimal pH of enzyme Z is 3.

B Enzyme W functions well in an alkaline environment.

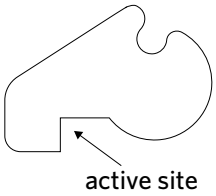
C At pH 5, enzyme X has greater activity than enzyme W.

D Enzyme Y is more likely to be found in the human body than enzyme Z.

Adapted from VCAA 2014 Section A Q13

Question 6 (1 MARK)

A drug molecule has been designed to inhibit the activity of an enzyme. The shape of the enzyme is shown.

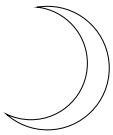


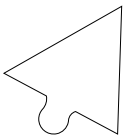
The position of the active site is labelled.

Which of the following is the most likely shape for a drug molecule that is capable of competitively inhibiting the enzyme?

A 

B 

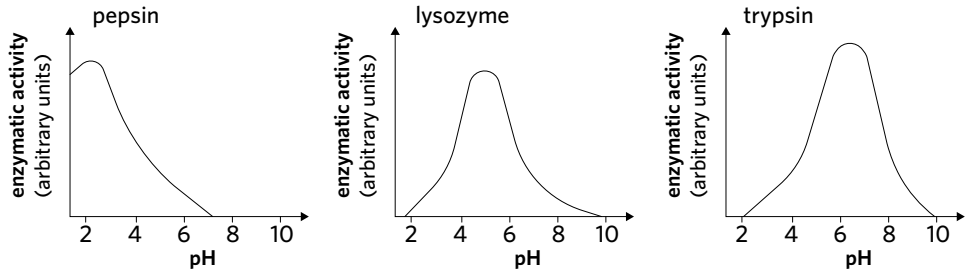
C 

D 

Adapted from VCAA 2013 Section A Q1

Question 7 (1 MARK)

Examine the following graphs.



From these graphs, it is reasonable to infer that at a pH of 6

A all the lysozyme would be denatured.

B trypsin converts a large amount of substrate.

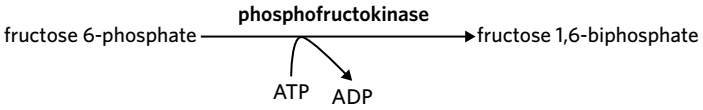
C all three enzymes would lack a functional active site.

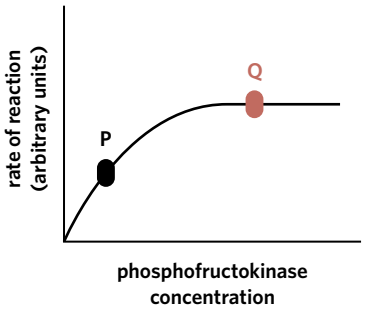
D the active site of pepsin would bind well to the substrate.

Adapted from VCAA 2011 Exam 1 Section A Q16

Use the following information to answer Questions 8 and 9.

The biochemical pathway of glycolysis involves nine intermediate reaction steps. One of these steps is represented in the diagram, as well as a graph displaying the rate of the reaction. In this reaction, the environmental temperature and pH are optimal.





Question 8 (1 MARK)

As the concentration of phosphofructokinase is increased from point P,

A the rate of reaction will increase.

B ADP concentration will decrease.

C the rate of reaction will not change.

D fructose 6-phosphate concentration will increase.

Question 9 (1 MARK)

Based on the information in the graph, what can be concluded about point Q?

A Fructose 6-phosphate is the limiting reactant.

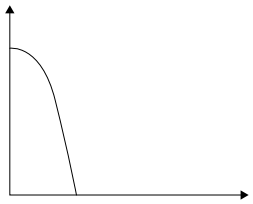
B The enzyme phosphofructokinase has denatured.

C Fructose 1,6-biphosphate is the limiting reactant.

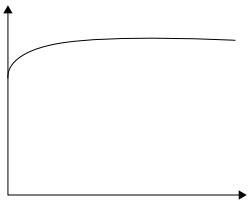
D ADP has become the limiting reactant of the reaction.

Use the following information to answer Questions 10 and 11.

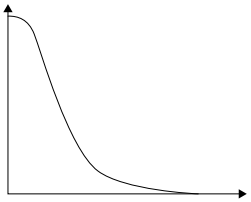
Four students performed a series of experiments to investigate the effects of four different variables on the rate of an enzyme-catalysed reaction. In each experiment, the students increased one of the following variables: temperature, pH, enzyme concentration, and the concentration of a known enzyme inhibitor. Each experiment started at a pH and temperature value known to be within the optimal range of the enzyme. When starting each experiment, one student made the mistake of not recording the data for the first several minutes. The students still displayed their results in a series of graphs, as shown, but the reaction rate was high when recording started. Each graph is a line of best fit.



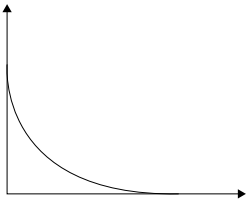
Variable 1



Variable 2



Variable 3



Variable 4

Question 10 (1 MARK)

The students did not label the horizontal axis on any of their four graphs. The next day, the students could not agree on which variable should be labelled on the horizontal axis of each graph. The students made the following suggestions as to what each variable could be.

Student	Variable 1	Variable 2	Variable 3	Variable 4
Alester	Temperature	Enzyme concentration	pH	Inhibitor concentration
James	Enzyme concentration	Inhibitor concentration	pH	Temperature
Alexis	pH	Enzyme concentration	Inhibitor concentration	Temperature
Riley	Temperature	pH	Enzyme concentration	Inhibitor concentration

Which student correctly identified all four variables on the horizontal axes?

A Alester
B James
C Alexis
D Riley

Adapted from VCAA 2018 Section A Q7

Question 11 (1 MARK)

What type of error was made by not recording data at the beginning of the experiment?

A systematic error
B theoretical error
C personal error
D random error

Section B (23 MARKS)

Question 12 (9 MARKS)

A group of students wanted to investigate the activity of an enzyme that catalyses the breakdown of hydrogen peroxide into water and oxygen.

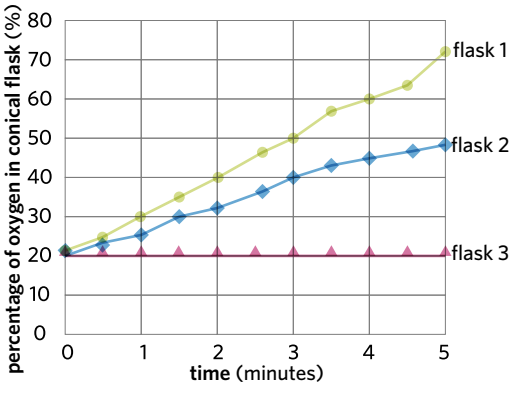
The students measured oxygen concentration using an oxygen sensor. The oxygen sensor fits into the top of a conical flask, as shown in the diagram.

The students set up three conical flasks with the contents listed in the table.

Flask	Contents of flask
1	50 mL of 3% hydrogen peroxide solution 2 mL of enzyme solution 50 mL of neutral pH buffer solution
2	50 mL of 3% hydrogen peroxide solution 2 mL of enzyme solution 50 mL of high pH buffer solution
3	50 mL of 3% hydrogen peroxide solution 2 mL of enzyme solution 50 mL of low pH buffer solution

The buffer solutions and the distilled water did not react with the hydrogen peroxide, and all conical flasks were at room temperature.

The students recorded the concentration of the oxygen over a five minute period. The results of the experiment are shown in the graph.



a State the dependent and independent variables in this experiment. (2 MARKS)

b The students hypothesised that the enzyme would have the highest activity in a high pH buffer.

Do the results support their hypothesis? Justify your response. (2 MARKS)

c The class teacher told the students they should use a control.

Describe how a control could be implemented and outline its purpose. (2 MARKS)

d The students were required to write a report on their experiment.

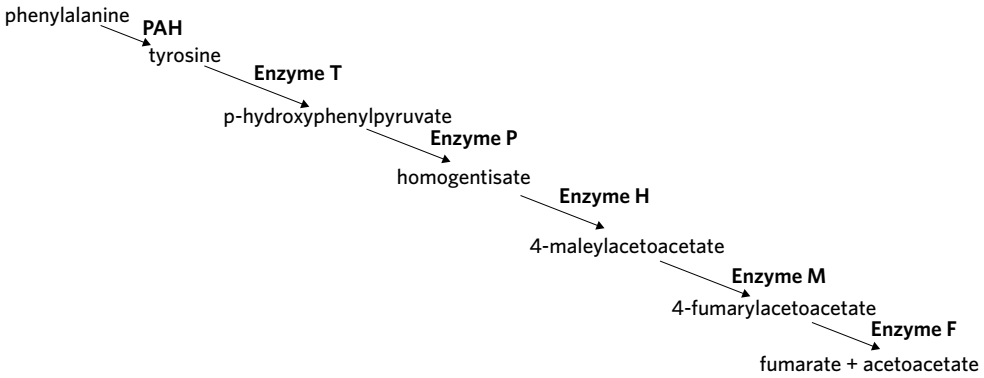
What conclusion could they draw from their experiment about the enzyme's activity?

In your response, refer to the variables identified in part a, the accuracy of the students' hypothesis in part b, and the results seen in the graph. (3 MARKS)

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q11

Question 13 (6 MARKS)

A genetic disease called phenylketonuria (PKU) may occur in babies. Affected individuals produce little or none of the enzyme phenylalanine hydroxylase (PAH). PKU is caused by the effects of too much of the amino acid phenylalanine building up in the body. Phenylalanine enters the body because it is abundant in a normal protein-rich human diet, and is metabolised in the biochemical pathway shown.



a Explain how the concentration of PAH affects the concentrations of fumarate and acetoacetate in unaffected individuals. Justify your response. (2 MARKS)

b Enzymes are biological molecules that catalyse reactions but are susceptible to several types of inhibition. Describe the difference between competitive and non-competitive enzyme inhibitors. (2 MARKS)

c If a known competitive inhibitor of PAH was introduced to a baby's system, would they be more or less likely to suffer the symptoms of PKU? Justify your response (2 MARKS)

Adapted from VCAA 2017 Sample Exam Section B Q2a

Question 14 (8 MARKS)

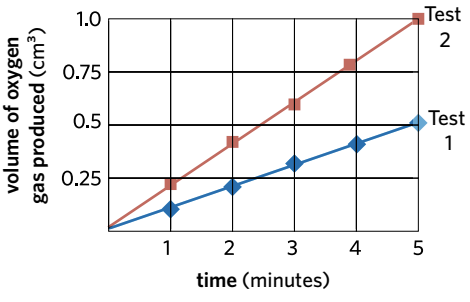
Hydrogen peroxide is a toxic by-product of many biochemical reactions. Human cells break down hydrogen peroxide into water and oxygen gas with the help of the intracellular enzyme catalase. The optimal pH of catalase in humans is 7.

A biology student, Student Z, measured the activity of catalase by recording the volume of oxygen gas produced from the decomposition of hydrogen peroxide when a catalase suspension was added. The catalase suspension was produced from human liver samples. The student performed two tests and graphed the results.

In Test 1, the student used 5 mL of 3% hydrogen peroxide solution and 0.5 mL of catalase suspension. The test was conducted at 20 °C in a buffer solution of pH 7.

Test 2 was carried out under identical conditions to Test 1, except for one variable that the student changed.

Student Z forgot to record which variable was changed in Test 2. The next day, Students A and B came across their unfinished results. Student A stated that the change in Test 2 was likely to be a change in the buffer solution's pH. Student B stated that the change was more likely to be a change in the temperature under which the experiment was conducted.



a Using the data, identify which test had the higher rate of oxygen production and describe the overall trends in the graph. (2 MARKS)

b Explain whether the results support Student A or Student B. Justify your response. (2 MARKS)

c Catalase is a common enzyme found in nearly all living organisms including humans.

i Given catalase is at its optimum temperature when functioning in a human, what would its optimum temperature be? (1 MARK)

ii Explain why catalase is able to catalyse more reactions at this temperature. (1 MARK)

d The students set up a third test that used 5 mL of 3% hydrogen peroxide solution and 0.5 mL of catalase suspension but was conducted at 5 °C in a buffer solution of pH 7.

Describe where the results for Test 3 would lie on the graph. Justify your response. (2 MARKS)

Adapted from VCAA 2017 Section A Q6

CHAPTER

4

DNA manipulation

Key knowledge

- the use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA, and endonucleases to cut DNA
- the function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome
- amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling
- the use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin
- the use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease

4A ENZYMES THAT MANIPULATE DNA

*Everyone knows that one of the best animals to keep as a pet is the axolotl a.k.a. Mexican walking fish (*Ambystoma mexicanum*) – they can regenerate parts of their body that fall off, grow extra legs, and can even slightly alter their colour for camouflage. The only thing they can't do is glow in the dark... until modern biology came along. Yes, that's right – we can make Mexican walking fish even more awesome by making them glow in the dark. How?*

What do you call a Mexican walking fish with a broken leg?A Mexican't walking fish.

Image: Carter Charles Johnson/ Shutterstock.com

Lesson 4A

In this lesson you will learn how genetic material can be cut by endonucleases, joined by ligases, and synthesised by polymerases.

◀ *Prerequisite knowledge*

Years 7–10
DNA is the blueprint for controlling the characteristics of organisms.

Year 11
Nucleotides, which are made up of a phosphate group, sugar group, and nitrogenous base, are joined together with phosphodiester bonds between adjacent sugar groups to create a polynucleotide chain.

Lesson 3A
Enzymes are proteins that act as biological catalysts.

Future applications ▶

Chapter 4
DNA manipulation uses endonuclease, ligase, and polymerase enzymes to analyse, alter, and/or amplify a sample of DNA.

Lesson 5D
Improving crop yields through genetic modifications often requires the use of enzymes that manipulate DNA.

Study design dot point

- the use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA, and endonucleases to cut DNA

Key knowledge units

Endonucleases	3.1.8.1
Ligases	3.1.8.2
Polymerases	3.1.8.3

Endonucleases 3.1.8.1

OVERVIEW

Scientists use a range of 'molecular scissors' known as endonucleases to cut DNA.

THEORY DETAILS

Endonucleases refer to a broad range of enzymes responsible for cutting strands of DNA. When these enzymes target specific **recognition sites**, they are known as **restriction endonucleases**. To 'cut' the DNA, these enzymes cleave the phosphodiester bond of the sugar-phosphate backbone that holds DNA nucleotides together. Some scientists refer to this 'cutting' process as 'restriction endonuclease digestion'.

Restriction endonucleases are often sourced from bacteria, where they are naturally produced as a defence mechanism against invading viral DNA that could harm the bacteria. The names of restriction endonucleases are based on the bacteria in which they were discovered (e.g. EcoRI was discovered in *E. coli*).

The recognition site of a restriction endonuclease, which is usually four to six nucleotides in length, is specific to each enzyme. Generally, recognition site sequences are palindromes, which means the 5' to 3' sequence of the template strand is the same as the 5' to 3' sequence of the non-template strand (Figure 1).

Table 1 Restriction sites for some common restriction endonucleases. EcoRI and HindIII create sticky ends, while AluI and HaeIII create blunt ends.

Restriction endonuclease	Recognition sequence (5' to 3' where * is the cut site)
EcoRI	5' G* A A T T C 3' 3' C T T A A *G 5'
HindIII	5' A* A G C T T 3' 3' T T C G A *A 5'
AluI	5' A G* C T 3' 3' T C *G A 5'
HaeIII	5' G G* C C 3' 3' C C *G G 5'

Endonucleases either create **sticky ends** or **blunt ends**. Blunt end endonucleases, such as AluI, cut DNA in the middle of the recognition site, which results in a straight cut and no **overhanging nucleotides**. Sticky end endonucleases, such as EcoRI, do not cut in the middle of the recognition site, resulting in a staggered cut with overhanging, unpaired nucleotides (Figure 2). They are called 'sticky' because the unpaired nucleotides will be attracted to, or want to stick to, a complementary set of unpaired nucleotides. Sticky end endonucleases have the advantage of ensuring an inserted gene is orientated correctly when manipulating DNA.

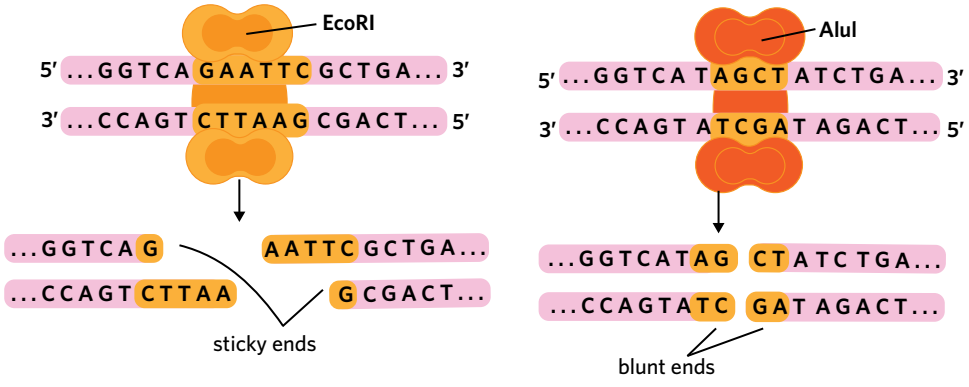


Figure 2 The action of restriction endonucleases EcoRI and AluI at their recognition sites on a fragment of linear DNA. Note that EcoRI creates sticky ends, but AluI creates blunt ends.

endonuclease an enzyme that breaks the phosphodiester bond between two nucleotides in a polynucleotide chain

recognition site a specific target sequence of DNA upon which restriction endonucleases act

restriction endonuclease any enzyme that acts like molecular scissors to cut nucleic acid strands at specific recognition sites. Also known as a **restriction enzyme**

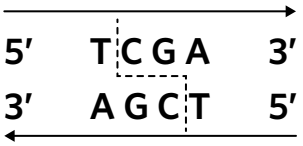


Figure 1 Recognition site for TaqI. Notice when reading from 5' to 3' on both strands, the sequence is the same – TCGA – since recognition sites are typically palindromes.

Lesson link

In ***lesson 3A*** you learned about how the specificity of enzymes arises due to the 3D shape of their active site. It is important to remember that because endonucleases are enzymes, their active site is complementary to a specific recognition site (the substrate).

sticky end the result of a staggered cut through double-stranded DNA by an endonuclease resulting in overhanging nucleotides

blunt end the result of a straight cut across the double-stranded DNA by an endonuclease resulting in no overhanging nucleotides

overhanging nucleotides unbonded nucleotides on the ends of the DNA strand resulting from a staggered cut

Memory device

Endonucleases are like the ☒ +X or Ctrl+X on your laptop.

Figure 3 Using the cut function while Edrolo n chilling

Examiners' tip

Question 39 in section A of the 2019 VCAA Biology exam asked students to determine how many fragments a piece of DNA will be cut into given a certain number of recognition sites. Be careful! For circular DNA like plasmids, the number of fragments will equal the number of recognition sites. For linear DNA, the number of fragments will equal the number of recognition sites plus one.

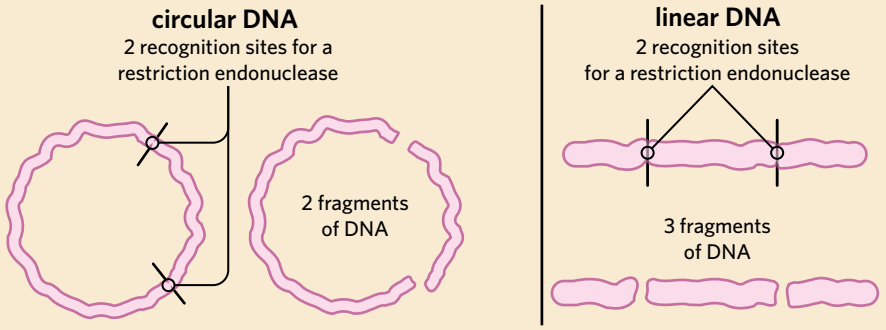


Figure 4 For circular DNA, two recognition sites lead to two fragments. For linear DNA, two recognition sites lead to three fragments.

Lesson link

Cas9 is an endonuclease crucial to the manipulation of genomes with CRISPR technology, which you will learn about in ***lesson 4B.***

Ligases 3.1.8.2

OVERVIEW

Ligases are enzymes that join two fragments of DNA or RNA together.

THEORY DETAILS

Ligases are enzymes that join two fragments of DNA or RNA, acting like molecular glue. To do this, the enzyme will catalyse the formation of phosphodiester bonds between the two fragments to merge them together. There are two main types of ligase enzymes: DNA ligase, which joins two DNA fragments, and RNA ligase, which joins two RNA fragments.

Essentially, ligase enzymes function as the reverse of endonucleases. However, they lack the specificity of restriction endonucleases – meaning they can join together any blunt or sticky ends. This is because the substrates for this enzyme are the sugar and phosphate groups of the DNA or RNA, rather than specific nitrogenous bases which is the case for restriction endonucleases.

ligase an enzyme that joins molecules, including DNA or RNA, together by catalysing the formation of phosphodiester bonds

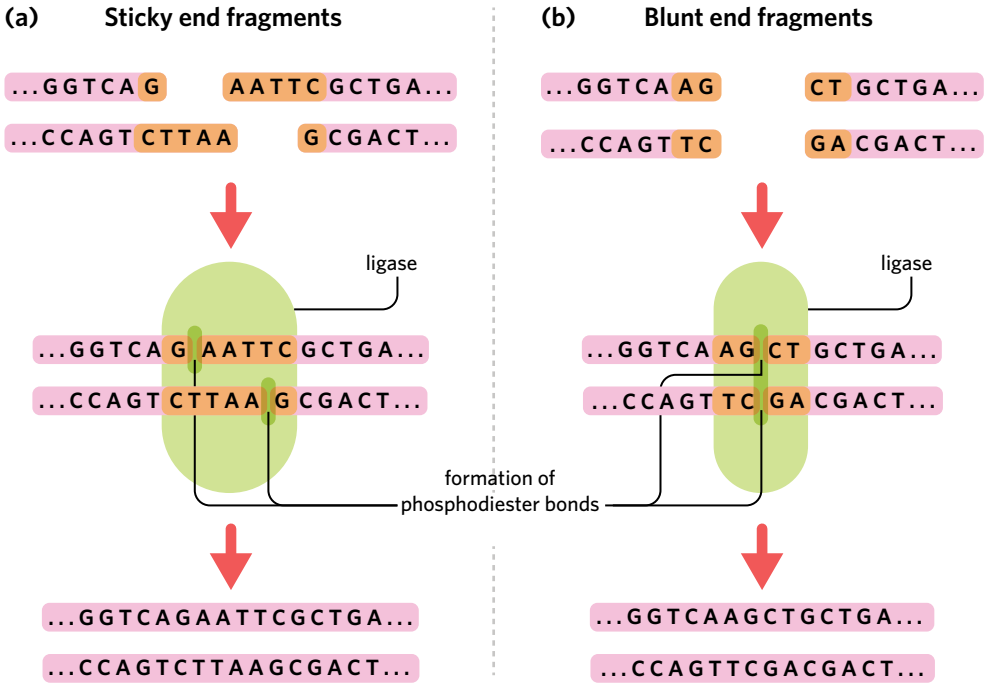


Figure 5 DNA ligase joining **(a)** two sticky end fragments together and **(b)** two blunt end fragments together.

Memory device

Ligases are like the ☒+V or Ctrl+V on your laptop.

Figure 6 Using the paste function while Edrolo n chilling

Polymerases 3.1.8.3

OVERVIEW

Polymerases add nucleotides to DNA or RNA, which can lead to copying entire genes.

THEORY DETAILS

Polymerases synthesise polymer chains from monomer building blocks. There are two particular polymerases used for gene manipulation, RNA polymerase and DNA polymerase (Table 2).

Table 2 Types of polymerase enzymes used for gene manipulation

Polymerase	Monomer	Polymer
RNA polymerase	RNA nucleotide	RNA strand
DNA polymerase	DNA nucleotide	DNA strand

While RNA polymerase is primarily used in the transcription of genes, DNA polymerase is used in the replication or amplification of DNA. For example, in forensic medicine, when scientists are testing a sample they often have a very small amount of DNA available. DNA polymerase can be used to synthesise more strands of DNA, thereby amplifying the DNA.

Polymerases require a **primer** to attach to the start of a template strand of DNA. Primers are short single-stranded chains of nucleotides that are complementary to the template strand. Once attached to the primer, the polymerase enzyme can read and synthesise a complementary strand to the template strand in a 5' to 3' direction.

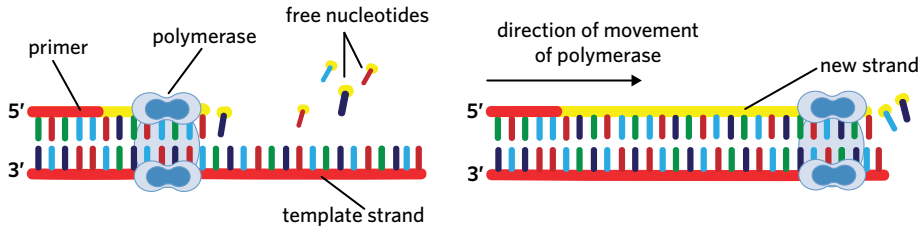


Figure 7 DNA polymerase synthesising a new strand from a template strand

polymerase an enzyme that synthesises a polymer from monomers, such as forming a DNA strand from nucleic acids

primer a short, single strand of nucleic acids that acts as a starting point for polymerase enzymes to attach

Lesson link

In ***lesson 2D***, you learned about the function of RNA polymerase and its involvement in the process of transcription. Its role is to amplify a gene by transcribing DNA into RNA for ribosomes to translate proteins.

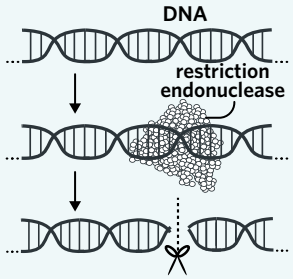
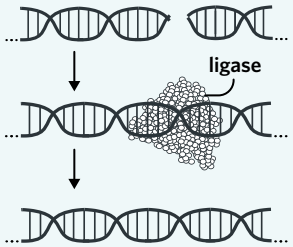
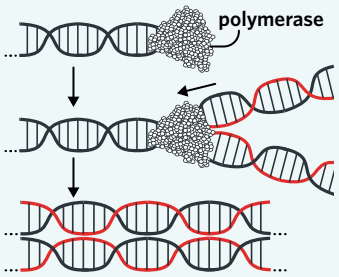
Memory device

Polymerases are like the ⊠ +C or Ctrl+C on your laptop.

Figure 8 Using the copy function while Edrolo n chilling

Theory summary

Table 3 Summary of enzymes used in DNA manipulation

Enzyme	Restriction endonuclease	Ligase	Polymerase
Action	Cut DNA or RNA at specific restriction sites	Join (or paste) fragments of DNA or RNA together	Amplify (or multiply) sections of DNA or RNA
Diagram			

How do we make Mexican walking fish glow in the dark? Through the use of enzymes! With the help of endonucleases, a protein called green fluorescent protein (GFP) is removed from a species of jellyfish called Aequorea victoria*, amplified by polymerases, and placed into the genome of the Mexican walking fish using ligase enzymes. This protein produces bright green fluorescence when exposed to certain wavelengths of light. Our Mexican walking fish will now express GFP and glow in the dark like a soothing aquatic night light!*

4A QUESTIONS

Theory review questions

Question 1

Endonucleases, ligases, and polymerases are all examples of

A enzymes.

B DNA.

Question 2

Fill in the blanks in the following sentence.

_____ are responsible for catalysing the formation of the phosphodiester backbone, _____ act as 'molecular scissors', and _____ are used to amplify fragments of DNA.

Question 3

Categorise the following restriction endonucleases as **sticky end** or **blunt end**.

	Restriction endonuclease	Recognition sequence (5′ to 3′ where * is the cut site)	Sticky end or blunt end
I	EcoRI	G*A A T T C C T T A A*G	_____
II	HaeIII	G G*G G C C*C C	_____
III	BamHI	G*G A T C C C C T A G*G	_____
IV	AluI	A G*C T T C*G A	_____
V	HindIII	A*A G C T T T T C G A*A	_____

Question 4

A primer

A synthesises a new strand of DNA.

B creates a starting point for a polymerase enzyme.

C is the specific sequence of nucleotides that endonucleases act upon.

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–9.

In the microscopic world, bacteria face many threats from the external environment. Indeed, although invisible to the naked eye, bacteria are constantly being attacked by viruses known as bacteriophages. Bacteriophages are viruses composed of genetic material consisting of either DNA or RNA surrounded by a protein coat. Bacteriophages inject viral DNA or RNA into the bacterial cell, altering the bacteria's genome. In doing so, the bacterial cell begins to produce viral proteins and contributes to the assembly and replication of more bacteriophages. Eventually, due to the build-up of viral particles within the bacterial cell, it lyses and releases thousands of bacteriophages into the external environment. Even though it may seem that bacteria are primitive and defenceless organisms, they've actually developed a series of techniques to protect themselves!

With the help of restriction endonucleases, which target specific sequences of DNA or RNA, bacteria can defend themselves against bacteriophages. When a bacteriophage attempts to infect a bacterium, the restriction endonucleases will locate and inactivate the injected viral genetic material, preventing its incorporation into the bacterial genome.

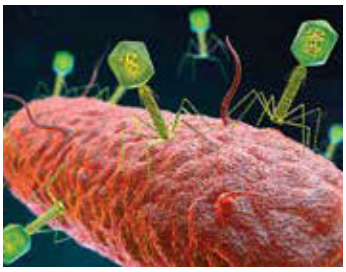


Image: Tatiana Shepeleva/Shutterstock.con

Research into the use of bacteriophages has increased dramatically during the last decade. For example, researchers at Flinders University, Adelaide, are currently experimenting with the use of bacteriophages to combat bacterial diseases in humans. In the past, bacterial infections could be easily treated with the use of antibiotics, but with the rise of highly resistant strains of bacteria, scientists must develop new therapies to combat bacterial infections.

Question 5

Bacteriophages are

A prokaryotic organisms.

B made up of genetic material and a protein coat.

Question 6

Which of the following enzymes would be effective at defending against a bacteriophage?

A ligase

B polymerase

C endonuclease

Question 7

Restriction endonucleases can only target the genetic material of

A specific bacteriophages with a complementary DNA sequence.

B all bacteriophages, due to the universal nature of the phosphate sugar backbone.

Question 8

Endonucleases are a unique feature of bacteria that are not naturally found in animal cells because animal cells

A do not contain plasmid DNA.

B are not vulnerable to viral attacks.

C have developed more complex defence mechanisms.

Question 9

How can bacteriophages be used in research to treat human diseases?

A by studying bacteriophages, researchers can design treatments targeting viral infections

B by editing the genome of bacteriophages, scientists could create a treatment against antibiotic-resistant bacteria

Exam-style questions

Within lesson

Question 10 (1 MARK)

The role of DNA ligase is to

A synthesise a strand of DNA complementary to its template.

B act as molecular scissors and cut DNA at a specific sequence.

C unravel double-stranded DNA to allow polymerase to read the template strand.

D join fragments of DNA together by catalysing the formation of phosphodiester bonds.

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q9d

Question 11 (1 MARK)

Enzymes can be used to cut, insert, and amplify genes into circular pieces of DNA known as plasmids. Which of the following options shows the correct function of each enzyme?

	Cuts plasmid	Inserts gene into plasmid	Amplifies plasmid DNA
A	endonuclease	DNA polymerase	DNA ligase
B	endonuclease	DNA ligase	DNA polymerase
C	DNA ligase	endonuclease	DNA polymerase
D	DNA polymerase	DNA ligase	endonuclease

Use the following information to answer Questions 12 and 13.

Genetic engineers use restriction endonucleases to cut DNA into smaller fragments. The recognition sites of several restriction endonucleases are shown in the table. The symbol * denotes the recognition site (position of the cut).

Restriction endonuclease	Recognition sequence (5′ to 3′ where * is the cut site)
EcoRI	G*AATTC CTTAA*G
HindIII	A*AGCTT TTCGA*A
AluI	AG*CT TC*GA
HaeIII	GG*GG CC*CC

Question 12 (1 MARK)

Consider a fragment of linear double-stranded DNA with the sequence

5′ **GGCCTATGAAGCTTGAA** 3′

3′ **CCGGATACTTCGAACTT** 5′

Adding HindIII to a solution containing one copy of this double-stranded DNA produces

A four fragments of single-stranded DNA, each with blunt ends.

B two fragments of double-stranded DNA, each with blunt ends.

C four fragments of single-stranded DNA, each with sticky ends.

D two fragments of double-stranded DNA, each with sticky ends.

Question 13 (1 MARK)

Now consider a different length of linear double-stranded DNA with the sequence

5′ **G A A T T C G A A G G T T T A A T G G C T** 3′

3′ **C T T A A G C T T C C A A A T T A C C G A** 5′

Which enzyme(s) will cut this piece of DNA?

A EcoRI only

B HindIII only

C AluI and HindIII only

D AluI, HindIII, and HaeIII only

Adapted from VCAA 2013 Section A Q30

Multiple lessons

Question 14 (6 MARKS)

GloFish are fish that have undergone modifications to their genome by adding a gene encoding green fluorescent protein (GFP) from a fluorescent jellyfish that produces the protein in order to glow. Once this gene is part of the fish's genome, cellular functions occur to produce the protein.

a Name the process by which a complementary mRNA strand is synthesised from the DNA template strand. (1 MARK)

b Outline the steps of translation in the synthesis of GFP. (3 MARKS)

c In order to insert the gene encoding GFP into fish, it first needs to be isolated from the jellyfish genome. Identify the enzyme that acts as molecular scissors and is required to isolate the gene encoding GFP from the jellyfish genome. (1 MARK)

d Scientists also use DNA ligase enzymes. Describe the role of DNA ligase. (1 MARK)

Adapted from VCAA 2018 Section B Q1a

Key science skills and ethical understanding

Question 15 (1 MARK)

The manipulation of DNA is a contentious area of study in society and raises many ethical considerations. One argument is that if we allow scientists to manipulate DNA, humans could alter their genomes to create a generation of 'super-humans' which could affect natural selection and reduce genetic diversity. This argument is an example of

A a virtues-based approach to bioethics.

B the bioethical concept of non-maleficence.

C a consequences-based approach to bioethics.

D a duty- and/or rule-based approach to bioethics.

Question 16 (12 MARKS)

Ahmed and Sunitha are investigating the recognition sites of an unknown restriction endonuclease. They have a singular piece of DNA, four different known restriction endonucleases, and an unknown enzyme. The sequence of the DNA is:

5′ **A T C G A T C T T A A G C T T C G A A G G A T C C A T T C C C G G G** 3′

3′ **T A G C T A G A A T T C G A A G C T T C C T A G G T A A G G G C C C** 5′

a Given a template strand of DNA, what enzyme would aid the process of amplification of a complementary strand? (1 MARK)

b Ahmed sourced the following table from a research article that shows the recognition sites of their four known restriction endonucleases. Restriction endonucleases can produce either a sticky end or a blunt end.

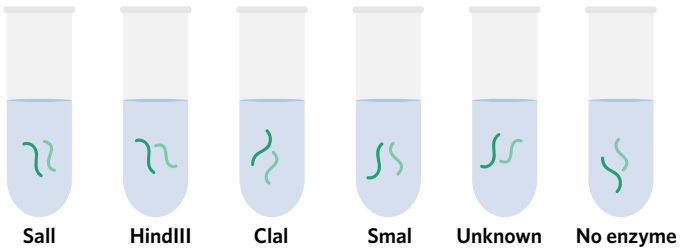
i Explain what sticky end restriction endonucleases are and how they can be useful. (2 MARKS)

ii From the four known restriction endonucleases, identify which create sticky ends. (1 MARKS)

Restriction endonuclease	Recognition sequence (5′ to 3′ where * is the cut site)
SalI	G* T C G A C C A G C T *G
HindIII	A* A G C T T T T C G A *A
ClaI	A T* C G A T T A G C *T A
SmaI	C C C* G G G G G G* C C C

c Ahmed and Sunitha successfully multiplied the number of DNA strands and have enough to complete the following experiment. They set up six test tubes with identical amounts of DNA buffer solution. The experimental set up is shown.

They added each of the four restriction endonucleases into the first four test tubes and the unknown enzyme into the fifth. For the final test tube, no enzyme was added. All of the test tubes were then incubated for one hour to allow the restriction endonucleases to digest the DNA samples.



Assume neither Ahmed nor Sunitha made any mistakes when running the experiment.

i Explain the purpose of the test tube without an enzyme and how it improves the validity of the results. (2 MARKS)

ii Apart from the amount of DNA buffer solution and incubation time, list two factors that must be kept constant across the test tubes for the digestion stage of the experiment. (2 MARKS)

iii Ahmed hypothesised that the HindIII sample will not cut the DNA as the HindIII recognition site is not present in the sample. Sunitha disagrees and believes there will be two fragments of DNA produced from the HindIII sample. Identify who is correct. Justify your response. (1 MARK)

d Two DNA fragments were produced in the unknown sample. The sequences of these fragments are shown.

Fragment 1
5′ **ATCGATCTTAAGCTTCGAAG** 3′
3′ **TAGCTAGAATTCGAAGCTTCCTAG** 5′
Fragment 2
5′ **GATCCATTCCCGGG** 3′
3′ **GTAAGGGCCC** 5′

i Explain whether or not Ahmed and Sunitha are able to identify the unknown restriction endonuclease. (1 MARK)

ii From the fragments, explain what information Ahmed and Sunitha can extract about the type of restriction endonuclease and its recognition site. (2 MARKS)

4B CRISPR-Cas9

What if having a baby was like online shopping? You could choose the gender, height, or hair and eye colour. But you could also add in some bonuses like resistance to malaria, a lower chance of developing cancer, or even a slower aging rate. But don't get too excited, this technology doesn't exist... or does it?

Image: fizkes/Shutterstock.com

Lesson 4B

In this lesson you will learn about the precise technology known as CRISPR-Cas9, which is radically changing the process of genetic engineering.

◀ *Prerequisite knowledge*

Year 11
Prokaryotic organisms, such as bacteria and archaea, are unicellular, simple organisms that do not contain membrane-bound organelles.

Lesson 4A
Cas9 is an endonuclease that creates blunt end cuts in both strands of the DNA it cuts.

Future applications ▶

Lesson 4F
CRISPR-Cas9 has many ethical considerations as it can be used to genetically engineer organisms.

Lesson 5D
CRISPR-Cas9 can be used to improve crop yields and increase the rate of photosynthesis.

Lesson 7A
Bacteriophages are a type of virus that specifically target prokaryotes.

Study design dot point

- the function of CRISPR-Cas9 in bacteria and the application of this function in editing an organism's genome

Key knowledge units

CRISPR-Cas9 in bacteria	3.1.9.1
CRISPR-Cas9 in gene editing	3.1.9.2

CRISPR-Cas9 in bacteria 3.1.9.1

OVERVIEW

CRISPR is a naturally occurring sequence of DNA found in bacteria that plays an important role in their defence against viral attacks.

THEORY DETAILS

What is CRISPR?

Just as humans can get a cold from infection by a **virus**, bacteria are also susceptible to viral attacks from **bacteriophages** (Figure 1). Unlike humans, when a virus infects a bacterium it won't leave the bacterium with the sniffles that go away after a few days. Their mode of action involves inserting viral DNA or RNA into a bacterium, then hijacking the cell's machinery to produce their own proteins and nucleic acids. Over time, the virus replicates and causes the bacterium to lyse and die, spreading viral particles to infect other cells. To protect themselves against bacteriophages, generations of bacteria slowly evolved the **CRISPR-Cas9** system.

virus a non-cellular, infectious agent composed of genetic material enclosed in a protein coat that requires a host cell to multiply

bacteriophage a virus that infects prokaryotic organisms

CRISPR-Cas9 a complex formed between gRNA and Cas9 which can cut a target sequence of DNA. Bacteria use this complex for protection from viruses and scientists have modified it to edit genomes

When a bacterium encounters a virus, it takes a 'mugshot' of it by storing some of the viral genetic material within the bacterium's own genome. Next time the virus invades, the bacterium transcribes the 'mugshot' DNA and attaches it to an **endonuclease** called **Cas9**. The transcribed mugshot is complementary to the viral DNA, so it ensures that the Cas9 only destroys the invading virus rather than any bacterial nucleic acids.

Scientists discovered this system in 1987, although they didn't recognise its potential applications until many years later. The system is relatively easy to recognise in the bacterial genome: it is a section of DNA with short, repeated sequences of nucleotides that have the same forward and reverse read. In other words, **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)** (Figure 2). The clustered repeats are interrupted by **spacer** DNA, which is the viral 'mugshot'. CRISPR sequences are always downstream of the gene for Cas9.

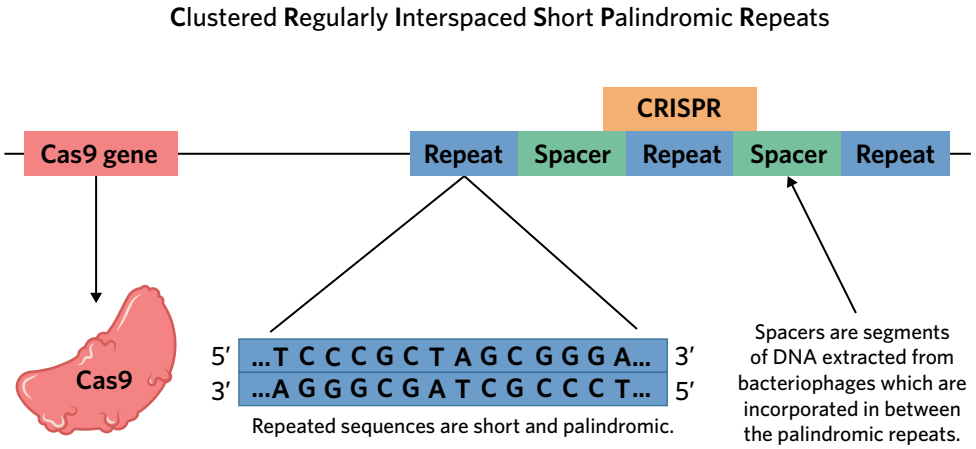


Figure 2 The CRISPR-Cas9 genetic architecture found in the bacterial genome

How the CRISPR-Cas9 defence system works

When a bacterium is infected by a bacteriophage, there are three steps to fighting the virus with the CRISPR-Cas9 system: exposure, expression, and extermination.

1 Exposure – the bacteriophage injects its DNA into a bacterium, which identifies the viral DNA as a foreign substance. Cas1 and Cas2 are both CRISPR-associated enzymes like Cas9, but they serve a different purpose. These enzymes cut out a short section of the viral DNA (typically ~30 nucleotides long), known as a **protospacer**. This protospacer can then be introduced into the bacterium's CRISPR gene and become a spacer (Figure 3).

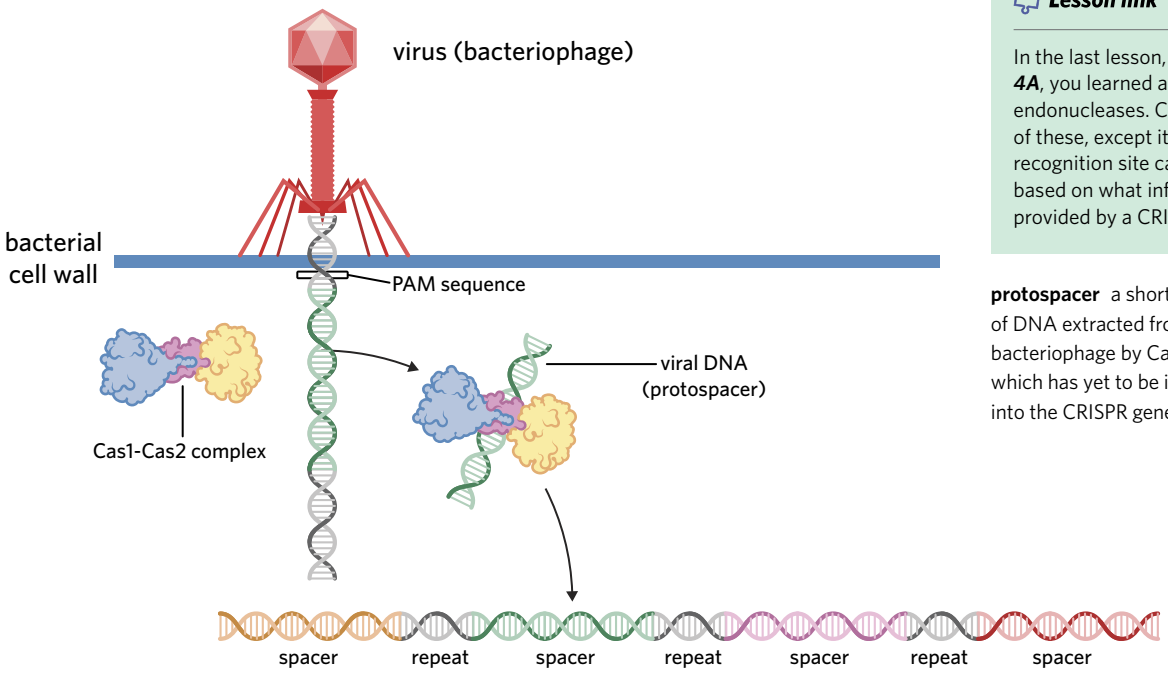


Figure 3 The exposure of a bacterium to bacteriophage DNA

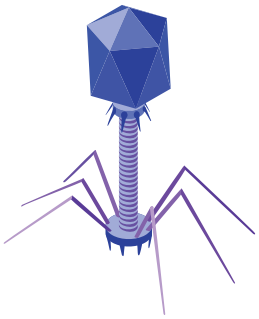


Image: Igorda/Shutterstock.com

Figure 1 No, it's not one of the aliens from Chicken Little or Dr Who. This is a bacteriophage.

endonuclease an enzyme that breaks the phosphodiester bond between two nucleotides in a polynucleotide chain

CRISPR-associated protein 9 (Cas9) an endonuclease that creates a blunt end cut at a site specified by guide RNA (gRNA)

CRISPR short, clustered repeats of DNA found in prokaryotes which protect them against viral invasion

spacer short sequences of DNA obtained from invading bacteriophages that are added into the CRISPR sequence

Lesson link

You will learn more about viruses in ***lesson 7A*** when you study the immune system in humans.

Lesson link

In the last lesson, ***lesson 4A***, you learned about endonucleases. Cas9 is one of these, except its target recognition site can vary based on what information is provided by a CRISPR spacer.

protospacer a short sequence of DNA extracted from a bacteriophage by Cas1 and Cas2, which has yet to be incorporated into the CRISPR gene

Theory in context

PROTOSPACER ADJACENT MOTIF (PAM)

Introducing PAM! PAM, or the **protospacer adjacent motif**, is a short (2–6 nucleotides) sequence of nucleotides that Cas1 and Cas2 can consistently recognise. When Cas1 or Cas2 come across the PAM, they are signalled to extract a protospacer from invading DNA. The enzymes cut the viral DNA just before the PAM, so the PAM does not get included in the final protospacer.

protospacer adjacent motif (PAM) a sequence of two-six nucleotides that is found immediately next to the DNA targeted by Cas9

For example, in *Streptococcus pyogenes* the specific PAM sequence is NGG. This means that Cas1 and Cas2 search foreign DNA for any nucleotide followed by two consecutive guanine nucleotides, then cut the previous sequence, ensuring that NGG is not included in the protospacer (Figure 3).

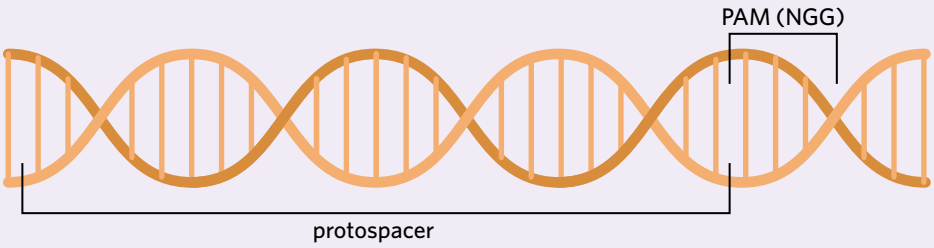


Figure 4 The PAM sequence found in bacteriophage DNA

Now PAM isn't just useful for Cas1 and Cas2: it serves a big role in increasing the efficiency of Cas9. If Cas9 has to search through every sequence of DNA inside a cell, unwrap the helix, scan the DNA for a match with the ~30bp spacer, then move on, it would be very time-consuming. To speed this process up, Cas9 only looks for PAMs (Figure 4).

Using the same example of *S. pyogenes*, Cas9 would search for NGG sequences, then check if the spacer aligns with the previous sequence. If it is a match, it will then cut both strands of the DNA, if not, it keeps searching.

The PAM sequence also has a protective role to play for bacteria. Bacteria never have a PAM sequence in the CRISPR repeats of their own DNA. This ensures that Cas9 cannot cut up a bacterium's own DNA.

2 Expression – the CRISPR spacers are transcribed along with half a palindrome from the repeat either side of it, and converted into an RNA molecule known as **guide RNA (gRNA)**. gRNA binds to Cas9 to create a CRISPR-Cas9 complex which is directed to any viral DNA inside the cell that is complementary to the gRNA (Figure 5). gRNA forms a hairpin loop-like structure from the transcribed palindromic repeats either side of the spacer.

guide RNA (gRNA) RNA which has a specific sequence determined by CRISPR to guide Cas9 to a specific site

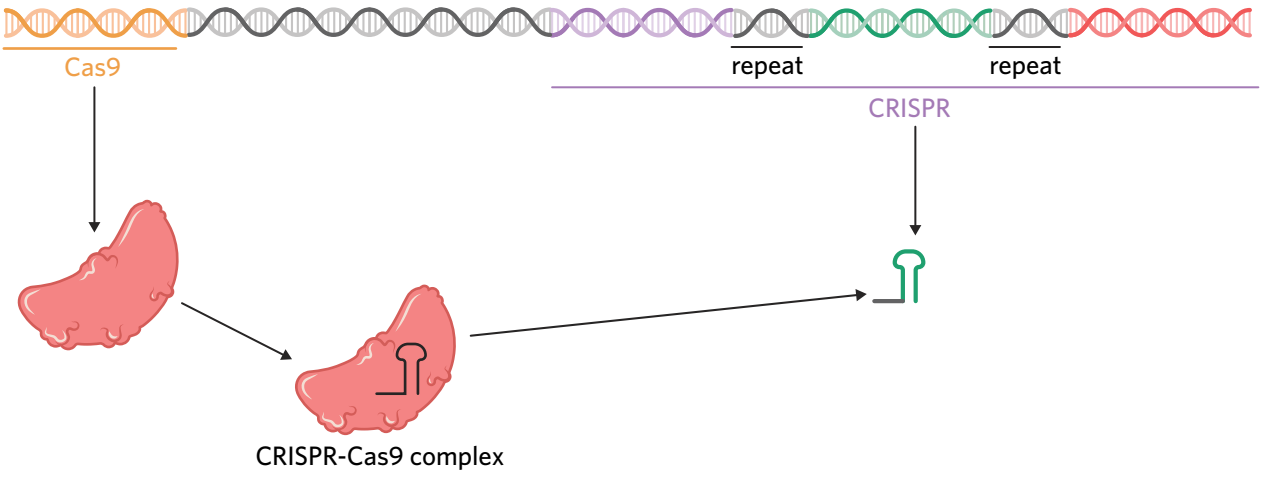


Figure 5 Expression of Cas9 protein and CRISPR gRNA

Theory in context

THE COMPONENTS OF GUIDE RNA

The gRNA molecule is formed by two smaller RNA molecules creating a hybrid of CRISPR RNA (crRNA) and trans-acting CRISPR RNA (tracrRNA).

crRNA is made up of a spacer and a repeat, which are transcribed and cleaved to produce the 'mugshot' for Cas9 to cut DNA. The tracrRNA consists of a complementary sequence to the crRNA repeat which enables the two molecules to bond and establish the final gRNA structure (Faure et al., 2018). Additionally, tracrRNA is responsible for binding tightly with Cas9 to establish the CRISPR-Cas9 complex.

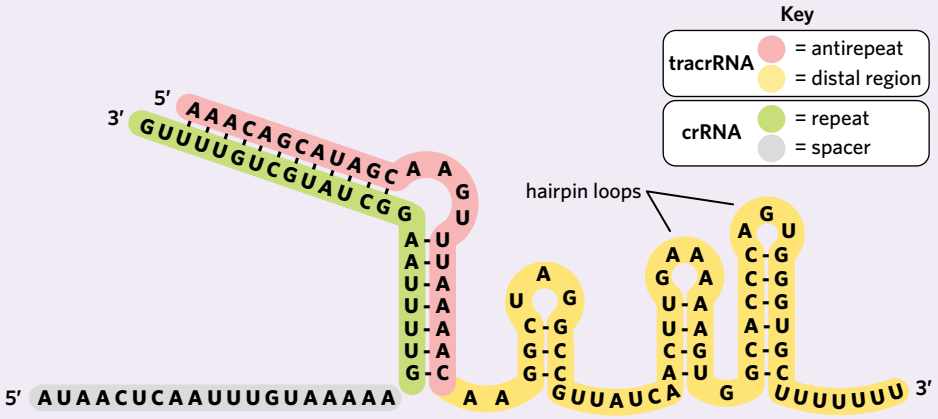


Figure 6 crRNA-tracrRNA hybrid creating gRNA

3 Extermination - The CRISPR-Cas9 complex then scans the cell for invading bacteriophage DNA that is complementary to the 'mugshot' on the gRNA. When it does, Cas9 cleaves the phosphate-sugar backbone to inactivate the virus. Cas9 contains two active sites to cut both strands of DNA and create **blunt ends** (Figure 7).

blunt end the result of a straight cut across the double-stranded DNA by an endonuclease resulting in no overhanging nucleotides

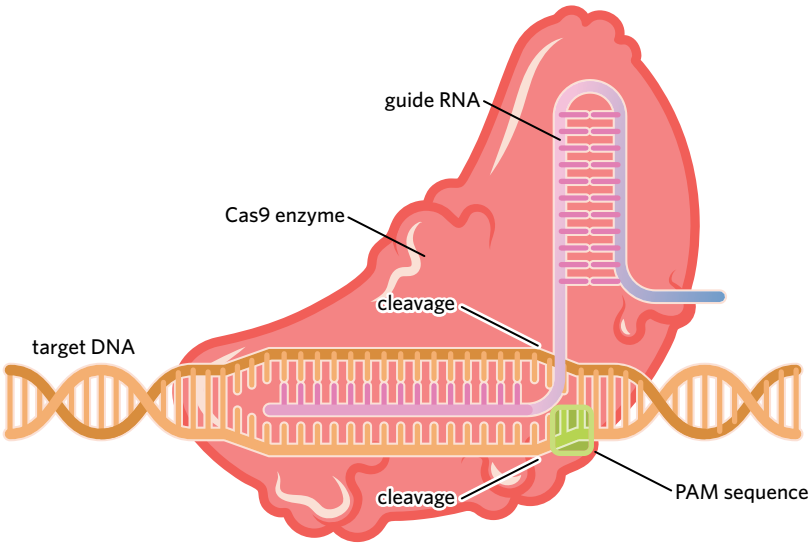


Figure 7 Cas9 has two active sites to cut both strands of DNA.

What happens when the viral DNA is cut?

When the viral DNA is cut, enzymes within the bacterium will naturally act to repair it. However, the repair mechanisms in a cell are prone to errors that can result in nucleotide additions, deletions, or insertions in the middle of the viral gene. This is advantageous in the case of bacteriophage infiltration because these mutations tend to render viral genes non-functional. If a mutation does not occur after the cut, the gRNA will find the gene again and repeat the whole process until the DNA repair mechanisms induce a mutation, inactivating the virus.

CRISPR-Cas9 in gene editing 3.1.9.2

OVERVIEW

Scientists have been able to adapt the CRISPR-Cas9 system to edit genomes with great precision.

THEORY DETAILS

The power of CRISPR-Cas9

Genetic modifications can amend **deleterious mutations** or introduce biologically advantageous alleles to an individual's genome. However, many **gene therapy** techniques lack precision and may inadvertently insert an introduced piece of DNA into the wrong part of the genome, interrupting a healthy and functioning gene. So, while gene therapy has enormous potential to solve a number of health problems, it has very rarely been used on humans outside of research and clinical trials.

CRISPR-Cas9 has been labelled as the future of genetic engineering with the potential to increase crop productivity, eliminate genetic diseases, and better understand the purpose of specific genes (Table 1). The CRISPR-Cas9 system can induce genetic changes by cutting DNA at a location specifically chosen by scientists, who make a synthetic gRNA to guide Cas9. This creates an opportunity for nucleotides to be added, removed, or substituted into the selected sequence. In turn, this can **knockout**, enhance, or change the function of a gene.

genetic modification the manipulation of an organism's genetic material using biotechnology

deleterious mutation a change in DNA that negatively affects an individual

gene therapy repairing genetic mutations by replacing a defective gene with a healthy one

gene knockout a technique in gene editing where scientists prevent the expression of a target gene to understand its function in an organism

How to use CRISPR-Cas9 for gene editing

CRISPR-Cas9 is not the only mechanism available for genetic engineering, however, it is currently the most precise and affordable option.

To use CRISPR-Cas9 for gene editing, the following steps must be taken (Figure 8):

1. Synthetic gRNA is created in a lab that has a complementary spacer to the target DNA that scientists wish to cut.
2. A Cas9 enzyme is obtained with an appropriate target PAM sequence.
3. Cas9 and gRNA are added together in a mixture and bind together to create the CRISPR-Cas9 complex.
4. The gRNA-Cas9 mixture is then injected into a specific cell, such as a **zygote**.
5. The Cas9 finds the target PAM sequence and checks whether the gRNA aligns with the DNA.
6. Cas9 cuts the selected sequence of DNA.
7. The DNA has a blunt end cut that the cell will attempt to repair.
8. When repairing the DNA, the cell may introduce new nucleotides into the DNA at this site. Scientists may inject particular nucleotide sequences into the cell with the hope that it will ligate into the gap.

zygote the diploid cell formed by the combination of two haploid gamete cells

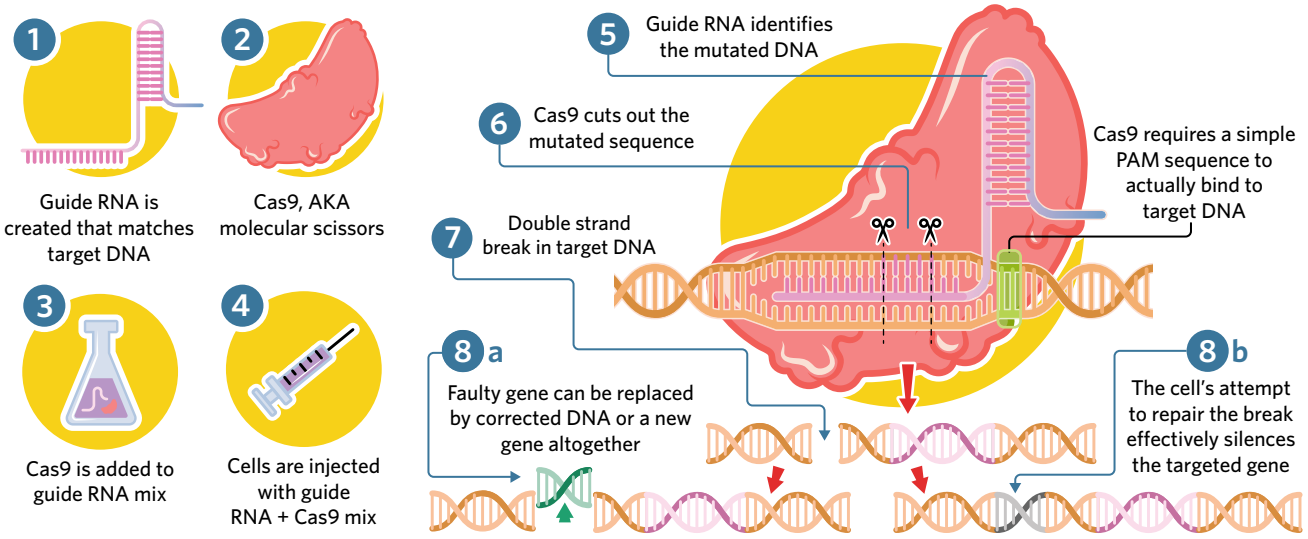


Figure 8 Steps in editing genomes using CRISPR-Cas9

Table 1 Applications of CRISPR-Cas9.

Uses	Examples
Research	• Attaching a fluorescent protein to Cas9 to locate a specific gene in the genome • Disrupting the expression of a gene to see the effect of that protein being knocked out. In turn, this helps scientists identify the function of specific genes
Dealing with diseases	• Replacing a deleterious allele with a healthy allele • Adding genes that code for proteins that decrease susceptibility to infectious diseases such as HIV/AIDS • Modifying cancer-promoting genes to make them less influential
Agriculture	• Introducing pest and herbicide-resistance genes to increase the yield of crops • Altering genes to promote increased growth rates to improve the yield of crops

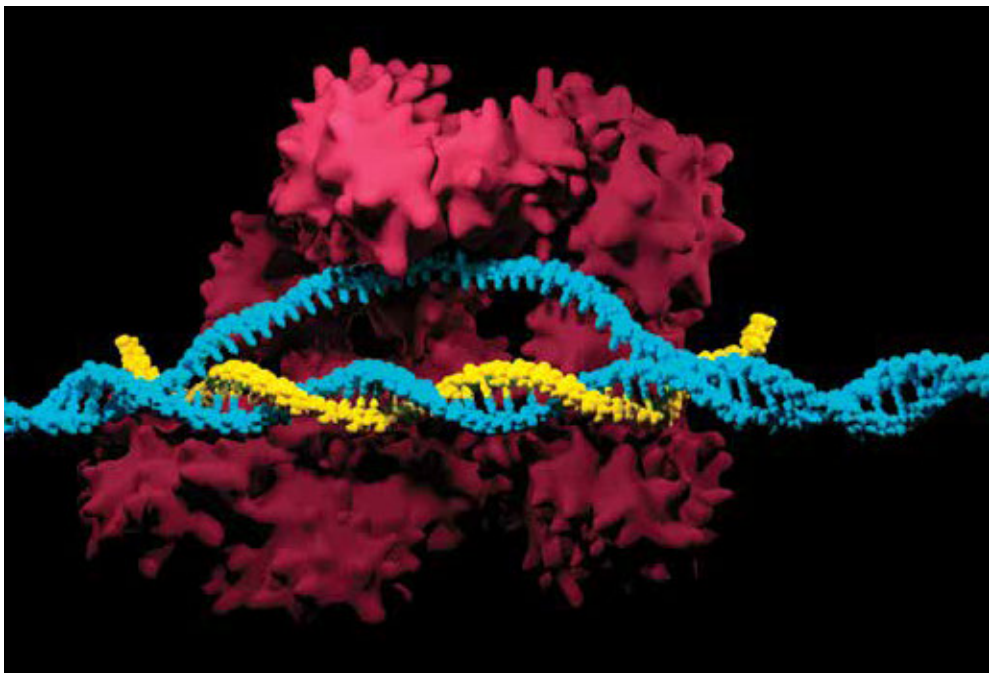


Image: Meletios Verras /Shutterstock.com

Figure 9 Visual representation of CRISPR-Cas9 finding the target site on a DNA molecule

Lesson link

You will learn more about genetic engineering in agriculture in ***lesson 4F*** and ***lesson 5D.***

Limitations of CRISPR-Cas9

Although CRISPR-Cas9 has great potential for use in medicine and research, there are many limitations to the technology. In animal studies, scientists have successfully eliminated genetic diseases such as muscular dystrophy in mice (Min, Bassel-Duby, & Olson, 2018). However, this success is yet to be seen in humans. This means that there is still quite a way to go before scientists can use CRISPR-Cas9 to reliably eliminate genetic diseases.

Additionally, CRISPR-Cas9 simply cuts DNA at a chosen site. To induce substitution mutations or **knock-in** a new segment of DNA, scientists must introduce the nucleotide sequence they wish to add into the cell and hope it is taken up by the DNA repair machinery. This can be difficult to achieve with precision and is not consistently successful.

One of the reasons why progress has been slow is because of the ethical implications of developing effective CRISPR-Cas9 technologies. To successfully alter an organism's genome using CRISPR-Cas9 technologies, scientists must treat an **embryo** prior to the cells **differentiating**, as this will ensure every cell in the organism is altered. Some groups are concerned that scientific research on embryos does not respect the sanctity of human life. In addition, it is currently illegal to implant genetically modified embryos into human females, and to allow the embryo to develop and be born. This law is in place partly to prevent unforeseen consequences or potential harm coming to pregnant women and unborn babies. You will learn more about the ethical considerations associated with genetic engineering in lesson 4F.

gene knock-in a technique in gene editing where scientists substitute or add nucleotides in a gene

embryo an early stage of development in an organism. In humans, used to refer to the organism during the first eight weeks of development

differentiation the process in which cells develop specialised characteristics, typically transforming them from one cell type to another more specialised cell type

In lesson 1B, you learned about the five bioethical concepts and three approaches to bioethics. Consideration of these can help you understand why some people might be in favour of or against the application of CRISPR-Cas9 technologies on humans or other living things.

For example, the bioethical concept of non-maleficence discourages causing harm wherever possible. According to this principle, an individual might oppose the law against the gestation of genetically modified embryos out of concern for unforeseen negative consequences on the pregnancy. On the other hand, imagine that a couple discovers their foetus has a debilitating genetic condition that could be fixed by CRISPR-Cas9 technology. The concept of non-maleficence could be used to argue for CRISPR-Cas9 in this case, as the application of this technology could reduce the disadvantage or pain faced by the child or parents.

Some other ethical concerns that could be analysed through the concepts and approaches include:

- Safety – the possibility of off-target cleavages (edits in the wrong place) and mosaics (some cells containing edited genomes, others not) mean that many scientists are hesitant to use CRISPR outside of research.
- Informed consent – scientists cannot get consent from embryos to edit their genes. If the embryo goes on to be born and one day has children of its own, these children also will never have consented to scientists interfering with their genome.
- Inequality – there is concern that only wealthy people will be able to afford to use CRISPR to treat genetic conditions or otherwise change their genes.
- Discrimination – CRISPR may be a threat to those who are judged by society as biologically inferior, when in fact those individuals do not feel they need 'fixing' at all.

Theory summary

- CRISPR refers to a series of short, palindromic, clustered repeats of DNA that are separated by spacer DNA. CRISPR is naturally found in prokaryotes, where it plays an important role in defending against bacteriophage invasions.
- CRISPR is transcribed into gRNA, which delivers Cas9 to a specific recognition site.
  - In bacteria, this recognition site is typically the genetic material of a virus that has invaded
  - In genetic engineering, gRNA is synthetic and complementary to a recognition site that researchers wish to genetically modify
- Cas9 is an enzyme with two active sites which cuts both strands of DNA to create a blunt end cut.
- CRISPR-Cas9 technologies are now being extensively studied, as they offer a precise and potentially affordable pathway to scientific breakthroughs, dealing with diseases, and improving agriculture.

Table 2 Comparison between CRISPR-Cas9 in prokaryotes and in artificial gene editing

	Naturally in prokaryotes	Artificially in gene editing
Purpose	To attack and destroy invading viral DNA	Induce mutations to alter genomic DNA
Production of gRNA	Naturally through the transcription and post-transcriptional modifications of the CRISPR gene	Synthetically produced in a laboratory
PAM sequence	Specific to each host organism	Cas9 enzyme can be altered to suit a specific gene
What happens after the cut	DNA repair mechanisms often induce a mutation that inhibits viral function	DNA can mutate to knock out, enhance, or otherwise change the function of genes

Before you start trying to shop online for a designer baby, it is important to remember that the current CRISPR-Cas9 technology has not yet come far enough to 'design the perfect baby'. It is important to remember the biological and ethical implications of CRISPR-Cas9 technology including whether there is equality of access to the technology and altering the evolutionary pathway of a species.

Fortunately for this baby, their parents did consider the ethical and social consequences of inserting the jazz gene into its genome, setting it up for a life of smooth and chill vibes.

Image: Mini MooN/Shutterstock.com

4B QUESTIONS

Theory review questions

Question 1

CRISPR describes

A enzymes that cut DNA.

B short, repeated, palindromic clusters of nucleotides.

Question 2

Fill in the blanks in the following sentences.

Cas9 stands for _____-associated protein 9 and has _____ active sites which create a _____ cut in DNA.

Question 3

When invading bacteriophage DNA is incorporated into the CRISPR sequence, it is introduced as a

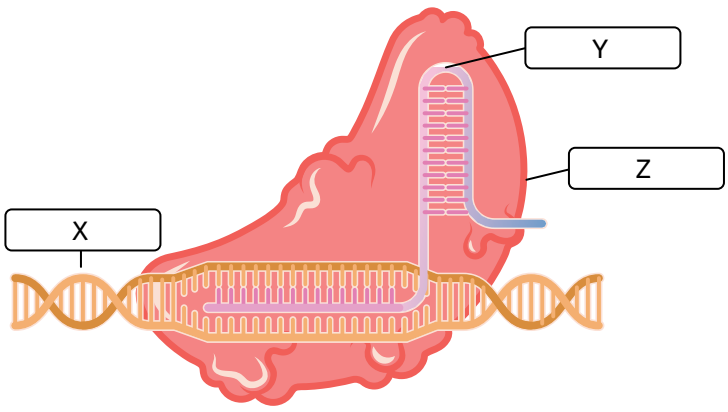
A spacer.

B repeat.

Question 4

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- Cas9
- gRNA
- CRISPR
- viral DNA
- bacteriophage



Question 5

What are the applications of CRISPR in eukaryotes? (*Select all that apply*)

I research

II agriculture

III genetic diseases

IV adaptive immunity against bacteriophages

Question 6

A protospacer adjacent motif is

A an enzyme used to cut DNA at a target site dictated by a gRNA molecule.

B a sequence of twenty nucleotides that guides Cas9 to a particular target sequence.

C a few nucleotides found in bacteriophages which signal to Cas1 and Cas2 to remove a sequence of DNA.

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 7–10.

He Jiankui is a Chinese scientist currently serving a three-year prison sentence for his participation in modifying two human embryos using CRISPR-Cas9 and implanting them into a surrogate mother. The two embryos were edited to remove the *CCR5* gene, which is the target site of HIV on white blood cells. After their birth, He released a video online announcing the twins' birth to the world. He explained what precautions he took, such as:

- sequencing the entire genome of both embryos prior to implantation to check for genetic changes outside of the *CCR5* gene, and again once each baby was born.
- regular blood tests and ultrasounds with the mother to check on the twins' wellbeing.

He reiterated that there were no other changes in the DNA except the one created by CRISPR-Cas9. He completed his research in secret in 2018 and did obtain consent from the parents, however it was later found that he did not provide sufficient information to obtain their informed consent. Ultimately, Jiankui was fined for "forging ethical review documents and misleading doctors into unknowingly implanting gene-edited embryos into a woman". Additionally, the World Health Organisation halted all human genetic engineering research until they had created a global registry to prevent gene editing from occurring in secret.

Question 7

How might a duty/rules-based approach to bioethics have informed the decision to sentence He to three years imprisonment?

A The Chinese judicial system wants to punish He for acting against the values of good science, including his failure to obtain informed consent, which is seen as deceptive and unethical.

B The Chinese judicial system recognises its responsibility to protect the community and deter other researchers from conducting unapproved scientific experiments in the future. In removing He from the community, the judicial system fulfils these obligations of protection and deterrence.

Question 8

Some critics of He's work argue that he failed to uphold the bioethical concept of respect. Which of the following options support this argument?

A He adequately obtained informed consent from all participants.

B He failed to properly disclose all information to the participating doctors.

C He failed to protect the welfare of the mother during pregnancy through regular testing.

D He ignored the personal autonomy and values of the doctors by misleading them into participating in his study.

Question 9

With reference to a consequences-based approach, how might a proponent of He's research defend the study?

A At the conclusion of the study, both children appear to be healthy and therefore CRISPR-Cas9 applications could have the potential to eliminate certain lethal diseases in humans in the future.

B At the conclusion of the study, it was proven that both children were healthy and that CRISPR-Cas9 is a simple and risk-free means of eliminating most heritable diseases.

C At the conclusion of the study, He and his team have fulfilled their responsibility to science by making significant scientific advancements despite a lack of transparency with their research participants.

Question 10

Currently, scientific investigations that edit embryos in this way require that the embryos be destroyed after the experiment is concluded instead of being implanted into the mother. Which of the following options best describes the biological implication underpinning this protocol?

A It is uncertain how best to commercialise gene editing technology and establish large-scale industry around this new infrastructure.

B It is uncertain how best to control the long-term genetic consequences of this technology and ensure our evolution as a species continues in a natural manner.

C It is uncertain how best to ensure the equitable use of this technology, including those members of lower socio-economic communities who might not have equal access to gene therapies.

Exam-style questions

Within lesson

Question 11 (1 MARK)

Which one of the following is a correct statement?

A CRISPR-Cas9 introduces a mutation into a specific gene.

B CRISPR-Cas9 can disrupt the expression of a chromosome.

C Cas9 is responsible for guiding the gRNA to a specific site to cut.

D Bacteriophage DNA is incorporated as spacers in the CRISPR sequence.

Question 12 (1 MARK)

CRISPR is naturally found in

A bacteriophages.

B prokaryotes.

C eukaryotes.

D viruses.

Multiple lessons

Question 13 (1 MARK)

Which of the following correctly describes how CRISPR-Cas9 changes DNA?

A cutting DNA up to create a new chromosome

B substituting nucleotides by cutting and ligating the sugar-phosphate backbone

C by cutting an entire gene out of a cell and replacing it with an entirely new gene

D making a cut in the DNA and relying on DNA repair machinery to introduce a mutation

Question 14 (11 MARKS)

CRISPR-Cas9 is a gene editing technique that is one of the most accurate and affordable technologies available. It operates through guide RNA (gRNA) that leads Cas9 to a specific sequence in target DNA, then Cas9 cuts both strands.

a Identify the components that make up a monomer of gRNA. (1 MARK)

b Explain the effect temperature would have on the rate of a Cas9 reaction. (2 MARKS)

c CRISPR-Cas9 is naturally occurring in prokaryotic organisms. What is the purpose of this machinery in prokaryotes? (1 MARK)

d Cas9 is an endonuclease.

i What is meant by the term 'restriction endonuclease'? (1 MARK)

ii Suggest an advantage of using Cas9 instead of a restriction endonuclease. (2 MARKS)

e Non-coding DNA, or introns, are a key area of future scientific research, as they are now understood to play an important role in gene regulation.

i Introns are spliced out during post-transcriptional modifications. How can this splicing event give rise to different functioning proteins? (2 MARKS)

ii Describe one example of how CRISPR-Cas9 could be used in understanding the function of introns. (2 MARKS)

Key science skills and ethical understanding

Question 15 (6 MARKS)

Cystic fibrosis is an inherited disease involving a mutation in the *CFTR* gene causing the CFTR protein channel to malfunction. When working properly, CFTR controls the facilitated diffusion of chloride across membranes. When it doesn't work, CFTR is unable to help move chloride to the cell surface. As the average life expectancy for a person born with cystic fibrosis is around 40 years old, many scientists are researching ways to improve the quality and length of life of individuals with cystic fibrosis.

Scientists studied mutated alleles and determined the most common mutation was the deletion of three nucleotides, causing the loss of a phenylalanine amino acid. They created the following method to attempt to correct an embryos' DNA to fix the *CFTR* gene.

1. Extract ova and fertilise with father's sperm, then sequence the one-cell zygote.
2. Inject the single-celled zygote with Cas9 and synthetic gRNA that is complementary to the deletion mutation.
3. Observe as the cells multiply *ex vivo* then sequence their entire genome to detect any unintentional mutations.
4. Once complete, the sample is either deemed successful or unsuccessful and the embryo is destroyed.

a Why might an ethics board advise that this experiment has a small sample size? (1 MARK)

b Explain why the embryos should not be implanted into a surrogate mother after this experiment. (1 MARK)

c Suggest two reasons why this experiment was performed *ex vivo* on embryos rather than *in vivo* on adults. (2 MARKS)

d Justify whether this method is collecting quantitative or qualitative data and explain how this data is advantageous. (2 MARKS)

4C THE POLYMERASE CHAIN REACTION

Breaking news! Edward Rola has been found dead, with multiple gunshot wounds. Forensic detectives found very little evidence, however, right as they were about to leave the scene, they found a single hair. But how could one tiny piece of hair be the key to solving the case?

Image: felipe caparros/Shutterstock.com

Lesson 4C

In this lesson you will learn the purpose and process of the polymerase chain reaction.

◀ *Prerequisite knowledge*

Lesson 2C
Taq polymerase acts similarly to RNA polymerase, except that it synthesises a DNA strand rather than a strand of RNA.

Lesson 3A
Enzymes are organic catalysts that speed up chemical reactions. *Taq* polymerase is an enzyme that functions optimally at 72 °C.

Lesson 4A
PCR amplifies small samples of DNA using a polymerase enzyme.

Future applications ▶

Lesson 4D
Often PCR is performed before gel electrophoresis, to increase the amount of DNA available.

Lesson 10A
PCR can be used to amplify a DNA sample from some fossils, such as from teeth or bones, to provide an insight into the relatedness between organisms.

Study design dot point

- amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling

Key knowledge units

Purpose of the polymerase chain reaction	3.1.10.1
Process of the polymerase chain reaction	3.1.10.2

Purpose of the polymerase chain reaction 3.1.10.1

OVERVIEW

The polymerase chain reaction amplifies a sample of DNA by creating additional copies. It is a multistep process that involves thermal cycling.

THEORY DETAILS

The **polymerase chain reaction (PCR)** is a DNA manipulation technique that **amplifies** DNA by making multiple identical copies. It is used by scientists whenever there is an insufficient amount of a DNA sample for testing. After undergoing the polymerase chain reaction, scientists can run further analyses on the DNA such as:

- paternity testing
- forensic testing samples of bodily fluids
- analysing gene fragments for genetic diseases.

polymerase chain reaction (PCR) a laboratory technique used to produce many identical copies of DNA from a small initial sample

amplify to increase the quantity of a molecule by making many copies

When undertaking a polymerase chain reaction cycle, scientists do not usually copy the entire genome. Instead, they focus on certain genes through the use of **primers** or **restriction endonucleases** to make the process more efficient. After each cycle of the polymerase chain reaction, the amount of DNA present is doubled (Table 1).

primer a short, single strand of nucleic acids that acts as a starting point for polymerase enzymes to attach

restriction endonuclease any enzyme that acts like molecular scissors to cut nucleic acid strands at specific recognition sites. Also known as a **restriction enzyme**

Table 1 The number of double-stranded DNA molecules per PCR cycle. You can determine the number of double-stranded DNA molecules formed through the formula $x = 2^n$.

Number of cycles (n)	Number of double-stranded DNA molecules (x)
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1024

Examiners' tip

The VCAA has not stated that PCR is an approved abbreviation, therefore to use it in your responses, first write it out completely, then in brackets write the abbreviation – e.g. 'polymerase chain reaction (PCR)'.

Process of the polymerase chain reaction 3.1.10.2

OVERVIEW

The process of the polymerase chain reaction involves manipulating temperatures to cause denaturation and annealing in a four-step process.

THEORY DETAILS

The polymerase chain reaction requires the following materials to take place:

- a DNA sample that subsequently gets **denatured** and amplified through the polymerase chain reaction
- ***Taq* polymerase** is required in the **elongation** stage to bind complementary nucleotides to the single-stranded DNA
- nucleotide bases must be constantly available for *Taq* polymerase to create a new strand that is complementary to the single-stranded DNA
- sequence-specific DNA primers join to the 3' end of single-stranded DNA by complementary base pairing to form the first segment of double-stranded DNA, allowing *Taq* polymerase to attach and begin extending the DNA strand.

denature the disruption of a molecule's structure by an external factor such as heat

***Taq* polymerase** a heat-resistant DNA polymerase enzyme isolated from the bacteria *Thermus aquaticus*, which amplifies a single-stranded DNA molecule by attaching complementary nucleotides

elongate to synthesise a longer polynucleotide

To begin the polymerase chain reaction, a mixture of the above materials is placed into a **thermal cycler** (Figure 1), where it undergoes the following processes:

1. Denaturation – DNA is heated to approximately 90–95 °C to break the hydrogen bonds between the bases and separate the strands, forming single-stranded DNA.
2. **Annealing** – the single-stranded DNA is cooled to approximately 50–55 °C to allow the primers to bind to complementary sequences on the single-stranded DNA.
3. Elongation – the DNA is heated again to 72 °C, which allows *Taq* polymerase to work optimally. *Taq* polymerase binds to the primer, which acts as a starting point, and begins synthesising a new complementary strand of DNA.
4. Repeat – the cycle (steps 1–3) is repeated multiple times to create more copies of DNA.

thermal cycler a laboratory apparatus which alters the temperature in pre-programmed steps for temperature-sensitive reactions like PCR

anneal the joining of two molecules, for example two complementary DNA strands during the cooling phase of PCR

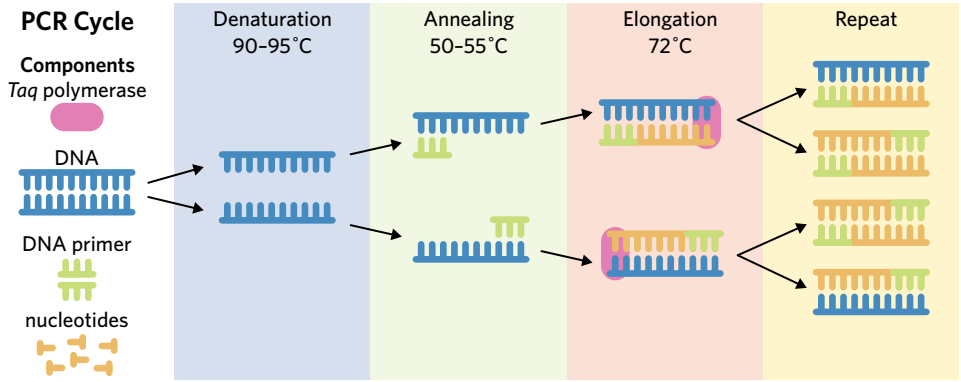


Figure 1 The polymerase chain reaction is a four-step process.

> **Examiners' tip**
>
> When answering questions about the steps in the polymerase chain reaction, you can state the temperature ranges or specific temperatures within these ranges. However, you must specifically state the elongation stage occurs at 72 °C as this is the optimal temperature for *Taq* polymerase.

Forward and reverse primers

In the polymerase chain reaction, there are two different DNA primers needed. This is because, during denaturation, the double-stranded DNA molecule has been separated into two single-strands – the template strand and the coding strand. The two primers are:

- the **forward primer**, which will bind to the start codon at the 3' end of the template strand. This causes *Taq* polymerase to synthesise a new DNA strand in the same direction that RNA polymerase would function.
- the **reverse primer**, which will bind to the stop codon at the 3' end of the coding strand. This causes *Taq* polymerase to synthesise a new DNA strand in the reverse direction that RNA polymerase would function.

Ultimately, having these two primers is necessary as the 5' ends of both the template and coding strands are different. As *Taq* polymerase only functions towards the 3' end, a primer is needed for both strands to facilitate this directionality (Figure 3).

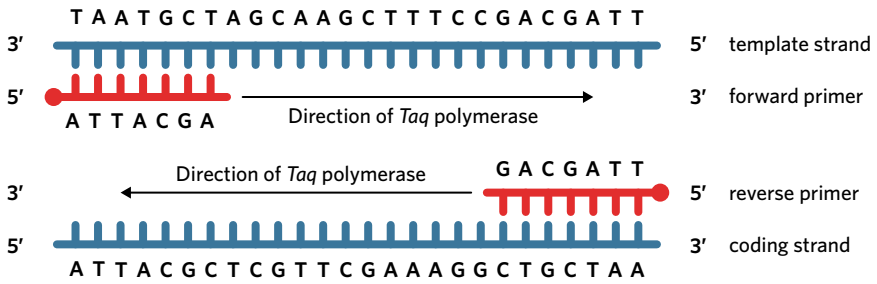


Figure 3 Comparison between forward and reverse primers for a gene

Theory summary

The polymerase chain reaction is used to amplify a sample of DNA. It requires a DNA sample, *Taq* polymerase, additional nucleotides bases, and forward and reverse primers. It operates by (1) denaturing the DNA, (2) annealing the primers, (3) elongating the new strands, and (4) repeating the process to make more copies.

Lucky for the detectives, the hair follicle at the base of the hair is coated in cells that contain DNA. This means scientists can amplify the DNA using the polymerase chain reaction to analyse it and find the perpetrator.

> **Lesson link**
>
> In ***lesson 4A***, you learned that DNA polymerase can be used to synthesise new strands of DNA. *Taq* polymerase is an example of DNA polymerase and is used specifically during the polymerase reaction due to it's high heat resistance. In comparison, human DNA polymerase would be unsuitable due to its significantly lower heat resistance – having an optimum around body temperature (37 °C).

Image: Vit Kovalcik/Shutterstock.com

Figure 2 A PCR thermal cycler. These are highly valuable instruments for researchers – for example, a standard sequence of 30 PCR cycles can create over one billion copies (2^{30}).

forward primer a DNA primer that binds to the 3' end of the template strand and reads the DNA in the same direction as RNA polymerase

reverse primer a DNA primer that binds to the 3' end of the coding strand and reads the DNA in the reverse direction to RNA polymerase

4C QUESTIONS

Theory review questions

Question 1

The polymerase chain reaction

A amplifies DNA.

B cuts DNA.

Question 2

Fill in the blanks in the following sentence.

The first step of the polymerase chain reaction is the _____ stage which occurs between 90–95 °C. The second step is the annealing stage which occurs between _____–_____ °C. The third step is the _____ stage which occurs at _____ °C.

Question 3

Starting with one fragment of DNA, after four polymerase chain reaction cycles, how many fragments would be produced?

A 4

B 8

C 12

D 16

Question 4

Which of the following is not required in the mixture of the polymerase chain reaction?

A DNA

B primers

C promoters

D nucleotide bases

Question 5

Order the steps to correctly describe the polymerase chain reaction.

I The cycle is repeated to produce more copies of the DNA.

II DNA is heated to 90–95 °C to break the hydrogen bonds between strands.

III *Taq* polymerase begins synthesising when the temperature reaches 72 °C.

IV DNA is cooled to 50–55 °C which allows the primers to anneal to the DNA strands.

SAC skills questions

Case study analysis

Use the following information to answer Questions 6–9.

During the COVID-19 pandemic, polymerase chain reaction (PCR) testing was crucial in diagnosing individuals. The test was undertaken by collecting a respiratory swab collected in the nose which was then sealed and delivered to the laboratory. From there, genetic material was extracted from the sample, including nuclear DNA from cells and SARS-CoV-2 RNA if the virus was present.

Since SARS-CoV-2 possesses RNA instead of DNA, an enzyme known as reverse transcriptase is used to convert SARS-CoV-2 RNA into DNA to allow PCR to occur. The DNA is then passed through a PCR machine with the standard materials in with the sample, with the addition of a fluorescent probe. The fluorescent probe binds to specific sequences on SARS-CoV-2 and emits light which can be detected from laboratory equipment, and thus, can diagnose an individual with COVID-19.

Question 6

What is the role of reverse transcriptase?

A To transcribe an RNA strand and produce a complementary DNA strand.

B To transcribe a DNA strand and produce a complementary RNA strand.

Question 7

If the fluorescent probe emits light when it binds to the RNA, why must the sample be run through PCR?

A PCR amplifies the volume of SARS-CoV-2 RNA to increase the chance of the probe binding to the sample.

B PCR is responsible for extracting SARS-CoV-2 RNA from the sample collected.

C PCR is required to purify the sample of genetic material collected.

Question 8

Why must the respiratory swab be sealed after collection?

A It prevents SARS-CoV-2 from replicating as there is no oxygen available.

B It prevents contamination from other microbes.

Question 9

The purpose of the type of PCR test described in the scenario is to

A amplify eukaryotic DNA.

B detect SARS-CoV-2 in adults only.

C detect individuals who are currently infected with SARS-CoV-2.

Exam-style questions

Within lesson

Question 10 (1 MARK)

The process known as the polymerase chain reaction (PCR) involves repeated cycles made up of several steps.

During PCR, the

A first step of each cycle involves heating the sample to 72 °C.

B second step of each cycle involves cooling the sample to 37 °C.

C third step of each cycle involves measuring the melting temperature of each sample.

D third step of each cycle involves *Taq* polymerase synthesising a complementary strand.

Question 11 (1 MARK)

In the polymerase chain reaction, primers are required to allow *Taq* polymerase to begin copying. Which of the following outlines why two different types of primers are necessary?

A The nucleotide sequence is different at the 5′ ends of the two DNA strands that are copied.

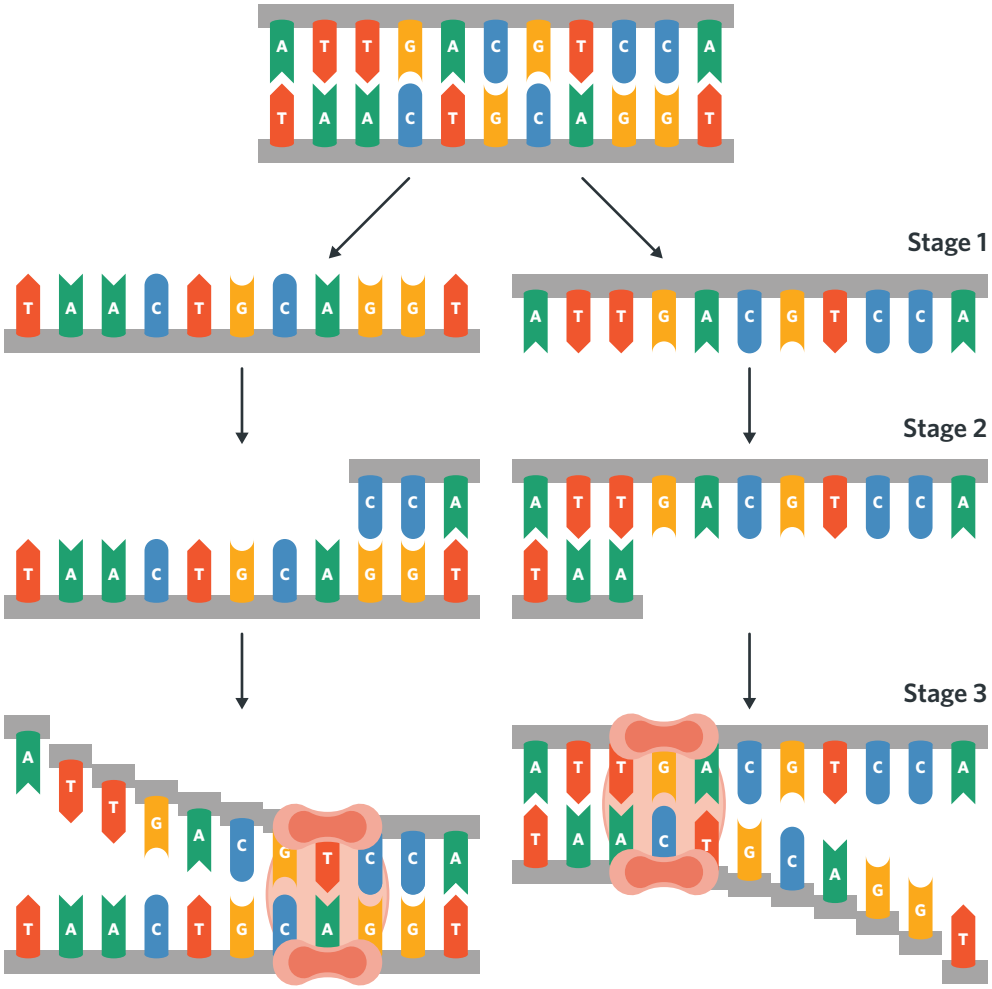
B Two primers help to show the relatedness between strands of DNA.

C The two primers are identical and bind the 5′ end of each strand.

D *Taq* polymerase requires both a start and stop primer.

Question 12 (6 MARKS)

The following diagram represents the polymerase chain reaction.



a Explain the purpose of the polymerase chain reaction. (1 MARK)

b Outline the process of Stage 1. (1 MARK)

c Explain the role of the small sections of DNA added in Stage 2. (2 MARKS)

d Justify why *Taq* polymerase must be used instead of human DNA polymerase in Stage 3. (2 MARKS)

Multiple lessons

Question 13 (5 MARKS)

The following diagram represents a DNA molecule and the positions of the recognition sites for the restriction endonucleases BamHI, EcoRI, HaeIII, and SalI.

a Explain the role of a restriction endonuclease. (1 MARK)

b EcoRI has an optimal temperature of 37 °C. Describe the effect that increasing and decreasing the temperature would have on enzyme function. (2 MARKS)

c Identify an enzyme used in a DNA manipulation technique that has an optimal temperature of 72 °C. (1 MARK)

d State one other factor which scientists must consider for EcoRI to function optimally. (1 MARK)

Adapted from VCAA 2017 Section A Q38

Key science skills and ethical understanding

Question 14 (11 MARKS)

Mohammad and Rashida are forensic scientists investigating a crime scene. They have found a small amount of DNA and amplified it. The DNA amplification mixture was made up of the specific DNA segment they found, a plentiful supply of four nucleotide bases, *Taq* polymerase, and DNA primers.

a The amplification of DNA is a four-step process.

i Name the process to amplify DNA. (1 MARK)

ii Describe the steps of this technique. (3 MARKS)

b Mohammad and Rashida completed the process separately. The table outlines the temperatures they used in each step.

Step	Mohammad	Rashida
1	37 °C	94 °C
2	55 °C	55 °C
3	72 °C	72 °C

Mohammad and Rashida compared their results of their DNA amplification and only one of them was successful.

i Identify whose method was unsuccessful. Justify your response. (1 MARK)

ii The temperature difference occurred because of faulty calibration of the thermal cycler. Given this information, identify the type of measurement error that has occurred. (1 MARK)

iii Describe how this error could be avoided in the future. (1 MARK)

c Rashida was teaching this process to Diego, an eager Year 12 student. Diego did not understand why two different DNA primers were necessary. Explain the role played by primers and why it is necessary to have two different primers. (3 MARKS)

d Out of embarrassment, Mohammad attempted to cover up his mistakes from his colleagues. Which bioethical concept did Mohammad contradict? (1 MARK)

4D GEL ELECTROPHORESIS

Welcome back to the chronicles of the murder of Edward Rola. We have obtained a sample of DNA from hair found at the scene of the crime and we have amplified it using the polymerase chain reaction... now what? Well, the detectives have taken five suspects in for questioning but want to know how they can test to see if any of the suspects' DNA matches the DNA from the hair.

Image: Skocko/Shutterstock.com

Lesson 4D

In this lesson you will learn how to separate pieces of DNA using gel electrophoresis and how to interpret the data from this process.

◀ *Prerequisite knowledge*

Lesson 4C

To run gel electrophoresis, you must first complete the polymerase chain reaction to ensure you have enough DNA fragments for bands to form.

Future applications ▶

Lesson 9A

Genetic testing uses gel electrophoresis to determine whether a mutation is present in an individual's DNA.

Study design dot point

- amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling

Key knowledge units

The process of gel electrophoresis	3.1.10.3
Interpreting gels	3.1.10.4
Applications of polymerase chain reaction and gel electrophoresis	3.1.10.5

The process of gel electrophoresis 3.1.10.3

OVERVIEW

Gel electrophoresis allows you to take DNA fragments prepared using restriction endonucleases or polymerase chain reaction and separate them based on their size.

THEORY DETAILS

Gel electrophoresis is a laboratory technique used by scientists to measure the size of DNA fragments. It is typically used after a sample of DNA has been cut up using restriction endonucleases or after a short sequence of DNA has been amplified using the polymerase chain reaction.

The process of gel electrophoresis is described in the following steps (Figure 1):

1 The DNA samples are placed in the **wells** at one end of the gel using a micropipette. A **standard ladder** of DNA fragments with known sizes is also typically loaded into one well. This is required for estimating the size of any unknown DNA fragments by comparing them to the known fragments in the standard ladder. The gel is made of **agarose**, a sponge-like jelly that is filled with tiny pores to allow movement of the DNA fragments. This agarose gel is immersed in a **buffer** solution which helps carry an electric current.

gel electrophoresis a technique that separates DNA fragments based on their molecular size

well an indent in the gel into which a DNA sample is loaded

standard ladder a mixture of DNA fragments of known length that are used to infer the size of fragments in a sample

agarose gel a sponge-like gel used in gel electrophoresis that contains pores for DNA fragments to move through

buffer an ion-rich solution that carries electrical current through the agarose gel

2 An electric current is passed through the gel using two **electrodes** – one positive, one negative. The negative electrode is positioned near the wells and the positive electrode is at the opposite end of the gel. Since DNA is negatively charged due to the phosphate backbone, it is attracted to the positive electrode. When the electrical current is applied, DNA fragments will move from the wells, through the tiny pores in the agarose gel, towards the positive electrode.

3 Smaller DNA fragments move faster through the gel and so travel further than larger fragments, which don't move as easily through the pores in the agarose. After a few hours, the current is switched off and the DNA fragments stop moving in the gel and settle into **bands**. The DNA fragments are now separated based on size.

4 DNA is difficult to see with the naked eye so the gel is stained with a fluorescent dye such as **ethidium bromide**, allowing the bands of DNA to be visualised under an ultraviolet (UV) lamp. This dye can be included in the gel before the experiment or applied after.

electrode conductors of electricity that are attached to both ends of a gel allowing an electrical current to pass through it

band a line seen in the gel after running gel electrophoresis that corresponds to a collection of DNA fragments of a specific size

ethidium bromide a fluorescent dye that binds to DNA fragments in a gel and allows them to be easily visualised under ultraviolet light

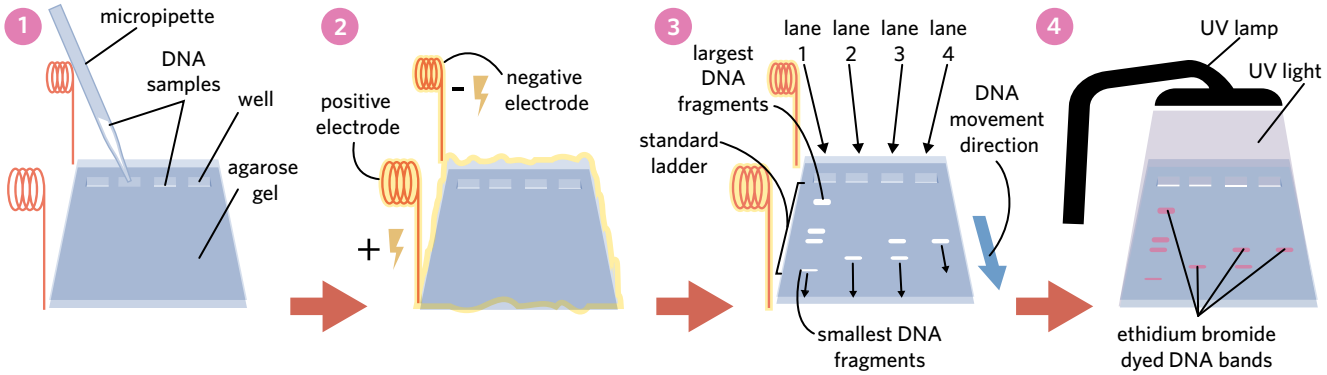


Figure 1 The process of gel electrophoresis

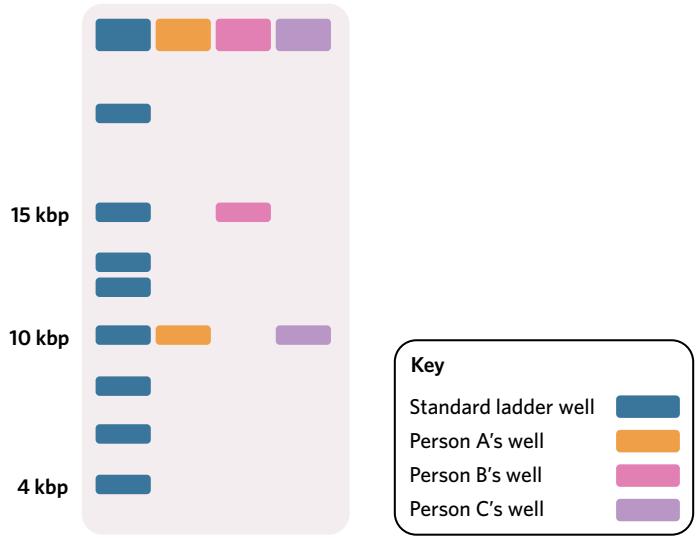


Figure 2 An example of a gel comparing the eye colour alleles of persons A, B, and C against a standard ladder

When gel electrophoresis separates DNA fragments based on size, long fragments of DNA collect in bands of DNA near the well, while shorter fragments form bands further from the well. So, if person A's allele for eye colour is 10 **kb** long, and person B's allele for eye colour is 15 kb long, person A's band will move further from the well (Figure 2). If they shared the same allele (e.g. person A and C), the bands would end up at the same distance from the well.

kilobase (kb) a unit of measurement that corresponds to one thousand nucleotides. May also be written as kbp

It's important to note that each band in the gel actually contains thousands of fragments of DNA, all of the same molecular size. The DNA sample loaded into the well is usually a mixture of different DNA fragments. This allows bands corresponding to a particular size of DNA to be isolated and cut out of the gel for use in other experiments (Figure 3).

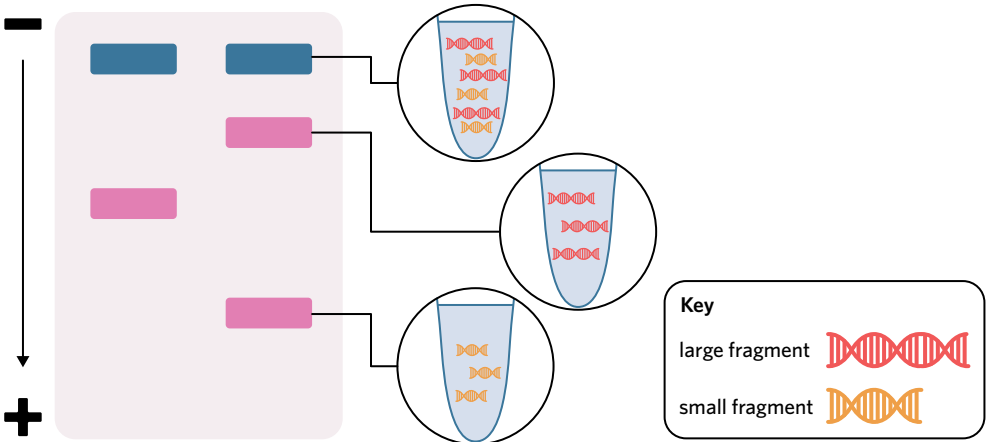


Figure 3 A closer look at how fragments separate in gel

Memory device

What would run through a dense forest faster: an elephant or a fox? While an elephant could plough through, it would be hindered by thick brush and densely packed trees. A fox, however, could squeeze between narrow gaps and dodge obstacles. The situation is the same for small and large DNA fragments in gel electrophoresis. The small fragments can slip through the pores in the agar gel with ease, while the large fragments get tangled up so travel less distance in the same amount of time.

Interpreting gels 3.1.10.4

OVERVIEW

Gel electrophoresis is a fundamental laboratory technique and being able to interpret these gels is essential for VCE Biology. Here you will learn how to interpret the results of a gel electrophoresis experiment to determine the size of DNA fragments. You will also look at genotyping as a practical application of gel electrophoresis.

THEORY DETAILS

A standard ladder contains a number of different DNA fragments with a known molecular size. Molecular size indicates the length of a nucleic acid sequence and is measured in **base pairs** (bp) or kilobases (kb).

base pair (bp) a unit of measurement that corresponds to one nucleotide

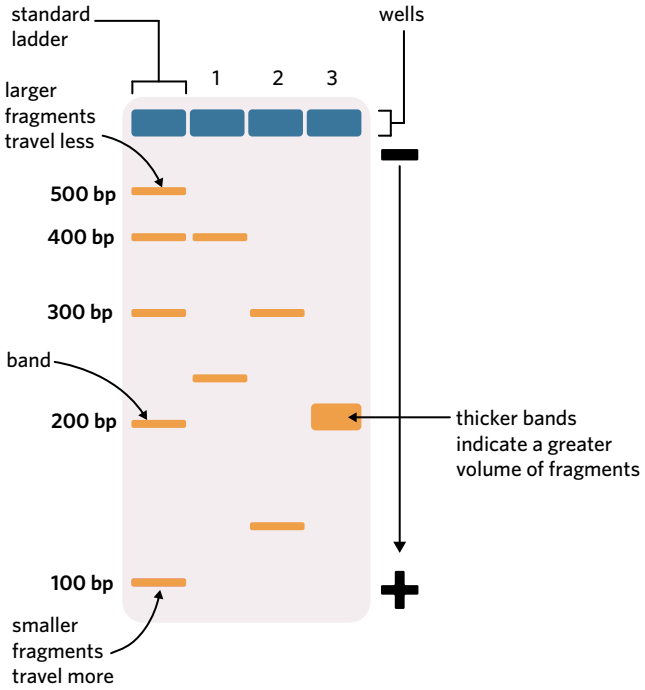


Figure 4 Features to note when interpreting a gel

In Figure 4, the standard ladder contains five fragments that are known to be 100, 200, 300, 400, and 500 bp. By comparing the other bands in the gel to the standard ladder we can estimate their molecular size. The single band in **lane** 3 'lines up' with the 200 bp band in the standard ladder. This indicates that the DNA fragments in this band are 200 nucleotides long. Since it is the only band present in this lane, we can also conclude that this is the only DNA fragment size that was initially present in the sample loaded into the lane 3 well. You may notice the band in lane 3 is also thicker than the bands in the other lanes. This means that a particular band contains more DNA than the other bands.

lane the column of the gel corresponding to each sample of DNA

Lanes 1 and 2 both contain two bands of DNA. Each band represents fragments of a particular size that were present in that DNA sample. The molecular sizes of the DNA in the two bands in lane 1 are approximately 250 bp and 400 bp and the two bands in lane 2 are roughly 140 bp and 300 bp.

Standard ladders are vital because DNA fragments of the same size don't always travel the same distance. Every gel type is different, and inconsistent experimental conditions will influence the distance moved by DNA fragments. For example, in one gel a 100 bp fragment may travel 7.8 cm whilst in another gel it may travel 8.6 cm, so distance travelled in a gel can't be used to directly measure molecular size. These variations are due to factors such as:

- voltage – the stronger the electric force generated by the electrodes the further DNA travels towards the positive electrode
- gel composition – gels with a greater density and agarose concentration increase the difficulty for larger fragments to move through
- buffer concentration – the greater the concentration of ions in the buffer the more the electric current is conducted through the gel, which causes DNA to move further down the lane
- time – the longer the electric current is applied, the further the DNA will travel. Note: if too much time passes, the DNA may move out of the gel.

Applications of polymerase chain reaction and gel electrophoresis 3.1.10.5

OVERVIEW

The polymerase chain reaction (Lesson 4C) and gel electrophoresis can be used for many purposes, such as the diagnosis of genetic disorders by microbiologists or the identification of a suspect in a crime by a forensic scientist.

THEORY DETAILS

You have learned how a gel is run and how to interpret its results – now you will look at how these techniques are used in practice. To understand this, you will look at how the polymerase chain reaction and gel electrophoresis can be used in **genetic testing** and **DNA profiling**.

genetic testing screening an individual's DNA for anomalies that may make them susceptible to a particular disease or disorder

DNA profiling the process of identification on the basis of an individual's genetic information

Genetic testing using gel electrophoresis

Genetic disorders occur when individuals possess mutated alleles which prevent parts of the body and its cells from functioning the way they should. These mutations can be as small as a change of a single nucleotide. For example, cystic fibrosis is a recessive genetic disorder that commonly involves the deletion of just three nucleotides on the *CFTR* gene which results in mucus in organs like the lungs becoming thick and sticky. This can cause difficulty breathing and ultimately reduces an individual's lifespan.

> ***Theory in action***
>
> Check out scientific investigation 4.1 to put this into action!

Cystic fibrosis can be diagnosed using a combination of the polymerase chain reaction and gel electrophoresis. In Australia, at birth, a routine test called a heel prick test is performed in which a blood sample is obtained from the heel of a newborn baby, extracting their genetic material. The sample undergoes the polymerase chain reaction using specific DNA primers that attach to either side of the *CFTR* gene mutation. The primers must be complementary to a section of the *CFTR* gene on either side of the mutation so that the sample will only include this part of the gene instead of the entire genome.

After amplifying the DNA sample at the *CFTR* mutation site, it can then be loaded into the wells of an agarose gel. However, for this test, we require three additional sample lanes: the standard ladder to help identify the size of the fragments, a sample of a known healthy *CFTR* gene (negative control), and a sample of a mutated *CFTR* gene (positive control).

It is important to remember humans have two copies of every gene since we are diploid organisms. This means that an individual could have two normal *CFTR* alleles (**homozygous** dominant), two mutated *CFTR* alleles (homozygous recessive), or one normal and one mutated *CFTR* allele (**heterozygous**). As this is a recessive disorder, affected individuals must have two copies of the mutated allele to develop cystic fibrosis.

homozygous having identical alleles for the same gene on homologous chromosomes

heterozygous having different alleles for the same gene on homologous chromosomes

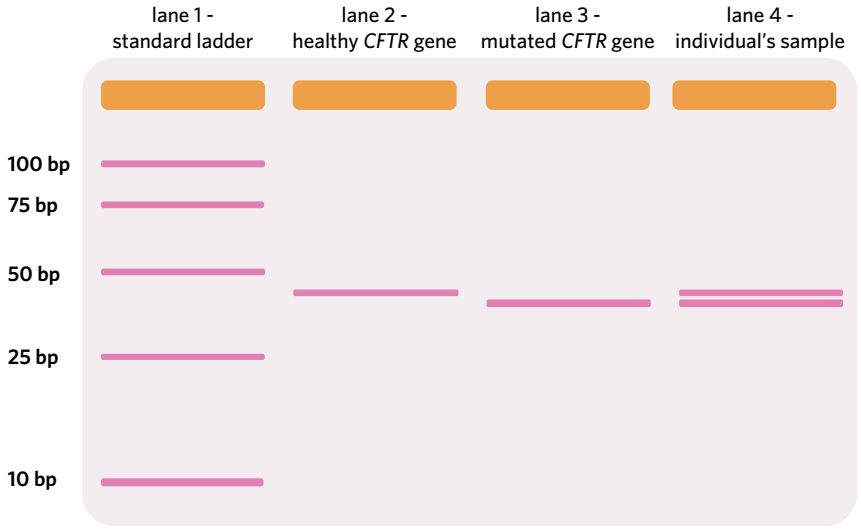


Figure 5 Example of gel electrophoresis used in *CFTR* genetic testing

What can be inferred from Figure 5 is that the isolated healthy *CFTR* gene is approximately 40 bp in length when compared to the standard ladder. Additionally, the individual in lane 4 is heterozygous as they have DNA which lines up with both the healthy and mutated allele, which means they would not suffer from cystic fibrosis as it is a homozygous recessive disorder.

DNA profiling

Imagine that you are an officer of the law who is investigating a murder. The crime scene is covered with blood and fingerprints, which you believe belong to the perpetrator. Before the recent advances in DNA technologies, you would be forced to utilise outdated and decidedly un-cool non-biological investigative techniques such as: following leads, talking to witnesses, or manually matching fingerprints. Nowadays, we can extract minute amounts of DNA from samples obtained at crime scenes and compare them to the DNA of any suspects. Whilst we cannot construct a DNA profile with only trace amounts of DNA, we can amplify DNA samples using the polymerase chain reaction until we have a larger total amount of testable genetic material.

short tandem repeats (STR) short, repeated sequences of nucleotides found in the non-coding regions of nuclear DNA

Even if we could map the entire genome from this material to identify a DNA sample, this method is both costly and time-consuming. Instead, it is currently much easier to analyse **short tandem repeats** (STRs) in a piece of DNA. STRs are small sections of repeated nucleotides that vary in length between people and are found in the non-coding areas of autosomal chromosomes. Because they are found in non-coding regions, they are not affected by natural selection, and many hundreds of variant STRs can be found in the DNA of each person due to their higher mutation rate than other areas of DNA. If the STRs in two samples of DNA match, we can say with confidence that the two pieces of DNA belong to the same person.

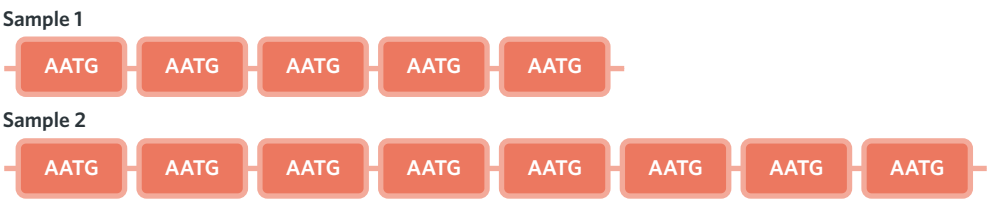


Figure 6 Two variants of the common TPOX STR. Sample 1 repeats the AATG nucleotide sequence five times while sample 2 repeats eight times, so it is unlikely the two belong to the same individual.

Using DNA profiling, we can also discover how related two people are. This is particularly useful in parental testing when the identity of one of the parents is not known. Because many of the STRs that we use are found exclusively on autosomal chromosomes, the child must inherit half of their STRs from each one of their parents. In addition to identifying criminals and parental testing, DNA profiling has been used to identify dead bodies, match potential organ donors, and find lost relatives.

One of the ways scientists construct a DNA profile is by using gel electrophoresis. Scientists perform the polymerase chain reaction and run a gel focusing on particular STRs, where each variant of an STR will separate according to size. If the individual is heterozygous for an STR their gel will have two bands, whereas if they are homozygous their gel will only have one thick band (Figure 7). In this way, scientists can accurately and efficiently match two samples of DNA.

Figure 7 Gel electrophoresis results of CSF1PO, TPOX, and THO1 STRs using DNA samples from a victim, crime scene, and four other individuals

Table 1 The DNA profiles of a victim, crime scene, and four other individuals using short tandem repeat (STR) analysis. Numbers indicate the number of repeats.

	victim	crime scene	1	2	3	4
CSF1PO	7, 13	7, 12, 12, 13	10, 14	12, 12	10, 14	8, 10
TPOX	12, 12	6, 7, 12, 12	10, 12	6, 7	10, 12	12, 12
THO1	5, 11	5, 9, 11, 13	7, 7	9, 13	7, 7	7, 11

From these results, we can conclude that:

- DNA from the victim and individual 2 were found at the crime scene
- DNA from individuals 1 and 3 match, so they are likely either from the same person or identical twins
- DNA from individuals 3 and 4 share at least one STR for every locus, and would likely be related by a parent-child or sibling relationship.

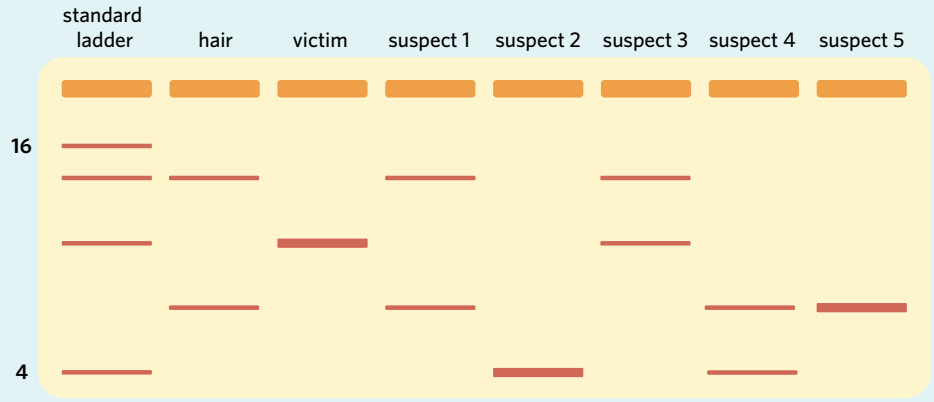
Theory summary

Gel electrophoresis uses electricity to separate pieces of DNA based on size. Separating DNA fragments is useful as it allows you to determine:

- how many different sizes of DNA fragments are in your sample
- the size of each fragment in your sample.

These two simple things can be used in a number of ways by scientists to gather and interpret genetic information.

Lucky you were there to tell the detectives to use gel electrophoresis to analyse the short tandem repeats present in the hair – otherwise the five suspects might have gotten away! From our gel, we have strong evidence to convict suspect 1 of Edward Rola's murder.



4D QUESTIONS

Theory review questions

Question 1

During gel electrophoresis, which type of fragments would travel further from the wells?

A larger fragments
B smaller fragments

Question 2

Prior to completing gel electrophoresis, which process must be completed to increase the amount of DNA in a sample?

A CRISPR
B endonuclease
C polymerase chain reaction

Question 3

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

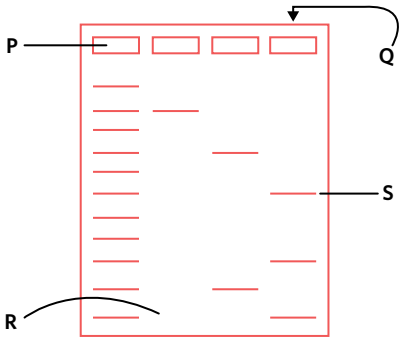
- UV
- sun
- buffer
- positive
- agarose
- negative
- ethidium bromide

The gel in gel electrophoresis is made of _____ that is immersed in a _____ solution. The _____ electrode is always closest to the wells in the gel since DNA has a _____ charge. The DNA is stained with _____ to allow the bands to be visualised under _____ light.

Question 4

Label the parts of this gel from the list of terms.

- well
- lane
- band
- piece
- loader
- agarose



SAC skills questions

Data analysis

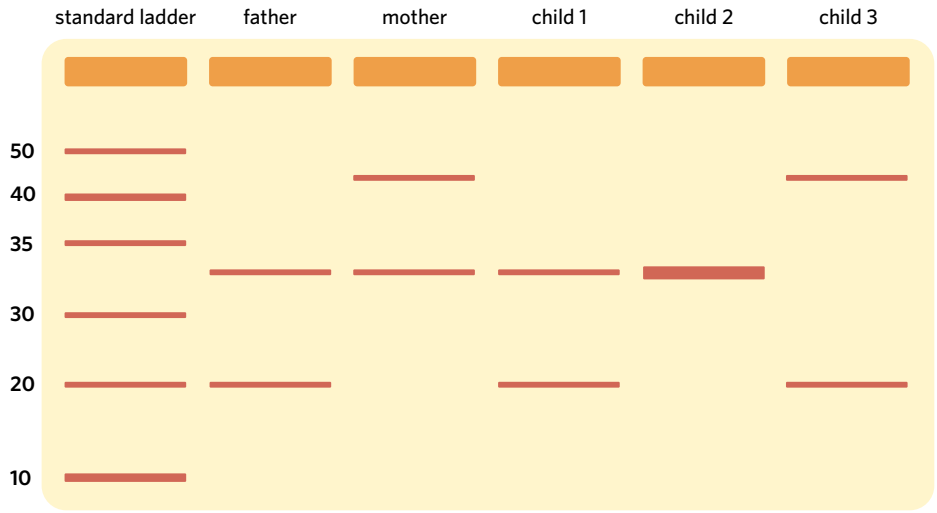
Use the following information to answer Questions 5–9.

Huntington's disease is a genetic neurological condition that typically does not show symptoms until 30–40 years of age, at which point it can cause a loss of cognitive and psychiatric function. It is an autosomal dominant disorder which means an individual only needs to carry one copy of the mutated gene to express the disease phenotype.

Huntington's disease is caused by mutations in the *HTT* gene where there are additional short tandem repeats (STR) of CAG trinucleotides. This means that laboratory technicians can take a DNA sample from an individual and test to see whether they will develop Huntington's disease using the polymerase chain reaction and gel electrophoresis.

When interpreting the results, individuals with 35 or fewer CAG repeats will not develop Huntington's disease, whilst individuals with 40 or more CAG repeats will develop the disease. If individuals have 36–39 CAG repeats, they have produced an uninformative test result meaning it is unknown whether they will develop Huntington's disease.

The following gel electrophoresis was produced showing the STRs on the *HTT* gene for a family of four.



Question 5

What is the purpose of performing the polymerase chain reaction?

A To increase the volume of DNA present in each sample.

B To purify the DNA and prevent it from mutating.

Question 6

Explain why this family likely got tested for Huntington's disease.

A The mother has Huntington's disease and likely wished to test whether her children would also suffer from the disease.

B The father has Huntington's disease and likely wished to test whether his children would also suffer from the disease.

C Both parents are carriers for Huntington's disease and wished to see if they had passed it onto their children.

D Neither parent suffers from Huntington's disease but wanted to be tested just in case.

Question 7

What is the most likely reason that child 2 only has one band present in their lane?

A The child has a different parent from those included in the gel electrophoresis.

B The laboratory technicians made a mistake during their extraction and amplification process.

C Both parents have an allele with the same number of CAG repeats which the child has inherited.

Question 8

Without the standard ladder, you could not identify

A which *HTT* alleles the children inherited from their parents.

B the number of CAG repeats to diagnose the disease.

Question 9

Considering Huntington's disease is an autosomal dominant disorder, which of the following statements are correct?

A All parents with Huntington's disease must have affected children.

B All children with Huntington's disease must have an affected parent.

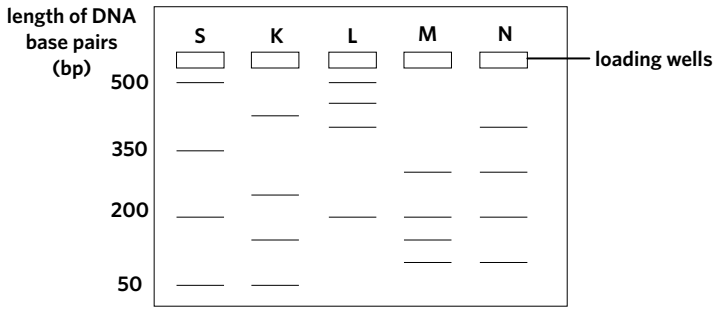
C All children with Huntington's disease must have two affected parents.

Exam-style questions

Within lesson

Use the following information to answer Questions 10 and 11.

Four samples of DNA were loaded into four different wells in lanes K, L, M, and N. A standard ladder was loaded into the well in lane S. The following gel electrophoresis shows the results.



Question 10 (1 MARK)

Which lane represents a sample that was loaded with DNA fragments of four different lengths: 100, 200, 300, and 400 bp?

A K

B L

C M

D N

Adapted from VCAA 2018 Section A Q30

Question 11 (1 MARK)

Which of the following lanes contains the band that is closest to the positive electrode?

A K

B L

C M

D N

Adapted from VCAA 2018 Section A Q31

Question 12 (1 MARK)

During a fight between a number of people, one person (the victim) was seriously injured. Blood samples were taken from the victim, the crime scene, and four suspects. DNA was extracted from white blood cells in each of the blood samples, amplified using the polymerase chain reaction, and gel electrophoresis of the samples was carried out. The results are shown in the following diagram.

The person most likely to have been at the crime scene is

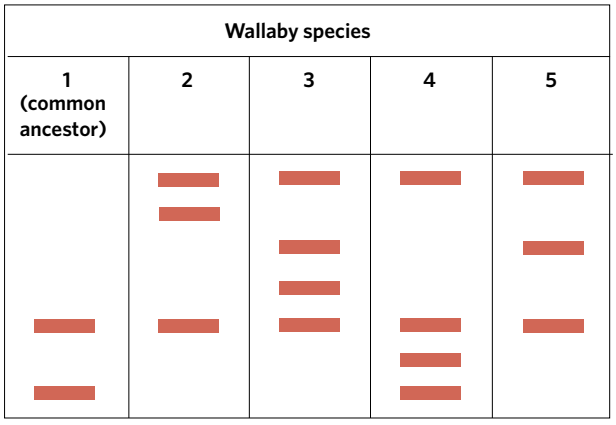
A suspect 1.
B suspect 2.
C suspect 3.
D suspect 4.

Adapted from VCAA 2013 Section A Q28

Question 13 (1 MARK)

Scientists analysed DNA markers from four wallaby species. Using gel electrophoresis, they compared these DNA markers to DNA extracted from the remains of a common ancestor.

Electrophoresis gel of wallaby species



Which wallaby species is most closely related to the common ancestor (wallaby species 1)?

A wallaby species 2
B wallaby species 3
C wallaby species 4
D wallaby species 5

Question 14 (9 MARKS)

There were three suspects in an assault case. A forensic scientist found blood at the site that didn't belong to the victim. DNA was extracted from five blood samples:

- the victim
- blood found at the crime scene (not the victim's)
- the three suspects.

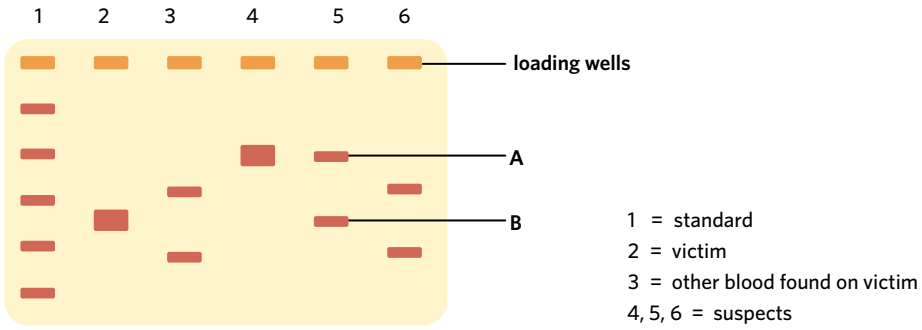
Short tandem repeat (STR) sequences were used in forensic analysis. An STR of four bases called D18S51 is located on chromosome 18. This STR has many alleles which differ from each other by the number of times the nucleotide sequence AGAA is repeated.

DNA from each sample was amplified using the polymerase chain reaction and loaded into a gel and electrophoresis was performed to separate the fragments of DNA.

a Name two properties of DNA fragments that allow them to be separated from each other during gel electrophoresis. (2 MARKS)

b Other than factors relating to the DNA sample, identify one factor that will impact the rate of movement of DNA fragments through the agarose gel. (1 MARK)

c The following diagram shows the results of the gel electrophoresis.



i Why is there only one band in lanes 2 and 4 but two bands in lanes 3, 5, and 6? (2 MARKS)

ii How many different alleles at the D18S51 locus are represented on the gel in individuals 2–6? (1 MARK)

iii Which fragment of DNA, A or B, has the greater number of the AGAA repeat sequence? (1 MARK)

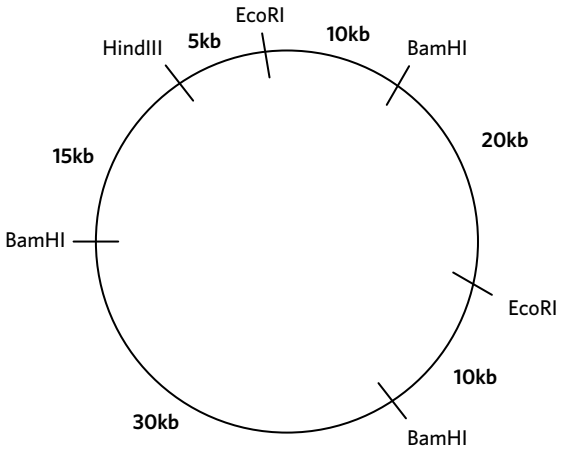
iv Based on the data, which of the suspects committed the assault? Justify your response. (2 MARKS)

Adapted from VCAA 2002 Exam 2 Section B Q5

Multiple lessons

Use the following information to answer Questions 15 and 16.

The following diagram represents a plasmid and the position of recognition sites for the restriction endonucleases BamHI, EcoRI, and HindIII.



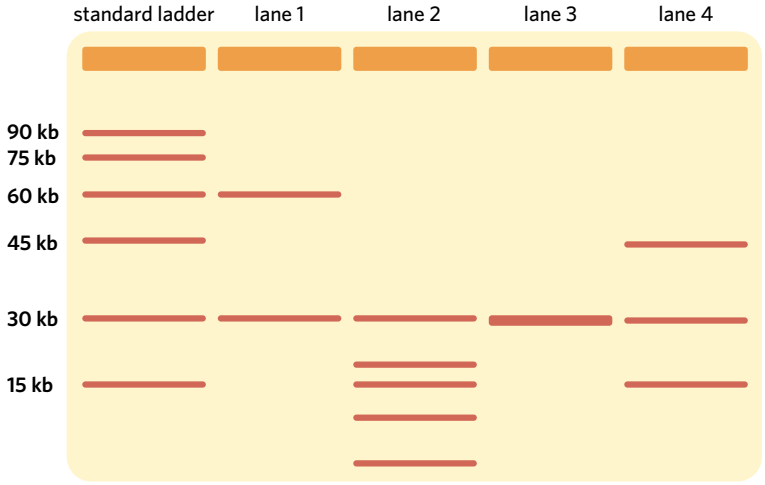
Question 15 (1 MARK)

A sample of the plasmid is incubated in a solution with EcoRI and HindIII. How many unique DNA fragments would be produced?

A 1
B 2
C 3
D 4

Question 16 (1 MARK)

The following diagram shows a gel electrophoresis of four different samples run against a standard ladder.

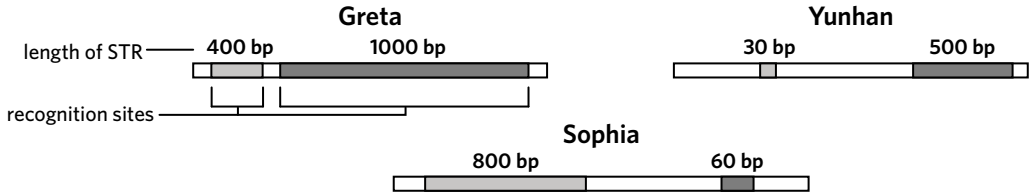


Which of the following lanes shows the results of a gel electrophoresis when this plasmid is digested by BamHI?

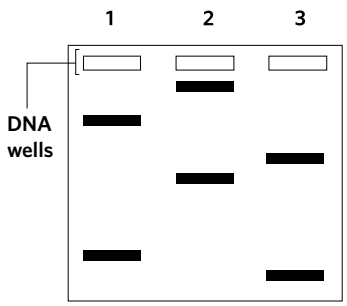
A lane 1
B lane 2
C lane 3
D lane 4

Question 17 (1 MARK)

Scientists analysed the DNA samples of three students: Greta, Yunhan, and Sophia. Two short tandem repeats (STRs) that are unique to each individual were investigated. Each student's DNA was digested using a restriction endonuclease. The length of each STR for each student is shown in the following diagram.



The DNA of each student was separated using gel electrophoresis and the positions of the STRs were observed. STRs were marked with a fluorescent probe and were the only visible bands in the gel. The results are shown in the following diagram of the electrophoresis gel.



From the results and the information provided for each student, it can be concluded that the DNA in wells 1, 2, and 3 respectively belong to

A Yunhan, Greta, and Sophia.

B Yunhan, Sophia, and Greta.

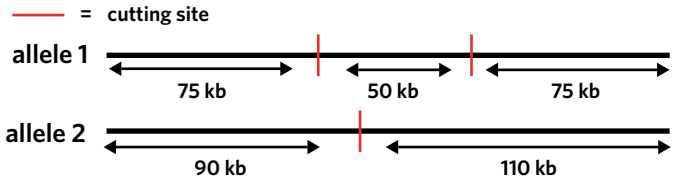
C Sophia, Yunhan, and Greta.

D Sophia, Greta, and Yunhan.

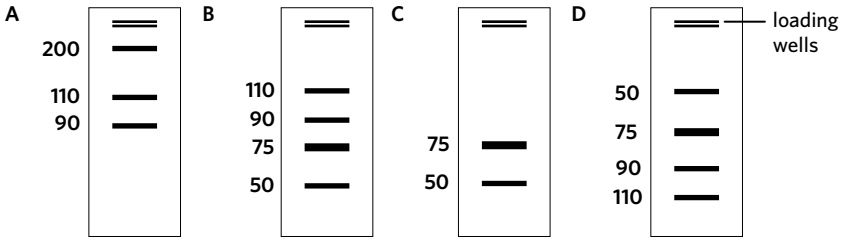
Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q26

Question 18 (1 MARK)

Cutting sites for a particular restriction endonuclease vary in a 200 kb region of human chromosome 2. The cutting sites for alleles 1 and 2 of a certain gene on this chromosome are shown in the following diagram.



The DNA of a person heterozygous for these alleles would have which of the following gel patterns after digestion with this restriction endonuclease?

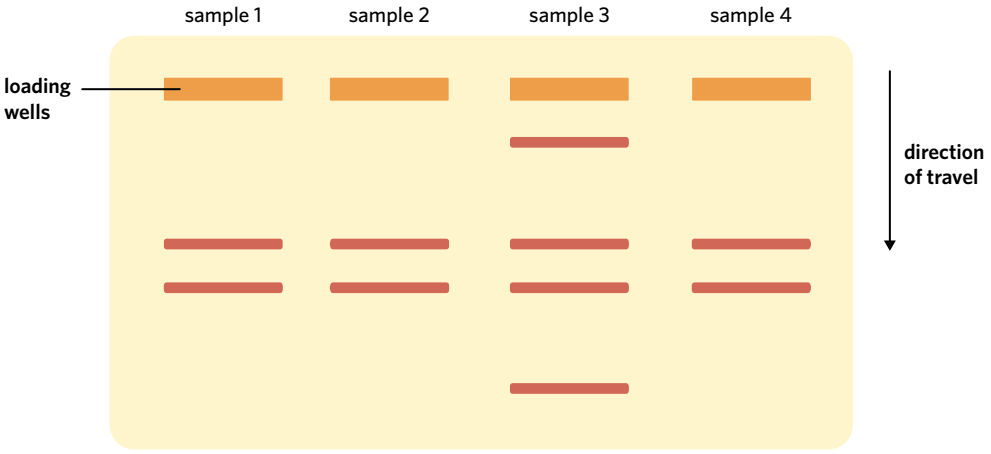


Adapted from VCAA 2005 Exam 2 Section A Q18

Key science skills and ethical understanding

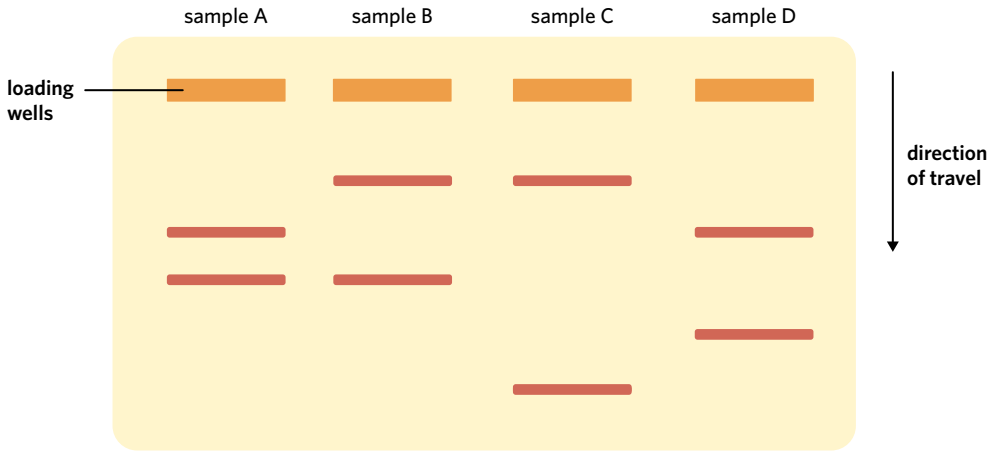
Question 19 (7 MARKS)

Riku wanted to set up an experiment to test the relatedness between him and his family members by using restriction fragment length polymorphism (RFLP) analysis. He first used gel electrophoresis to test four samples of his own DNA taken from mouth swabs. Before running his DNA samples on the gel, Riku needed to amplify the amount of DNA in each of the four samples using the polymerase chain reaction. The results of his gel are shown in the following diagram.



a Suggest a possible reason why the results for Sample 3 are different from the results for the other three samples. (1 MARK)

b Riku also obtained DNA samples from three different family members (his mother, his father, and his maternal grandfather) and separated out the alleles at the RFLP locus using gel electrophoresis. The results of Riku and his three family members are shown, where sample A is Riku's own DNA.



i Based on these data, identify which family member each of the three other DNA samples belongs to. (1 MARK)

ii Riku is considering running the experiment again but with a standard ladder. What is the purpose of a standard ladder in gel electrophoresis experiments? (1 MARK)

iii Would including a standard ladder improve the results of Riku's experiment? Justify your response. (1 MARK)

c Outline two safety guidelines that should have been followed by Riku. (2 MARKS)

d Before taking samples of his family members' DNA, Riku must obtain informed consent from them. Which bioethical concept does this satisfy? (1 MARK)

4E RECOMBINATION AND TRANSFORMATION

Diabetes is estimated to affect 1.2 million Australians and is caused by the inability of the pancreas to adequately provide the body with insulin, a hormone responsible for the uptake of glucose into cells from the bloodstream. There is currently no cure for diabetes, however it is treatable through regular injections of insulin. How do we obtain enough insulin for 5% of the Australian population to use daily?

Image: Pixel-Shot/Shutterstock.com

Lesson 4E

In this lesson you will learn how DNA manipulation techniques can produce recombinant plasmids which are used to transform bacteria.

◀ *Prerequisite knowledge*

Year 11
Bacteria contain a circular chromosome and independent plasmids.

Lesson 4A
To create a recombinant plasmid, enzymes such as restriction endonucleases and DNA ligase are required.

Future applications ▶

Lesson 4F
Manipulating DNA raises many important ethical issues which must be considered.

Lesson 8B
Introducing antibiotic resistance genes into bacterial populations risks spreading them into wild populations and creating highly resistant pathogens.

Study design dot point

- the use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin

Key knowledge units

Why transform bacteria?	3.1.11.1
Making a recombinant plasmid	3.1.11.2
Transforming bacteria	3.1.11.3
Insulin	3.1.11.4

Why transform bacteria? 3.1.11.1

OVERVIEW

Genetically modifying bacteria to produce human proteins has revolutionised modern medicine and agriculture.

THEORY DETAILS

Bacteria are simple prokaryotic organisms that replicate their **plasmid** DNA independently from their circular chromosome. The number of plasmids in each bacterium varies – one bacterium may have many plasmids whereas another may have none. Bacterial plasmids vary in length and can be anywhere up to 200 kbp.

plasmid a small, circular loop of DNA separate from the chromosome, typically found in bacteria

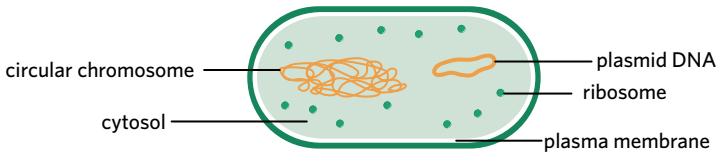


Figure 1 A prokaryotic organism contains plasmid DNA and a circular chromosome.

The fact that bacteria possess independently replicating plasmids means that humans can genetically modify bacteria to synthesise large amounts of protein in a simple process. This involves editing a plasmid to incorporate a target gene of interest. Once a plasmid is edited to integrate a target gene, it is referred to as a **recombinant plasmid**. Bacteria then take up these recombinant plasmids from the environment in a process called **bacterial transformation,** which is arguably the most important step in the entire **genetic modification** process. Once this has occurred, bacteria can synthesise specific proteins. In this lesson, you will learn about each of these steps involved in genetic modification.

Bacterial transformation has had many uses in the medical and food industries, usually enabling cheaper and more efficient methods of production. These include the large-scale production of the following proteins:

- **insulin** to manages **diabetes**
- erythropoietin to treat anaemia
- chymosin for cheese production
- interferon to treat some cancers
- growth hormone to manage growth disorders
- hepatitis B surface antigen for use in the hepatitis B vaccine
- alpha-amylase for ethanol and high fructose corn syrup production.

Later in this lesson, you will learn the specifics about insulin production as it has been specified by the VCAA in the study design dot point.

Making a recombinant plasmid 3.1.11.2

OVERVIEW

Genetic engineers are often interested in introducing DNA into an organism where it doesn't naturally occur. A simple way of doing this is to insert foreign DNA into a plasmid that can then be taken up by bacteria. The bacteria will then express the protein encoded by that foreign DNA.

THEORY DETAILS

Plasmids are excellent cloning vectors because they can self-replicate, are small, can be taken up by bacteria, and it is easy to include antibiotic resistance genes, recognition sites, and expression signals. In order for scientists to create recombinant plasmids, they require a **gene of interest**, a **plasmid vector**, a **restriction endonuclease**, and DNA **ligase**.

Gene of interest

A sequence of DNA encoding the protein we wish to produce is generated, which is referred to as the gene of interest (Figure 3). There are several ways of generating a specific sequence of DNA but these are not assessed by the VCAA so will not be covered in depth here. In short, the DNA sequence of a human protein is isolated and amplified using the polymerase chain reaction before it can be inserted into a **vector**. Despite the gene of interest coming from another organism, bacteria are able to use their DNA to synthesise an identical protein because the genetic code is universal – a CUU codon encodes leucine no matter which organism expresses the codon.

Plasmid vector

A **plasmid vector** is selected into which the gene of interest will be inserted. Many different plasmid vectors have been designed by scientists, but most contain the following four important DNA sequences (Figure 4):

- Restriction endonuclease sites – a site on the plasmid that can be recognised and cut by a restriction endonuclease, allowing the gene of interest to be inserted.

recombinant plasmid a circular DNA vector that is ligated to incorporate a gene of interest

bacterial transformation the process by which bacteria take up foreign DNA from their environment. Scientists use this process to introduce recombinant plasmids into bacteria

genetic modification the manipulation of an organism's genetic material using biotechnology

insulin a hormone secreted by the pancreas to control blood glucose levels

diabetes a disease where the body cannot properly produce or respond to insulin

Image: Shane Christopher/Shutterstock.com

Figure 2 Chymosin was traditionally obtained by killing newborn calves and extracting the enzyme from their fourth stomach. Nowadays, we can produce this enzyme in a laboratory from bacteria.

gene of interest a gene scientists want to be expressed in recombinant bacteria. This gene often encodes a protein we wish to produce in commercial quantities. Also known as the **desired gene**

restriction endonuclease any enzyme that acts like molecular scissors to cut nucleic acid strands at specific recognition sites. Also known as a **restriction enzyme**

ligase an enzyme that joins molecules, including DNA or RNA, together by catalysing the formation of phosphodiester bonds

vector a means of introducing foreign DNA into an organism. Plasmids are a popular vector in bacterial transformation

plasmid vector a piece of circular DNA that is modified to be an ideal vector for bacterial transformation experiments

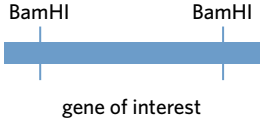


Figure 3 Gene of interest

- **Antibiotic resistance genes** – e.g. *amp*^{R} which confers ampicillin resistance or *tetA* which confers tetracycline resistance.
- **Origin of replication** (ORI) – a sequence that signals the start site for DNA replication in bacteria.
- **Reporter gene** – genes with an easily identifiable phenotype that can be used to identify whether a plasmid has taken up the gene of interest.

Restriction endonuclease

The gene of interest and our plasmid are both cut with the same restriction endonuclease to generate identical sticky ends on either end of the DNA sequence. Figure 5 shows the use of the restriction enzyme BamHI to isolate the gene of interest and create an opening in the plasmid vector. The overhanging nucleotides of the gene of interest will be complementary to the overhanging nucleotides on the plasmid vector, allowing them to form hydrogen bonds with each other easily. Blunt end restriction enzymes may also be used but are less targeted compared to sticky end restriction enzymes, as a blunt end can bond with any other blunt end.

DNA ligase

DNA ligase is added to join the gene of interest to the plasmid vector by forming phosphodiester bonds in the sugar-phosphate backbone. This creates a circular piece of DNA called a recombinant plasmid (Figure 6). Not every plasmid will take up the gene of interest – in fact, most plasmids will simply ligate back with themselves and are termed non-recombinant plasmids. The result of this procedure creates a mixture of both recombinant and non-recombinant plasmids. It is the role of the reporter gene to distinguish between a recombinant and non-recombinant plasmid. However, bacteria must first undergo transformation.

Transforming bacteria 3.1.11.3

OVERVIEW

Many bacteria will naturally take up free-floating DNA from their environment into their cytosol via transformation. Biologists are able to take advantage of this process to make bacteria take up recombinant plasmids.

THEORY DETAILS

Uptake of recombinant plasmids

The uptake of a recombinant plasmid involves the recombinant plasmid being inserted into the cytoplasm of bacteria in a process known as bacterial transformation. The two primary methods of promoting recombinant plasmid uptake are **heat shock** and **electroporation**.

The heat shock method first requires bacteria and plasmids to be placed in a calcium ion solution on ice. The positive calcium ions help make the plasma membrane more permeable to the negatively charged plasmid DNA. The solution is then heated to around 37–42 °C for 25–45 seconds, before being returned to the ice. This sudden change in temperature makes the plasma membrane even more permeable and allows plasmid vectors to cross the phospholipid bilayer and enter the bacteria's cytoplasm (Figure 7).

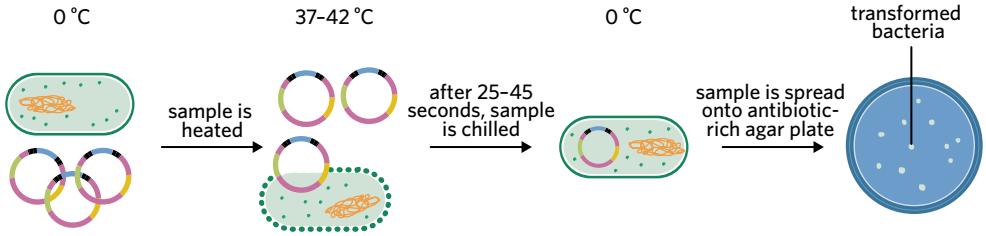


Figure 7 Heat shock to induce plasmid uptake

antibiotic resistance gene gene which confers antibiotic resistance

origin of replication (ORI) a sequence found in prokaryotes that signals the start site of DNA replication

reporter gene gene with an easily identifiable phenotype that can be used to identify whether a plasmid has taken up the gene of interest

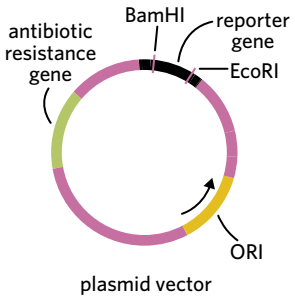


Figure 4 Example of a plasmid vector

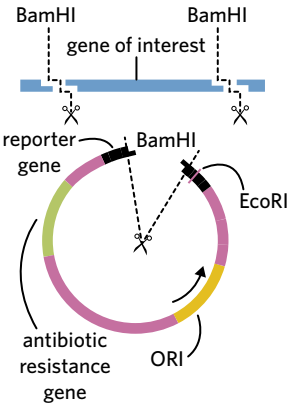


Figure 5 Restriction endonucleases cutting a gene of interest and a plasmid

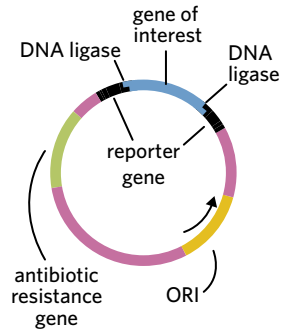


Figure 6 DNA ligase integrating the gene of interest into a plasmid to create a recombinant plasmid

Electroporation is a similar process to the heat shock method but instead of heat, an electrical current is passed through a solution containing bacteria and plasmid vectors. The electrical current causes the plasma membrane to become more permeable, allowing plasmid vectors to cross the phospholipid bilayer and enter the bacteria's cytoplasm (Figure 8).

heat shock a method that involves rapidly increasing and decreasing the temperature to increase membrane permeability in order to enhance the likelihood of bacterial transformation

electroporation a method that involves delivering an electric shock to bacterial membranes to increase their membrane permeability and increase the likelihood of bacterial transformation

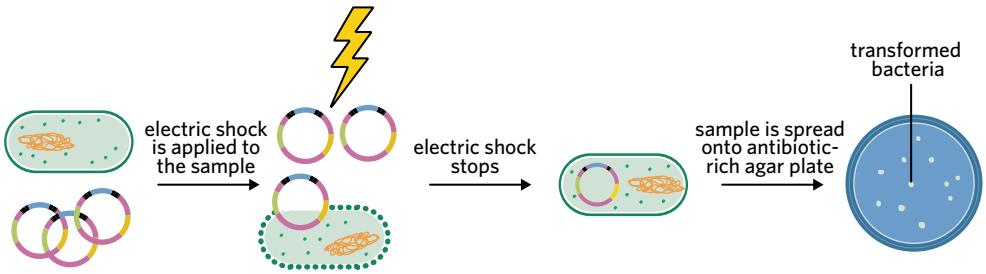


Figure 8 Electroporation to induce plasmid uptake

Antibiotic selection

In order to distinguish between transformed and untransformed bacteria, the mixture is cultured onto an antibiotic-rich plate. Since only transformed bacteria contain the gene necessary for antibiotic resistance, all untransformed bacteria will be killed off. This means that each colony visible on a plate represents a transformation event whereby a single bacterium has taken up a plasmid, allowing it to survive, multiply, and form a colony of identical daughter cells.

It is important to remember that transformed bacteria take up both recombinant and non-recombinant plasmids which need to be distinguished from one another. This is carried out by a reporter gene. An example is *gfp* which encodes for the green fluorescent protein which fluoresces green under UV light when fully expressed. In non-recombinant plasmids, the reporter gene is continuous and therefore completely expressed, which enables bacteria that have been transformed with non-recombinant plasmids to glow under UV light. In recombinant plasmids however, the reporter gene is split by the gene of interest and therefore non-continuous. In this scenario, bacteria that have taken up recombinant plasmids cannot glow under UV light.

Protein production and extraction

The transformed bacteria are cultured and induced to produce the target protein. As the bacteria make lots of different proteins, the protein of interest is extracted and purified (Figure 9).

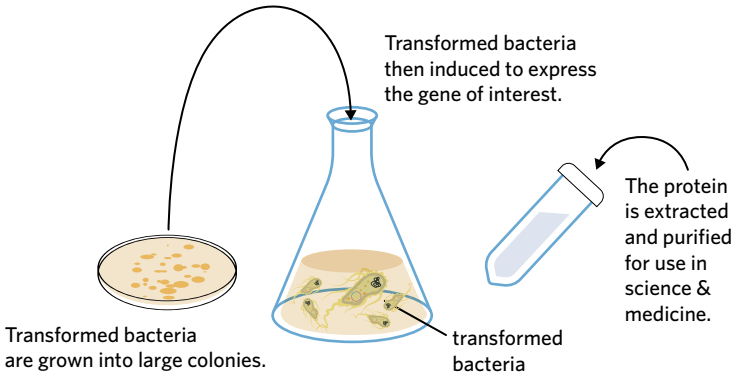


Figure 9 Protein production and extraction

Insulin 3.1.11.4

OVERVIEW

Insulin is an important hormone that is responsible for regulating our blood glucose levels. People living with diabetes do not naturally produce or respond to insulin and require it to be administered artificially into their body. Luckily, insulin can be produced by transformed bacteria.

THEORY DETAILS

Prior to the discovery of gene cloning techniques, porcine (pig) or bovine (cow) insulin was extracted and administered to diabetics as it shares a similar structure to human insulin. However, this resulted in the killing of many animals to extract insulin from their pancreas and was not as effective at blood glucose regulation as human insulin. In the 1980s, human insulin was first produced in bacteria containing recombinant plasmids. This has been the method of choice ever since as it is significantly cheaper and more effective than extracting insulin from animals.

The insulin protein has a quaternary structure consisting of two polypeptide chains known as the alpha and beta subunits (not to be confused with alpha helices and beta-pleated sheets). This means to produce insulin, we require two different recombinant plasmids and thus two different transformed bacteria samples – one producing the alpha subunit and one producing the beta subunit. For insulin to function properly in the human body, these two chains must first fold individually then be joined together by a disulphide bridge.

The process of producing recombinant human insulin is as follows:

Creating the recombinant plasmid

1. Plasmid vectors are prepared which contain the amp^R gene to encode for antibiotic resistance and *lacZ*, which acts as a reporter gene and has a specific recognition site to the restriction endonuclease used in step 2 inside it. *lacZ* produces β-galactosidase, an enzyme that converts X-gal from a colourless to a blue compound.
2. Two plasmid vectors are used – one for insulin subunit A and one for insulin subunit B. Using a restriction endonuclease such as BamHI, both plasmid samples, the insulin A subunit gene, and insulin B subunit gene, are cut to form sticky ends. DNA ligase is then used to reestablish the sugar-phosphate backbone and create two different recombinant plasmids.

Creating transformed bacteria

3. The plasmids are added to a solution of *E. coli* bacteria and then either heat shock or electroporation can be completed to increase the uptake of the plasmids into the bacteria.
4. The bacteria cultures are spread and incubated onto agar plates containing X-gal and the antibiotic ampicillin. Colonies that form which are colourless can then be determined to be transformed bacteria with the recombinant plasmid, as their *lacZ* gene is dysfunctional since the gene of interest is located inside it.
5. Recombinant plasmids will produce an insulin subunit with a β-galactosidase tail which is formed from the half of the *lacZ* gene which is transcribed and translated.

Protein production and extraction

6. Transformed bacteria that contain the recombinant plasmid are then placed into conditions to exponentially reproduce before their membranes are broken down, and the human insulin they have produced is isolated and purified.
7. The two insulin chains have their β-galactosidase tails removed and are mixed together, which allows the connecting disulphide bonds to form and create functional human insulin.

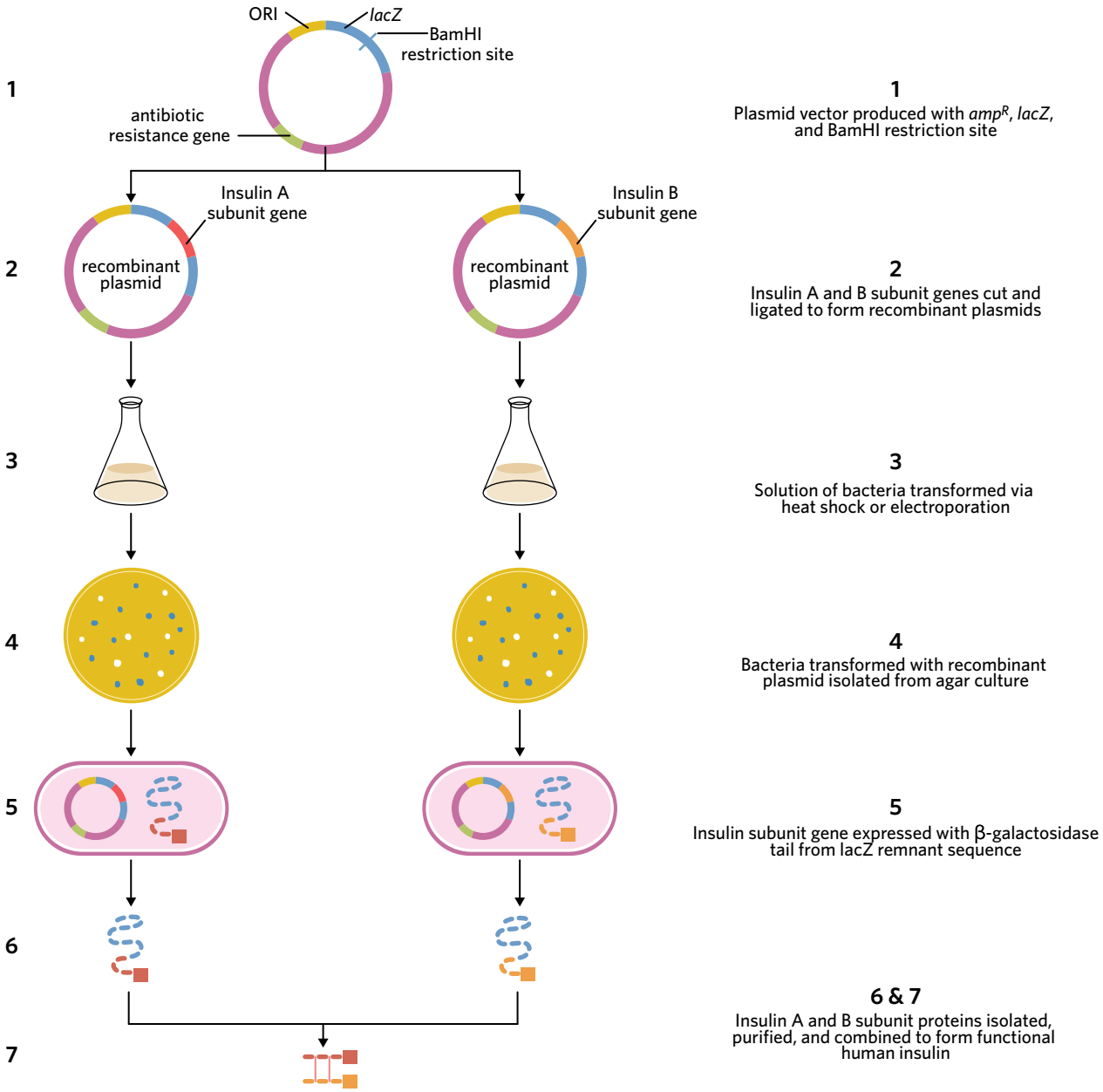


Figure 10 Production and extraction of recombinant insulin

Theory summary

Bacterial transformation occurs through the extraction of target genes, formation of recombinant plasmids, and plasmid uptake by bacteria. To distinguish transformed bacteria from non-transformed bacteria, an antibiotic resistance gene is included in the plasmid vector which is expressed solely by the successfully transformed bacteria. These bacteria can then be isolated, and the proteins they produce, such as human insulin, can be purified.

How do we make enough insulin for 1.2 million people? We get bacteria to do the work for us! Through recombination and transformation, non-pathogenic strains of bacteria pump out insulin. These insulin proteins can be extracted, purified, and converted into a serum to form a life-saving drug for diabetics which can be injected into their bodies.

Image: MedstockPhotos/Shutterstock.com

4E QUESTIONS

Theory review questions

Question 1

One reason scientists use plasmids for bacterial transformation is because

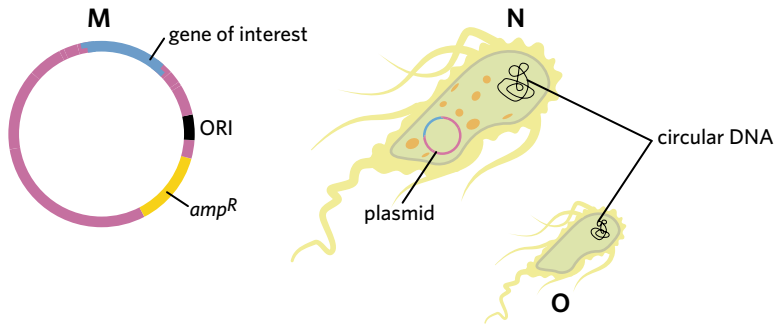
A plasmid DNA replicates independently from chromosomal DNA.

B plasmid DNA is smaller.

Question 2

Label the parts (M–O) of the following diagram from the list of terms. Terms may be used multiple times or not at all.

- transformed bacteria
- recombinant plasmid
- untransformed bacteria
- non-recombinant plasmid



Question 3

Order the steps to correctly describe the process of bacterial transformation.

I Recombinant plasmids and bacteria are mixed together in a solution. Electroporation or heat shock increases the bacteria's membrane permeability, allowing plasmids to pass into the cytoplasm.

II The gene of interest is generated and an appropriate plasmid vector is chosen.

III Bacteria are cultured on an antibiotic-containing medium. Only transformed bacteria are able to grow and form colonies.

IV Some restriction enzymes are used to create complementary sticky ends on both the gene of interest and the plasmid vector.

V DNA ligase is added to join the gene of interest and plasmid vector by sealing the sugar-phosphate backbone.

Question 4

The purpose of including an antibiotic resistance gene in the plasmid vector is to

A determine which bacteria have been transformed.

B determine whether the gene of interest has been correctly inserted into the plasmid.

SAC skills questions

Scientific methodology comparison

Use the following information to answer Questions 5–10.

Heat shock and electroporation are two methods to increase the uptake of recombinant plasmids by bacteria. These two methods increase the permeability of the bacterial plasma membranes to encourage uptake of plasmids from the environment.

Two groups were set up to measure and compare the transformation efficiency of heat shock and electroporation. All samples used by each group had the same initial amount of recombinant plasmids and bacteria. Their results are shown in the following tables.

Group 1 – Heat shock

Sample number	Number of colonies
1	91
2	93
3	99

Group 2 – Electroporation

Sample number	Number of colonies
1	604
2	440
3	778
4	597
5	880

Question 5

Bacterial transformation is

A the uptake of a recombinant plasmid by a bacterium.

B the addition of a gene of interest to a plasmid vector.

Question 6

What is the independent variable?

A method to enhance transformation

B number of colonies present on agar plates

Question 7

Which group has a more reliable methodology?

A Group 1 as there is less variation between each sample's results.

B Group 2 as there were more repeats performed.

Question 8

Which group recorded more precise results?

A group 1

B group 2

Question 9

Based on these results, which method likely has a greater transformation efficiency?

A heat shock

B electroporation

Question 10

Identify a potential consequence of prolonging the application of heat or electricity to a bacterial cell.

A There would be less uptake of plasmids.

B It could cause permanent damage to the bacterial cell.

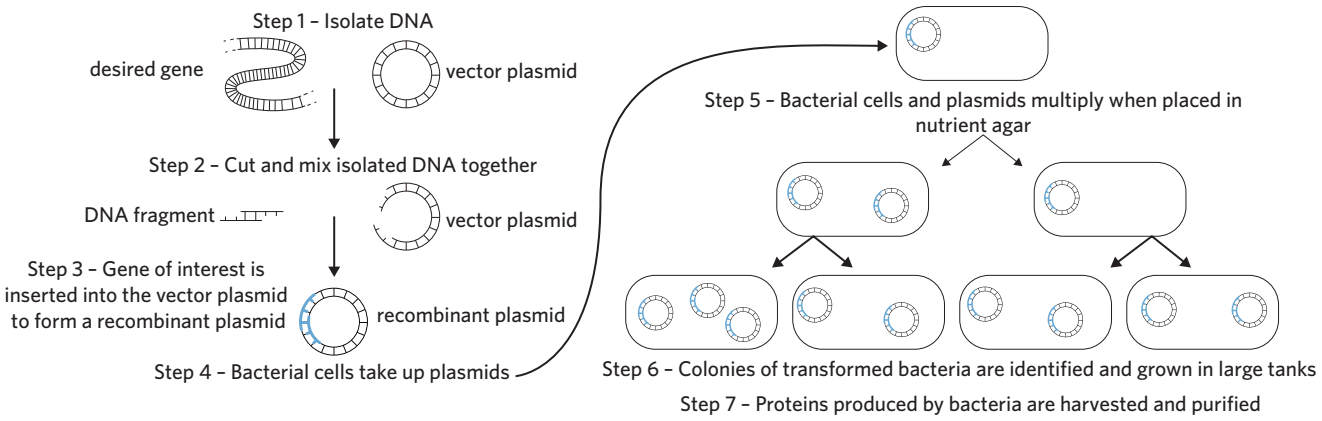
C It could remove the gene of interest from recombinant plasmids.

Exam-style questions

Within lesson

Use the following information to answer Questions 11–13.

A molecular biologist performed the procedure outlined in the flow chart.



Question 11 (1 MARK)

Which one of the following correctly shows the enzymes required in Steps 2 and 3?

	Cuts plasmid and gene of interest	Joins gene of interest with plasmid
A	DNA ligase	DNA polymerase
B	DNA ligase	restriction endonuclease
C	restriction endonuclease	DNA polymerase
D	restriction endonuclease	DNA ligase

Adapted from VCAA 2014 Section A Q25

Question 12 (1 MARK)

Which one of the following is a correct statement about the procedure outlined?

A In Step 1, the gene of interest and the plasmid vector are always isolated from the same organism.

B In Step 2, the overhanging nucleotides of the gene of interest and plasmid vector are complementary to each other.

C In Step 3, the transformed bacteria are able to replicate to generate more copies of the gene of interest.

D Multiplication of DNA in Step 5 is due to the polymerase chain reaction.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q37

Question 13 (1 MARK)

One application of the process outlined is to

A use plants containing recombinant plasmids to produce large quantities of human proteins, such as erythropoietin.

B produce human organs inside pigs that can be transplanted into humans who require an organ transplant.

C grow tissues in culture from human stem cells for laboratory models.

D produce commercial quantities of proteins such as human insulin.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q38

Use the following information to answer Questions 14 and 15.

Bacteria can be transformed with an artificial erythropoietin gene and cultured to make the erythropoietin protein in commercial quantities.

The steps taken to produce genetically engineered erythropoietin are summarised. The order of the steps has been mixed up.

I Harvest and purify erythropoietin protein from transformed bacteria.

II Add recombinant plasmids to bacteria and induce heat shock of the bacterial membrane to enhance uptake of plasmids.

III Identify successfully transformed bacteria.

IV Add DNA ligase to join the erythropoietin gene and plasmid vector.

V Culture transformed and untransformed bacteria on antibiotic-containing nutrient agar.

VI Use restriction endonucleases to generate cuts in the erythropoietin gene and plasmid vector.

Question 14 (1 MARK)

The correct sequence of steps involved in producing erythropoietin using bacterial transformation is

A IV, VI, II, III, V, I

B V, VI, IV, II, III, I

C VI, IV, II, V, III, I

D VI, IV, V, II, I, III

Adapted from VCAA 2013 Section A Q34

Question 15 (1 MARK)

The enzyme used to cut the erythropoietin gene and plasmid DNA at step VI is also known as

A a protease.

B DNA ligase.

C DNA polymerase.

D a restriction enzyme.

Adapted from VCAA 2013 Section A Q35

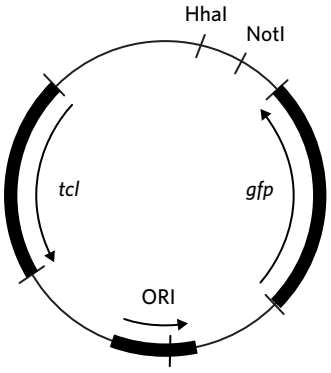
Multiple lessons

Question 16 (7 MARKS)

Scientists use recombinant plasmids as vectors to transform bacteria for a range of purposes, particularly research and biotechnology.

a What is meant by the term 'transform' when creating transformed bacteria? (1 MARK)

b A particular bacterial plasmid contains recognition sites for the restriction enzymes HhaI and NotI, along with the gene encoding tetracycline resistance (*tcI*), a reporter gene encoding green fluorescent protein (*gfp*), and an origin of replication (ORI). Cells expressing *gfp* fluoresce green under UV light.
The diagram shows the positions of these recognition sites, *tcI*, and *gfp* as well as the position of the origin of replication within this plasmid.



Recombinant bacterial plasmids are often used to produce bacteria capable of synthesising human proteins. This may be achieved by inserting the gene encoding the human protein into the bacterial plasmid.

The gene can be inserted into the bacterial plasmid by using restriction enzymes.

i Describe how restriction enzymes, such as HhaI and NotI, are used to help insert a gene coding for a human protein into this plasmid. (1 MARK)

ii Describe how DNA ligase is used to help insert a gene coding for a human protein into this plasmid. (1 MARK)

c After the scientists carried out the steps required to make and isolate recombinant plasmids with the inserted human gene, these plasmids were mixed with a culture of bacteria. This mixture was treated so that these plasmids would move into the bacterial cells. Not all bacteria took up these plasmids.

Describe the results scientists would expect to see if they cultured the treated bacterial cells on nutrient agar containing the antibiotic tetracycline. Explain the meaning of these results. (3 MARKS)

d What characteristic of the genetic code enables a human protein to be made by bacterial cells? (1 MARK)

Adapted from VCAA 2017 Section B Q9c

Key science skills and ethical understanding

Use the following information to answer Questions 17–19.

To replicate a gene of interest, the following four steps are performed:

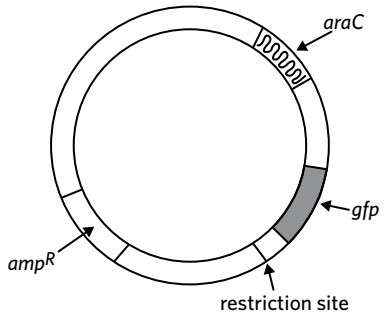
1 A plasmid and gene of interest are cut with an enzyme

2 The gene of interest is ligated into the plasmid

3 Plasmids are transferred to bacteria

4 Bacteria are grown on four nutrient agar plates (labelled W, X, Y and Z) that are coated with or without ampicillin and arabinose.

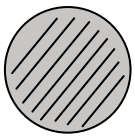
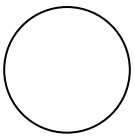
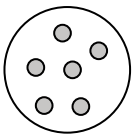
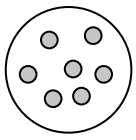
An example of a plasmid used in cloning is shown in the diagram.



This plasmid contains a restriction site and the following three genes:

- amp^R – confers resistance to the antibacterial agent ampicillin
- *gfp* – encodes the green fluorescent protein (GFP), which fluoresces under UV light
- *araC* – when arabinose is present, this gene expresses a protein turns *gfp* expression on.

The results from a bacterial transformation experiment are shown in the table.

Plate	**W** **untransformed bacteria only**	**X** **untransformed bacteria only**	**Y** **transformed bacteria only**	**Z** **transformed bacteria only**
Diagram of plate				
Added to plate	nutrient agar only	nutrient agar and ampicillin	nutrient agar, ampicillin, and arabinose	nutrient agar and ampicillin
Description of result	lawn of bacteria	no growth	bacterial colonies present	bacterial colonies present

Question 17 (1 MARK)

The purpose of the enzyme in the first step of this experiment is to

A reduce the effects of confounding variables due to different restriction enzymes.

B give both the plasmid and gene of interest complementary sticky ends, allowing them to ligate.

C give both the plasmid and gene of interest blunt ends, allowing segments of DNA to ligate together in various conformations.

D randomly cut DNA into fragments so that they can ligate and create a new recombinant plasmid that may potentially be beneficial.

Adapted from VCAA 2015 Section A Q25

Question 18 (1 MARK)

The results of plate W and X suggest that

A untransformed bacteria are unable to form colonies.

B the transformation efficiency of bacteria is quite low.

C untransformed bacteria are unable to grow in the presence of ampicillin.

D only transformed bacteria are able to grow in the presence of arabinose.

Adapted from VCAA 2015 Section A Q25

Question 19 (1 MARK)

Which of the following statements is correct in regards to plate Z?

A Bacterial colonies have evolved antibiotic resistance, allowing them to grow in the presence of ampicillin.

B The presence of the *gfp* gene allows colonies to fluoresce under UV light when arabinose is present.

C Untransformed bacteria would be able to grow if arabinose was present.

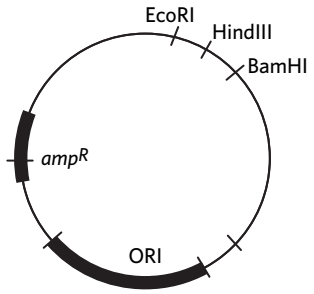
D mRNA for *gfp* would be absent in the bacterial colonies.

Adapted from VCAA 2015 Section A Q25

Question 20 (14 MARKS)

Chymosin is an enzyme used to manufacture cheese. Found in rennet, chymosin was traditionally extracted from the fourth stomach of newborn calves. Because of the difficulties in sourcing chymosin, scientists engineered a non-pathogenic strain of recombinant *E. coli* that can generate large quantities of chymosin in a laboratory. Currently, about 60–90% of hard cheese in the USA and UK is made with genetically engineered chymosin.

The *E. coli* plasmid contains recognition sites for the restriction enzymes *EcoRI*, *HindIII*, and *BamHI*, along with the gene for ampicillin resistance (amp^R) and an origin of replication (ORI). The diagram shows the positions of these recognition sites and the ampicillin-resistance gene, as well as the position of the origin of replication within this plasmid.



a Explain what is meant by 'non-pathogenic strain of recombinant *E. coli*'. (1 MARK)

b Before inserting the gene for chymosin into *E. coli*, scientists must amplify the section of calf DNA that codes for chymosin. Name the process that scientists use to amplify DNA. (1 MARK)

c Once the scientists have isolated and amplified the gene of interest, they can insert it into the plasmids. To do this, they use the restriction enzyme HindIII.

Draw and label a diagram to show the position of the chymosin gene in this plasmid when HindIII is used. Include the relative position of the recognition sites for the restriction enzymes EcoRI, HindIII, and BamHI on the plasmid. (1 MARK)

d Next, scientists must introduce the recombinant plasmids into non-pathogenic *E. coli*. To do this, they use the heat-shock method which involves causing a sudden temperature change that makes the cell membrane of the bacteria more permeable to the recombinant plasmids.

Identify the 'vector' in this experiment. Justify your response. (2 MARKS)

e Not all the *E. coli* will take up recombinant plasmids. To test which bacteria are transformed, the scientists set up four Petri dishes.

Plate A	Nutrient agar + *E. coli* not exposed to plasmids
Plate B	Nutrient agar + ampicillin + *E. coli* not exposed to plasmids
Plate C	Nutrient agar + *E. coli* exposed to plasmids + heat-shocked
Plate D	Nutrient agar + ampicillin + *E. coli* exposed to plasmids + heat-shocked

The Petri dishes were incubated overnight at 37 °C. The scientists found bacterial growth on plates A, C, and D but no growth on plate B.

i Explain the scientists' results for each Petri dish, including whether any results were unexpected. (4 MARKS)

ii After incubation, which Petri dish(es) will contain only transformed bacteria? Justify your response. (2 MARKS)

iii What is the purpose of petri dishes A and B? Explain the significance of the results obtained from each of these dishes. (2 MARKS)

f Which bioethical concept supports the scientists' attempts to synthetically create chymosin using bacteria instead of harvesting it from calves? (1 MARK)

4F GENETIC ENGINEERING

Spider silk has some incredible properties. Not only is it highly elastic and flexible, but it is also incredibly strong and able to resist extreme temperatures. Dragline silk, the kind that spiders spin to catch themselves when they fall, is said to be stronger than kevlar and has a combined strength that is several times that of steel on a per millimetre basis.

Now look at this pretty lady. A farmyard goat. Could you imagine a parallel universe in which this goat's milk produced spider silk? Elastic, bio-compatible dragline silk every time she lactates? This is exactly what researchers created in the late 2000s, hoping to harness the farming of goat's milk to capitalise on the commercial applicability of spider silk.

Why was this done, and how? (Spider goat. Spider goat. Does whatever a spider goat does. Can she swing, from a web? No she can't, she's a goat.)

Image: yevgeniy11/Shutterstock.com

Lesson 4F

In this lesson you will learn about the difference between genetically modified organisms and transgenic organisms, including their use in agriculture to increase crop productivity and disease resistance.

◀ *Prerequisite knowledge*

Years 7–10
You learned about the transmission of heritable characteristics across generations, which can be investigated and understood in regard to their impact on natural selection.

Chapter 4
Across the earlier parts of this chapter you have learned about various DNA manipulation techniques, including CRISPR and PCR.

Future applications ▶

Lesson 9E
Selective breeding is an ancient form of selecting for desirable traits in species.

Chapter 8
DNA manipulation technology is useful in the field of immunology, especially in relation to drug development.

Study design dot point

- the use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease

Key knowledge units

Genetically modified organisms (GMOs) vs transgenic organisms (TGOs)	3.1.12.1
How we use GMOs in agriculture	3.1.12.2
Issues surrounding GMOs	3.1.12.3

Genetically modified organisms (GMOs) vs transgenic organisms (TGOs) 3.1.12.1

OVERVIEW

Here, you will learn the difference between genetically modified organisms and transgenic organisms.

THEORY DETAILS

Introducing GMOs

Genetic engineering refers to the alteration of an organism's genome using **genetic recombination technologies**. Scientists will often use these techniques in order to confer the organism with any number of desirable traits, such as increased size, a higher drought resistance, or brighter colours. The alteration might be for research purposes, or perhaps for commercial reasons (which we will revisit later in this lesson), and may involve genes:

genetic engineering the process of using biotechnology to alter the genome of an organism, typically with the goal of conferring some desirable trait

- being **silenced**
- inserted into the genome
- removed from the genome
- altered by replacing nucleotides.

Any organism that has been altered using genetic engineering technologies is referred to as a **genetically modified organism (GMO)**. The organism that receives the altered gene/s is referred to as the **host organism**, with the goal typically being to confer the host with a desirable characteristic that was originally lacking from its genome.

Transgenic organisms: a type of GMO

Because of the range of ways in which we can create GMOs, it is useful to think of GMOs as an umbrella term under which many different types of genetically modified organisms can be categorised. The two main GMOs are:

- **Cisgenic organisms**: a genetically modified organism that has genes from the same species inserted into its genome. This process, known as cisgenesis, involves transferring genes between organisms that could otherwise be bred together.
- **Transgenic organisms**: a genetically modified organism that has genes from a different species inserted into its genome. This process, known as transgenesis, results in an organism that contains foreign DNA transplanted from a separate species.

Transgenic organisms are able to produce proteins that were not previously part of their species' proteome due to their genome being altered with foreign DNA (Figure 1). Later in the lesson, we will cover the usefulness of transgenic organisms, especially in agriculture.

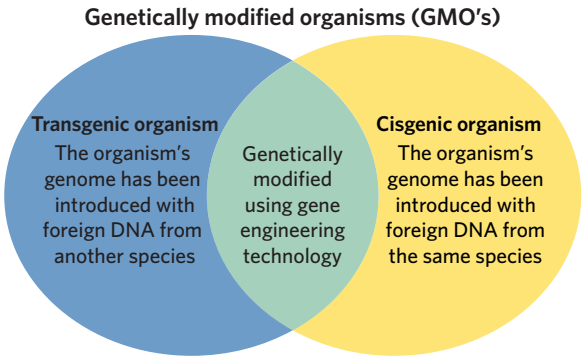


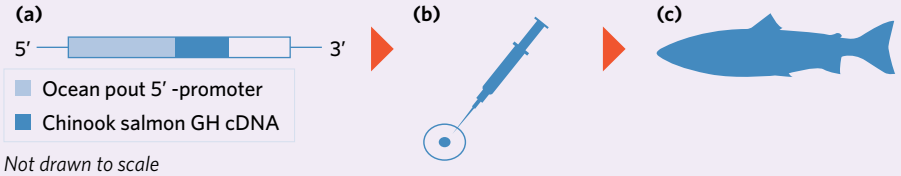
Figure 1 The relationship between transgenic organisms, cisgenic organisms and genetically modified organisms. Note that this representation is highly simplified. For example, transgenic organisms may also include those with DNA from the same species, provided that the DNA has been manipulated prior to being introduced. Similarly, cisgenesis can also involve separate species, so long as they are very closely related and able to breed.

Theory in context

TRANSGENIC SALMON

In 1989, a population of salmon were genetically modified (GM) so that they could grow all year-round, rather than only during spring and summer, which had been the norm. The goal of increasing the growth rate of GM salmon was to allow them to be sold earlier, which would in turn make them cheaper to grow compared to non-GM salmon.

Scientists created the GM salmon by inserting a foreign DNA construct composed of (1) a growth hormone gene from another salmon species, which was attached to (2) a promoter gene from another fish called a pout. The promoter gene increases the gene expression of the growth hormone (Figure 2a). The DNA construct was injected into newly fertilised salmon eggs, where it was incorporated into their genome (Figure 2b) and resulted in higher expression of growth hormone in all tissues.



Not drawn to scale

Figure 2 **(a)** The DNA construct containing a promoter from pout and a growth hormone-regulating gene from salmon. **(b)** Injection of the DNA construct into a fertilised salmon egg. **(c)** A GM salmon with increased growth ability.

genetic engineering technologies refers to the artificial alteration of an organism's genome via the exchange of foreign genetic material, typically from another organism. This is often done external to the organism via the use of a transfer vector such as a plasmid. Also known as **genetic recombination technologies**

silenced describes a gene that is prevented from being expressed

genetically modified organism (GMO) an organism with genetic material that has been altered using genetic engineering technology

host organism the organism which researchers wish to genetically modify

cisgenic organisms a genetically modified organism that contains foreign genetic material from a sexually compatible donor organism, typically from the same species

transgenic organism a genetically modified organism that contains foreign genetic material from a separate species (or recombinant DNA from the same species that has been manipulated before introduction)

Lesson link

Later in the course we will be covering selective breeding (***Lesson 9E***), which is a process that humans have been using for tens of thousands of years. The process involves breeding animals or plants that possess desirable traits to increase the prevalence of these traits in subsequent generations. For example, humans may only allow the fastest horses to mate to increase the number of fast horses in the population.

Selective breeding differs from genetic engineering in that, despite there still being an emphasis on increasing desirable traits, it does not require the genetic engineering techniques covered in this lesson.

Examiners' tip

In the past, the VCAA have typically shown that they do not explicitly test your knowledge of how GMOs are made (i.e. insertion, deletion, silencing of genes). However, they do expect you to be able to identify whether an organism is a GMO, TGO, or non-GMO based on a description of how it was made.

Consider the following prompt from the 2020 exam. The questions that the VCAA asked in response to this are indicative of the knowledge traditionally examined surrounding the concept of GMOs (Figure 3).

Rice (*Oryza sativa*) is a staple food for billions of people worldwide, particularly in Asia. Although rice supplies energy, it is low in micronutrients, such as iron and zinc. Australian scientists created a strain of biofortified rice that has been trialled in the Philippines and has been recently introduced to Bangladesh. The table below compares the iron and zinc content of normal white rice to that of biofortified rice in parts per million (ppm).

	Iron (ppm)	Zinc (ppm)
Normal white rice	2–5	16
Biofortified rice	15	46

The biofortified rice was created when two particular genes were inserted into normal rice. The biofortified rice plants responded as if they were iron deficient by permanently 'switching on' another gene to take up iron and zinc from the soil. Details of the two inserted genes are given in the table below.

Inserted gene	Protein function	Source of gene
rice nicotianamine synthase (OsNAS2)	assists iron uptake by roots of rice plants	rice plants
soybean ferritin (Sfer-H1)	binds and stores large amounts of iron	soybean plants

Source: VCAA Biology Exam 2020 Section A Q36–37.

Figure 3 The prompt for Q36 and Q37 in the 2020 VCAA Biology Exam.

Students were asked two multiple choice questions in relation to this prompt: one regarding the genetic composition of the biofortified rice and the other about the overall purpose of the research. Overall, around 90% of students got each answer correct, identifying the crop as transgenic as well as its purpose as combating malnutrition.

How we use GMOs in agriculture 3.1.12.2

OVERVIEW

GMOs have a range of important uses, especially in agriculture. Two of these uses are to increase crop productivity and to increase the disease resistance of the crop.

THEORY DETAILS

Scientists have been genetically modifying organisms for a range of purposes since the early 1970s. These purposes span many different disciplines and have academic, medical, agricultural, and industrial applications. For example, many different cell-based therapies make use of GMO technology, such as those designed for fighting diseases like cancer. However, for the purposes of VCE Biology, we will be focussing specifically on the agricultural uses of GMOs in this lesson. Two of these agricultural uses in particular are:

- increased crop productivity
- increased disease resistance of the crop.

Producing transgenic plants

Before considering each of these uses, let us take a brief look at how GMO technology is used to create transgenic plants for agriculture. This process typically involves three stages (Figure 4):

1 Gene identification: firstly, a particular gene of interest must be identified and isolated. This gene of interest will usually be found in the genome of another species, and have some important characteristics that would be useful for the host organism. For example, the gene of interest may help with more efficient uptake of soil nutrients, less reliance on fertilisers, and/or improved drought tolerance.

2 Gene delivery: next, the isolated gene of interest must be delivered into the cells of the host organism. This delivery may occur either via direct insertion of the DNA into the genome of the plant itself or through the use of a bacterial plasmid that is able to successfully transfer DNA between itself and the plant.

3 Gene expression: the transformed cell is then grown repeatedly (regenerated) using **plant tissue cultures** under sterile conditions before being applied in the field for agricultural use. The GM host organism is now able to express the new **transgene** as a useful protein and can regenerate itself.

plant tissue culture a range of techniques used to grow plant cells, tissues, or organs under sterile conditions using a nutrient culture medium, such as an agar plate or nutrient broth of known composition. It is widely used to produce clones of a plant

transgene a gene that has been artificially introduced into the genome of a separate organism (usually of another species)

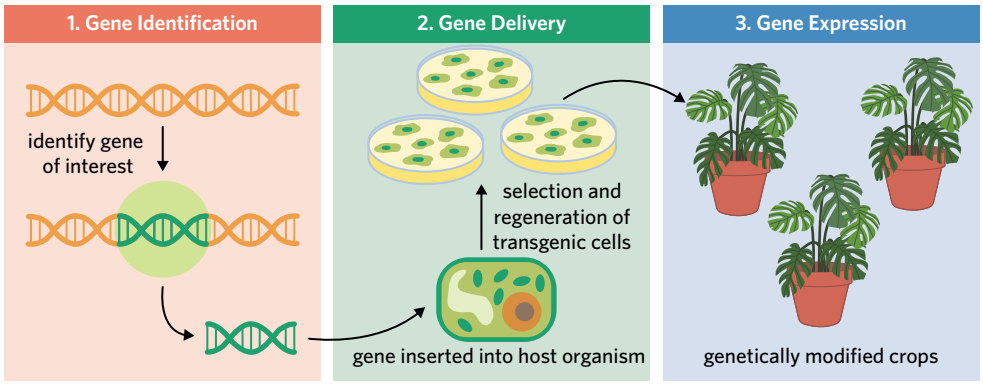


Figure 4 A simplified depiction of the production of transgenic crops: (1) a gene of interest is isolated and (2) delivered into the genome of a host organism, before being (3) cultured under sterile conditions and then applied in the field where they are able to produce proteins that were not previously part of their proteome.

Use in agriculture: increasing crop productivity

One important use of GMO technology in agriculture is to increase crop productivity. In other words, the agricultural industry uses GMO technology to increase its crop yield (how much crop is produced) per unit of farming land. The quality of crops can also be improved, for instance by increasing their nutritional value and their ability to grow in different conditions.

Why is this important? For starters, as the population of our species continues to grow, our demand for food will increase. At the time of writing this, the global human population is thought to be around 7.9 billion. Yet, according to the United Nations (2019), this number could rise to around 9.2 billion by 2040.

This increasing demand is compounded by a stagnating supply of grain and animal protein, especially as the availability of viable farmland decreases in the face of increasing environmental challenges. Additionally, a large proportion of the population growth is expected to occur specifically in developing countries, which already face a range of key economic, political, and social challenges that impact nutrition. Conventional breeding of crops is unlikely to be able to keep up with this situation indefinitely, and so the production of GMOs can help to fill this gap.

Theory in context

GOLDEN RICE – IMPROVING CROP PRODUCTIVITY

Golden rice is an example of a transgenic crop that was developed in response to vitamin A deficiency (VAD), which is a major cause of preventable blindness in children. This is especially the case in developing countries that don't have good access to expensive vitamin A-rich foods, such as eggs, dairy, and liver. Rice is a staple food in many of these countries, which inspired scientists to develop a strain of rice with increased levels of vitamin A to provide people with an easily accessible source of vitamin A.

Figure 5 Golden rice compared to regular rice

Golden rice was developed by inserting two genes into the genome of 'normal' rice: the *PSY* gene from a daffodil (*Narcissus pseudonarcissus*) and the *CRTI* gene from a soil bacterium (*Pantoea ananatis*). These genes cause the rice to store beta-carotene, a precursor of vitamin A, in the rice grains rather than in the leaves as normal rice would (Figure 5). This results in rice with a higher beta-carotene content, giving them their distinct yellow colour. Since the two genes inserted into the rice genome come from different species, golden rice is considered transgenic.

Use in agriculture: increasing disease resistance

A second important use of GMOs in agriculture is to increase a crop's resistance to disease. By developing crops that are less impacted by harmful plant pathogens, scientists can improve global food security by minimising crop destruction and the spreading of disease. Furthermore, not only can a crop's resistance to disease be increased, but so too can its resistance to other damaging environmental factors, such as drought and herbivorous pests.

Why is this considered important? Current estimates suggest that as much as 30% of global crop yield is lost as a result of plant pathogens and pests, and that these crop losses are highest in regions that already suffer from the most acute food insecurity (Savary et al., 2019). These losses are compounded by current estimates which suggest a need to increase food production by at least 60% using the same amount of land by 2050.

While sustained losses like those described above can occur year on year, there is also the very real danger of losing larger volumes of crops as a direct result of more widespread disease outbreaks. Consider Asian soybean rust, for example, which is a disease caused by the fungus *Phakopsora pachyrhizi* that was first reported in Brazil in 2001. Epidemics of the disease are common in the country and can cause crop yield losses of up to 90%. Diseases like this are devastating, especially for developing countries that rely heavily on agriculture. Genetically engineering plants that are resistant to disease is a path to reducing the risk of crop loss and ensuring a stable supply of food for the world.

Theory in context

BT CROPS – INCREASING PEST RESISTANCE

As mentioned, one use of GMOs in agriculture is to increase the protection afforded to certain crops against harmful pests. One example of this is Bt crops, which contain crystal toxin genes found in a particular bacterium known as *Bacillus thuringiensis* (Bt). The Bt bacterium naturally produces certain protein crystals that are toxic to many insect species that affect crop plants. Importantly though, these toxic protein crystals do not affect humans and are safe for consumption. If ingested by some insect species, however, the toxin will activate in the insect's intestines and cause it to die within a couple of days.

Scientists have cloned the crystal toxin genes from Bt and introduced them into crops, allowing plants to produce their own Bt toxin. This modification creates transgenic plants that are insect-resistant. The toxins are activated once they are ingested by insects, which stop feeding on the plant within a few hours. Plant species such as canola, cotton, maize, tobacco, rice, and eggplant have all been modified to contain Bt toxins.

Examiners' tip

The VCAA exams do not typically test your memory of facts relating to specific examples of GM crops, such as the golden rice and Bt crops described above. These are just examples that are commonly used in exams. Instead, the VCAA typically gives their own explanation of a GM crop and then tests your understanding of:

- whether the crop is transgenic or not
- why the GM crop was developed, including what issue it is aiming to address
- any biological, social, and ethical implications surrounding the GM crop.

Issues surrounding GMOs 3.1.12.3

OVERVIEW

As with many new biotechnologies, GMOs have been criticised and their use debated. Here we will consider a range of biological, social, and ethical implications that frame this debate.

THEORY DETAILS

While GMOs have proven to be incredibly beneficial in terms of increasing crop yields and disease resistance, they have also been criticised by some members of the community as potentially unsafe or unnatural. Genetically modified organisms, especially those designed for human consumption (i.e. foods and medicines), come with a range of biological, social, and ethical implications and are still hotly debated today. Some of these implications are discussed in Table 1.

Table 1 Summary of some of the biological, social, and ethical implications of GMOs

	Explanation
Biological implications	**Pros** • GM crops usually have better crop productivity than non-GM crops. This means that more food can be grown using less land, reducing habitat loss due to land clearing. • Insect-resistant GM plants require fewer pesticides, which is better for the environment. • GM foods can be made to have improved nutritional content, improving the health of individuals that consume them. **Cons** • GM crops may lose their effectiveness if weeds or pests evolve resistance. • Widespread use of GM crops could result in the loss of genetic diversity within crop populations. • Cross-pollination between GM crops and wild species or weeds may cause genes to spread and lead to unforeseen consequences.
Social implications	**Pros** • Increased crop productivity means more food can be produced, leading to better food security. • Crops that are able to grow in more adverse conditions (e.g. drought-tolerant corn) protect against famine, improving food security. • Herbicide-tolerant crops reduce labour demands as farmers don't need to pull weeds by hand, instead spraying chemicals that selectively kill weeds but not crops. • Increased crop yields result in larger profits for farmers. • GM foods can be made to have improved flavour and texture, giving consumers a more appealing product. • GM foods can be made to have improved nutritional content. This leads to a reduction in nutritional deficiencies, creating healthier populations. **Cons** • Having to buy new seeds each season may be costly for farmers. • Complex legal issues surrounding the use of GM products may cause farmers undue stress and anxiety related to regulation. • There are strict packaging and marketing regulations for GMO producers that may not be complied with if either the producer or consumer are undereducated on these regulations.
Ethical implications	**Pros** • Some people believe that using genetic modification is an ethical imperative given the potential for widespread benefits, including nutrition, wealth, and the overall health of humanity, especially in developing nations. **Cons** • Some people consider GMOs to be unnatural, or like we are 'playing God'. • Some people believe that GM foods are unsafe to eat and choose not to eat them as a result. This is especially true if there is lack of long-term evidence of healthy use. • Some people believe that genetically modifying animals for human benefit is inhumane – many anti-animal GMO arguments apply to animal agriculture in general. • The fact that companies can own the rights to GM crops is considered by some to be unethical due to companies possibly making unfair demands of farmers. This ownership power divide can materialise in a range of ways, including the following: – Cross-pollination of non-GM crops by nearby GM crops could result in the non-GM farmer being sued by the patent-owner. – Farmers can't reuse seeds from some GM crops and must buy new expensive seed supplies each year from biotechnology companies.

Theory in context

THE IMPLICATIONS OF GOLDEN RICE

Earlier in the lesson we discussed golden rice, a particular GMO designed to help improve crop productivity – particularly for developing countries which have limited access to vitamin A-rich foods. Let us consider the potential implications of this GMO crop in terms of the biological, social, and ethical implications discussed in this section of the lesson.

Pros:

- Golden rice has increased beta-carotene content, which may help people in developing countries avoid vitamin A deficiency (VAD).
- Golden rice seeds can be kept and replanted the next season, making it significantly cheaper than other GM seeds.
- Trials of golden rice have shown that it is safe to eat, and that cross-pollination between GM rice and non-GM rice is unlikely given that rice plants predominantly self-pollinate.
- Less VAD means a lower incidence of preventable blindness and fewer deaths.

Cons:

- Some groups argue that widespread use of golden rice could reduce crop biodiversity.
- Some groups argue that golden rice produces too little beta-carotene to eradicate VAD completely, and should therefore be avoided in favour of other alternatives, such as those that might better address the range of social, economic, and cultural factors that contribute to VADs in the first place.
- Golden rice programs might interfere with existing vitamin A supplementation programs and campaigns. For example, UNICEF implements a vitamin A supplementation program that has been said to improve a child's survival rate by 12–24%. This program costs only a few cents per child and is thought to be a much cheaper and well-proven alternative to GMO use.

Theory summary

In this lesson, you have learned that a genetically modified organism (GMO) is any organism whose genetic material has been altered using genetic engineering technology. This alteration often occurs via the transfer of target genes from one organism to another in order to confer some advantageous trait previously lacking in the host organism.

A transgenic organism is an example of a GMO that contains foreign genetic material from a separate species (or recombinant DNA from the same species that has been manipulated before introduction). Transgenic plants are often used in agriculture to improve crop productivity and disease resistance, particularly in developing nations, and are met with a range of interesting ethical, biological, and social implications that generate ongoing debate.

Researchers at Utah State University isolated the gene that codes for the production of dragline silk in orb-weaver spiders and inserted it into the genome of a goat, specifically amongst the DNA that prompts milk production in the udders. The transformed cells were inserted into an egg and implanted into a mother goat, who gave birth to a child whose milk was full of spider-silk protein.

A goat was used as an intermediary for farming dragline silk as opposed to the spiders themselves, who are very cannibalistic and difficult to farm together. While this research is ongoing and little commercial application has resulted thus far, the potential applications are exciting. For example, the technology could eventually be used to manufacture new ligaments for injured individuals, as well as defence materials like bullet-proof vests.

4F QUESTIONS

Theory review questions

Question 1

Which of the following best describes a genetically modified organism?

A A GMO is always characterised by the ability to produce proteins not part of their original proteome.

B Any organism that has had a gene inserted into its genome, with the goal of conferring a particularly advantageous characteristic such as drought tolerance.

C Any organism that has had its genome altered using gene engineering technologies, typically via the transferring of genes from one organism to another.

Question 2

Match the key terms from the lesson to their definition.

Key term

- genetic engineering
- host organism
- cisgenic organisms
- transgene

Definition

I _____ a genetically modified organism that contains genetic material from a sexually compatible donor organism

II _____ the process of using biotechnology to alter the genome of an organism, typically with the goal of conferring some desirable trait

III _____ a segment of DNA from one organism that is introduced into the genome of another organism, typically from a separate species

IV _____ the organism which researchers wish to genetically modify, typically via the alteration of its genome and/or the insertion of a transgene

Question 3

Order the steps to correctly describe the process of producing a transgenic plant. Note that this process has been highly simplified.

I The transgenic plant is put into the field and expresses new proteins.

II The transformed cells are repeatedly regenerated under sterile conditions.

III A gene of interest is identified and isolated.

IV The gene of interest is extracted and delivered into the cells of the host organism.

Question 4

Which of the following options contains all correct statements about golden rice?

A	It is a transgenic organism	It produces 100% of an adult's daily vitamin A intake	An ethical implication is that it improves nutrition by increasing vitamin A intake	A social implication is that farmers must buy new seeds each year, which can be expensive
B	It is not a transgenic organism	It is an accessible source of vitamin A	A social implication is that farmers can save rice to be used in the next harvest	The genes inserted into golden rice occur in other species naturally, therefore it is not transgenic
C	It is a transgenic organism	It contains high levels of beta-carotene	The *PSY* gene from the daffodil is considered a transgene when inserted into golden rice	A biological implication is that its large-scale adoption may pose a threat to crop biodiversity
D	It is not a transgenic organism	It is an accessible source of vitamin A	A biological implication is that herbicide-resistant genes may spread to weeds	An ethical implication is that creating GM foods is 'tampering with nature'

Question 5

Categorise the following statements as either **non-GMO**, **GMO**, and/or **TGO**. Terms may have multiple categories.

I A vaccine containing an attenuated virus is injected into a patient. _____

II A gene is removed from the genome of an organism by researchers. _____

III An organism is crossed with a closely related species to produce hybrid offspring. _____

IV An allele from a different individual of the same species is inserted into the genome of a host organism. _____

V A gene from a separate species is inserted into an organism's genome by researchers. _____

VI Scientists design a vector that inserts DNA from a different species into the genome of germ cells. _____

SAC skills questions

Case study analysis

Use the following information to answer Questions 6–9.

In Australia, the development of GMOs is regulated by the Office of the Gene Technology Regulator (OGTR), which is an independent statutory body responsible for administering the *Gene Technology Act 2000*. According to the OGTR, the current known uses of GMOs in Australia include (but are not limited to): (1) three genetically modified (GM) crops: canola, cotton, and safflower (with others undergoing field trials); (2) the development of important medicines such as insulin, and vaccines such as Gardasil; (3) genetic and proteomic research using genetically modified bacteria, plants, and animals in laboratories.

While only canola, cotton, safflower, and carnations are approved for commercial release in Australia, there are approximately 80 different types of GM crops grown worldwide. Most of these GM crops are modified canola, soybean, maize, and cotton. Some of the currently approved foods that have been produced using gene technology are summarised in the following table.

Crop	Food derived from	Commercial trait
Soybean	Herbicide-tolerant soybean line 40-3-2	Glyphosate tolerance
	Insect-protected soybean line MON87701	Lepidoptera protection
	Nematode-protected and herbicide-tolerant soybean line GMB151	Nematode protection, Glufosinate tolerance
Canola	Herbicide-tolerant canola line MON88302	Glyphosate tolerance
	Herbicide-tolerant canola line MON94100	Dicamba tolerance
Corn	Amylase-modified corn line 3272	Thermostable alpha-amylase production
	Drought-tolerant corn line MON87460	Drought tolerance
Cotton	Herbicide-tolerant cotton line 1445	Glyphosate tolerance
	Insect-protected cotton line MON88702	Hemiptera & Thysanoptera protection

Question 6

According to the information provided, which of the following is not a current known use of GMOs in Australia?

A Using genetically modified organisms in a lab to study the role and function of different genes.

B Using genetically modified organisms to replace the diet of undernourished communities with enhanced canola.

Question 7

According to the information provided, which of the following statements is most likely correct?

A In Australia, a range of foods that were produced using GMO technologies are available to the general public. These include soybeans, canola, corn, and cotton.

B In Australia, only a small proportion of approved GMOs are available for commercial use. Others, such as drought-tolerant corn, are approved by intergovernmental bodies such as The Organisation for Economic Co-operation and Development (OECD).

Question 8

Based on the information provided, glyphosate is most likely

A a herbicide.

B a protein for herbicide tolerance.

Question 9

In Australia, the use of GM foods is regulated by Food Standards Australia New Zealand (FSANZ). In this role, FSANZ is responsible for ensuring a full-scale safety assessment and subsequent approval of GM foods before they can be sold in Australia and New Zealand. They also mandate that all GM foods and ingredients must be labelled with the words 'genetically modified' when being sold.

Based on your understanding of bioethics, which of the following options most accurately matches the regulation with the corresponding bioethical concept?

	Safety assessment	Mandatory labelling
A	justice	integrity
B	non-maleficence	justice
C	non-maleficence	integrity
D	beneficence	respect

Exam-style questions

Within lesson

Question 10 (1 MARK)

All genetically modified organisms

A have some form of agricultural application.

B have at least one gene that is removed or silenced.

C contain at least some genetic material obtained from another species.

D have at least one section of their DNA that has been altered by scientists.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q31

Question 11 (1 MARK)

All transgenic organisms

A are genetically modified organisms.

B have at least one gene that is prevented from being transcribed.

C contain a gene from another species that gives them herbicide tolerance.

D have at least one gene that is silenced by genetic material obtained from another species.

Use the following information to answer Questions 12 and 13.

Cotton is an Australian crop grown for its fibres that may be spun to produce clothing, towels, and other fabrics. Due to the large number of insect pests that feed on cotton, particularly the cotton bollworm, insecticides must be extensively used in its production. These insecticides harm non-pest insect species, are expensive, and may have effects on human health too. A solution to this is Bt cotton, a strain of cotton that contains two genes from the soil bacterium *Bacillus thuringiensis*. These genes encode proteins that disrupt the digestive system of the cotton bollworm. If a cotton bollworm eats part of a Bt cotton plant, these proteins will enter its gut and kill it.

Question 12 (1 MARK)

Which of the following statements regarding Bt cotton is true?

A Bt cotton is not a genetically modified organism.

B Bt cotton requires less insecticide than regular cotton.

C Bt cotton is cisgenic because it contains genes from a bacterium.

D Yields from Bt cotton crops are lower than regular cotton due to the *B. thuringiensis* genes having a negative effect on plant growth.

Question 13 (1 MARK)

The role of the inserted genes from *B. thuringiensis* is to

A improve the fibre density of cotton.

B kill cotton bollworms that ingest Bt cotton.

C give Bt cotton resistance to insecticides that are routinely used.

D stimulate the plant's immune system to help fight off cotton bollworms.

Use the following information to answer Questions 14 and 15.

The *Anopheles* genus of mosquito are vectors for the malaria parasite *Plasmodium*. In an attempt to reduce the spread of malaria, researchers in the lab have developed sterile male *Anopheles* that are unable to produce sperm. These mosquitoes were created by removing a gene that is vital for sperm development. Female *Anopheles* only mate once, so mating with these sterile males would result in that female producing no offspring.

Question 14 (1 MARK)

Which of the following statements is true?

A The lab strain of *Anopheles* is not transgenic since no foreign DNA has been introduced.

B The *Anopheles* strain developed in the lab is considered transgenic since its genome has been altered.

C The *Anopheles* male mosquitoes developed in the lab are transgenic since they contain DNA from another species.

D The removal of the gene in lab *Anopheles* does not count as genetic modification since the fitness of the mosquito is not improved.

Question 15 (1 MARK)

A likely biological implication of introducing sterile male *Anopheles* into the wild is that

A the introduced DNA in the sterile males could spread to other *Anopheles* species.

B wild *Anopheles* may inherit the gene that makes males sterile resulting in extinction of the species.

C the spread of malaria will decrease due to the introduced gene found in sterile male mosquitoes.

D the wild *Anopheles* population would decrease due to non-viable matings between wild females and sterile males.

Question 16 (3 MARKS)

Roundup Ready™ Cotton is a herbicide-tolerant cotton product that is tolerant to glyphosate, the active ingredient in Roundup™ herbicide. The glyphosate-tolerant cotton (line 1445) was developed using the *Agrobacterium tumefaciens* transformation system to introduce a gene from a strain of common soil bacterium.

a Explain why an individual plant of Roundup Ready™ Cotton could be described as both a genetically modified organism and a transgenic organism. (2 MARKS)

Adapted from VCAA 2017 Sample Exam Section B Q9C

b Outline one economic advantage for farmers using Roundup Ready™ Cotton as opposed to regular cotton. (1 MARK)

Adapted from VCAA 2005 Biology Exam 2 Q6C

Multiple lessons

Question 17 (3 MARKS)

The Zika virus is a disease spread through mosquito bites, specifically by the bite of the *Aedes aegypti* female mosquito. Their male counterparts do not bite and therefore cannot spread the virus.

In order to control the spread of the virus, scientists decided to test ways of controlling reproduction in *A. aegypti* populations. One solution is to release genetically modified *A. aegypti* males who each carry a gene that makes their offspring die before reproductive age. The GM males will be released into wild *A. aegypti* populations and allowed to mate with females carrying the Zika virus.

a Explain how the GM mosquitoes will affect *A. aegypti* populations. (2 MARKS).

b The GM mosquitoes in this study are also examples of transgenic organisms.

What feature of the genetic code makes it possible for a gene to be transferred from one species to another and to be expressed in the second species? (1 MARK)

Question 18 (5 MARKS)

Corals are animals that build up calcium carbonate skeletons. Single-celled algae live within the coral tissues. Queensland scientists have reported that many of the corals in a region of the Great Barrier Reef have recently become bleached. Bleaching occurs when the single-celled algae leave the coral tissues due to environmental changes. Corals turn white without the algae present and may die. This bleaching has been attributed to an increase in water temperature and acidity.

Two approaches have been proposed to help reverse the bleaching occurring in the coral reefs.

Approach 1

Scientists introduce a particular gene into the genome of the algae that is taken from specific bacteria that can survive in highly acidic sulphur pits. Particular corals with modified algae are then reintroduced into the coral reef.

Approach 2

Scientists obtain algae of the same species that are adapted to warmer climates and release them onto the Great Barrier Reef.

a Explain how the first approach may help reverse coral bleaching. (2 MARKS)

b Suggest one possible social implication of the first approach. (1 MARK)

c Explain whether the second approach would be considered genetic modification or not. Justify your response. (2 MARKS)

Adapted from VCAA 2017 Northern Hemisphere Exam Section B Q11

Key science skills and ethical understanding

Question 19 (7 MARKS)

Citrus greening is a disease that affects citrus trees, such as orange trees. The disease is caused by the bacterium *Candidatus Liberibacter asiaticus*. These bacteria are transferred to the trees when insects called psyllids feed on the sap in their leaves. The bacteria live in the plants' nutrient-conducting tissues (phloem), causing slow death of the trees.

A solution to this disease uses a gene from a spinach plant, which codes for a defensin protein. The defensin protein binds to and 'punches holes' in the bacteria, breaking them apart. Genetic engineers have inserted the defensin gene into a viral vector which is taken up by the bacteria. The viral vector is a modified form of a virus that normally infects citrus trees.

The genetically engineered viral vectors were placed in many orange trees through small incisions in the trees' bark. They also kept several orange trees that did not receive the treatment to act as controls. After several years' growth, the scientists checked the trees for citrus greening. They found that none of the treated trees were affected by citrus greening while all of the untreated trees nearby were affected by citrus greening.

a Identify the independent and dependent variables in this experiment. (2 MARKS)

b State what the genetic engineers' hypothesis would be. (1 MARK)

c Outline two measures that would need to be taken to ensure the results of this experiment are reliable. (2 MARKS)

d Before governments allow the commercial production of these citrus greening-resistant orange trees, extensive field trials must be carried out by scientists.

With reference to the bioethical concept of non-maleficence, suggest one reason as to why extensive field trials are necessary in this scenario. (2 MARKS)

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q39

CHAPTER 4 SUMMARY

Enzymes that manipulate genetic material

- **endonuclease –** cuts fragments of genetic material
- **ligase –** joins fragments of genetic material
- **polymerase –** synthesises fragments of genetic material

DNA profiling

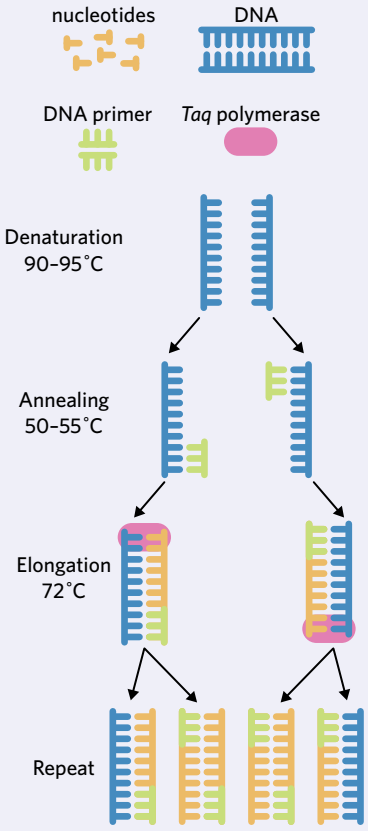
1. Obtain sample DNA

A DNA sample is extracted from blood, cheek or nasal swabs, hair, biopsies, or teeth samples.

2. Amplify sample using PCR

The polymerase chain reaction is a technique used to amplify a sample by synthesising new complementary strands of DNA.

PCR cycle components

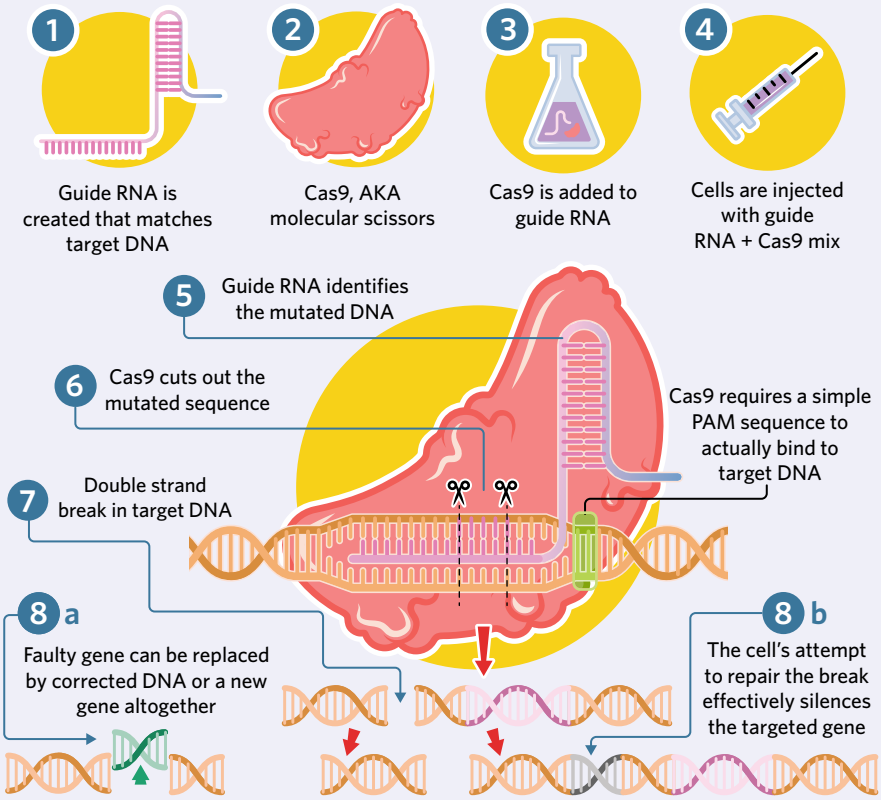


3. Sort DNA fragments by length in a gel

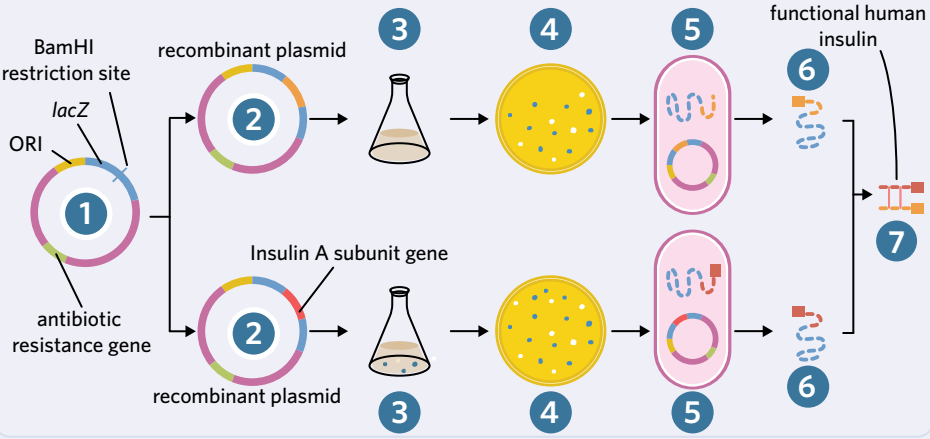
Gel electrophoresis is a laboratory technique which seperates DNA fragments by size and allows them to be measured and compared to other fragments. Comparison of fragments can be used in:

- paternity testing
- genetic screening
- crime scene analysis.

CRISPR-Cas9



Making recombinant bacteria to produce insulin



Agricultural applications of DNA manipulation

Editing the genomes of plants can increase crop productivity and improve disease resistance. These plants are known as genetically modified organisms (GMOs). If the gene included in their genome is from another species, they are also known as a transgenic organism (TGO). It is important to consider the biological, social, and ethical considerations of these applications.

Genetically modified organisms (GMOs)

Transgenic organism
The organism's genome has been introduced with foreign DNA from another species

Genetically modified using gene engineering technology

Cisgenic organism
The organism's genome has been introduced with foreign DNA from the same species

CHAPTER 4 SAC PRACTICE

SAC skills covered in this section:

✓ Bioethical deep dive ✓ Case study analysis ✓ Scientific methodology comparison

RECOMBINANT PRODUCTION OF HUMAN GROWTH HORMONE (20 MARKS)

Human growth hormone

Human growth hormone (hGH) is produced naturally by the pituitary gland, from where it can circulate freely in the bloodstream and interact with protein hormone receptors on a variety of cell membranes. In doing so, hGH plays an important role in your growth and development, as well as cellular repair and metabolism. Growth hormone deficiency is a rare genetic condition that leads to delayed growth and development, fine hair, weak nails, and often contributes to obesity.

Individuals with growth hormone deficiency are treated with recombinant human growth hormone. Whilst this drug is a necessity for those who are deficient in hGH, it is also illegally consumed by athletes wishing to gain performance-enhancing side-effects. hGH has been detected in athletes from many different sports including: bodybuilding, wrestling, swimming, baseball, track and field, soccer, weightlifting, and skiing.

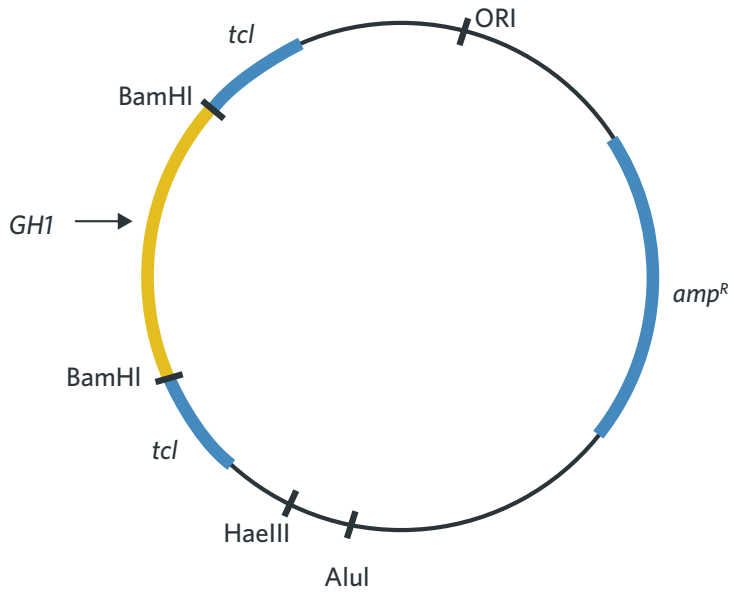
1 Identify the function of hGH. (1 MARK)

2 There are multiple disorders that prevent normal growth. Explain how a deficiency in hGH could be tested. (1 MARK)

3 By referring to the bioethical concept of justice, explain why hGH consumption in sport is banned. (2 MARKS)

Producing an hGH recombinant plasmid

hGH is coded for by the *GH1* gene. After obtaining a healthy version of the *GH1* gene, scientists amplify the gene using the polymerase chain reaction. From this, a recombinant plasmid can be created including the *GH1* gene, an origin of replication (ORI), and two antibiotic resistance genes: ampicillin resistance (*ampR*) and tetracycline resistance (*tcI*). Additionally, there are restriction sites for the AluI, HaeIII, and BamHI endonucleases as shown in the diagram.



4 Outline the steps in the polymerase chain reaction. (3 MARKS)

5 With reference to the diagram, explain whether a bacterium that takes up this plasmid would be resistant to tetracycline and ampicillin. (2 MARKS)

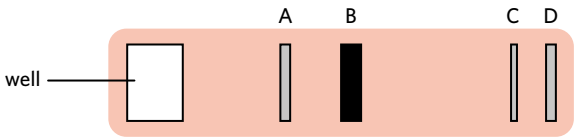
6 Explain how the *GH1* gene is likely incorporated into the plasmid vector. (2 MARKS)

7 What is a possible advantage of using restriction endonucleases instead of CRISPR-Cas9 for this experiment? (1 MARK)

To test whether plasmids have taken up the *GH1* gene, scientists can cut the plasmids with AluI then run the sample through gel electrophoresis. It was expected that four bands would be seen on the gel representing:

- a linear fragment cut by AluI which contains the gene of interest
- a linear fragment cut by AluI which does not contain the gene of interest
- a supercoiled fragment that was not cut by AluI and contains the gene of interest
- a supercoiled fragment that was not cut by AluI and does not contain the gene of interest.

It is known that supercoiled fragments pass through the agarose gel more quickly. The results of the gel can be seen in the following diagram.



8 Which band(s) shown on the gel represent plasmids that were not cut by the restriction endonuclease? (1 MARK)

9 With reference to the experimental results, justify whether the majority of plasmids took up the gene of interest. (2 MARKS)

Production of hGH

In order to produce recombinant hGH, the recombinant plasmids are mixed in an *E. coli* culture and, through the heat shock method, are taken up by some bacteria. The heat shock method involves cooling the bacteria and plasmid mixture on ice then transferring the mixture into a 40 °C water bath for 30 seconds, before returning it to the ice.

An alternative method to recombinant hGH is extracting growth hormone (GH) from animals like pigs and purifying it to be prescribed to humans. This involves killing the animal and harvesting hormones from its pituitary glands.

10 Describe the purpose of heat shocking the *E. coli* bacteria. (2 MARKS)

11 Using a virtues-based approach to bioethics, evaluate which method of hGH production is more appropriate. (2 MARKS)

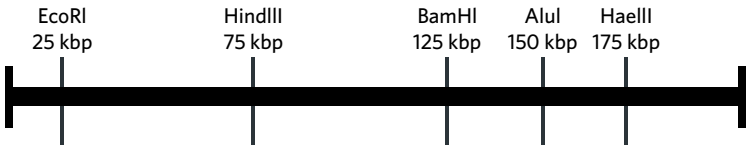
12 Outline a biological implication of using pig growth hormone in humans. (1 MARK)

CHAPTER 4 EXAM PRACTICE

Section A (15 MARKS)

Question 1 (1 MARK)

Consider the following linear section of DNA that has a total length of 200 kbp. The recognition sites of five different restriction endonucleases are shown. The linear section of DNA was treated with the three restriction endonucleases BamHI, HaeIII, and HindIII.



How many fragments of DNA would be produced and what would the lengths of these fragments be?

	Number of fragments	Length (kbp)
A	3	50, 50, 75
B	4	25, 25, 75, 75
C	4	25, 50, 50, 75
D	6	25, 25, 25, 25, 50, 50

Question 2 (1 MARK)

The CRISPR-Cas9 technique is a gene editing method. It involves a protein called Cas9 and a short piece of guide RNA (gRNA). The gRNA leads Cas9 to a gene in DNA that scientists wish to edit.

What action is Cas9 expected to perform?

A cut DNA fragments
B join DNA fragments
C sort DNA fragments
D amplify DNA fragments

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q9c

Question 3 (1 MARK)

Recombinant bacterial plasmids are vectors used to transform bacteria. In this context, 'vectors' are

A used as unedited genetic material that acts as a control in experiments.
B used as a means of transporting foreign DNA into a cell/organism.
C proteins that are produced by the transformed bacteria.
D agents that transmit disease.

Adapted from VCAA 2017 Section B Q9a

Question 4 (1 MARK)

In DNA manipulation, researchers often use polymerases.

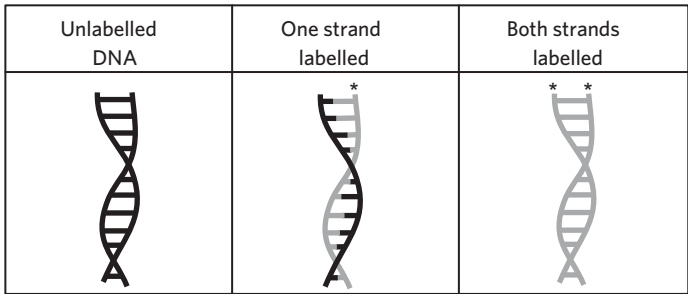
The function of a polymerase is to

A create nucleotides from organic and inorganic materials.
B join a target gene to plasmid DNA at complementary sticky ends.
C clone a plasmid in order to produce enough plasmids to ensure effective treatment.
D cut the DNA of the plasmid and a gene in the same manner in order to produce matching sticky ends.

Adapted from VCAA 2016 Section A Q36

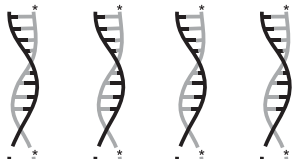
Question 5 (1 MARK)

Radioactively labelled nucleotides were incubated with an unlabelled molecule of double-stranded DNA. Appropriate substances were added and the DNA was allowed to replicate for three cycles of the polymerase chain reaction. Examine the following key.

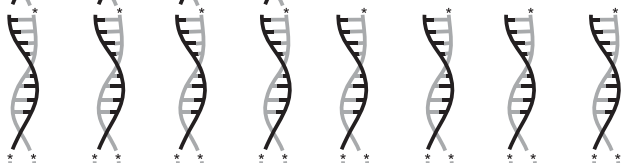


Given that three cycles of DNA replication occurred, which of the following depicts the end result?

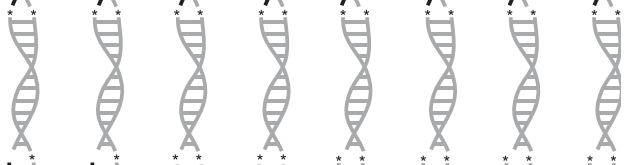
A



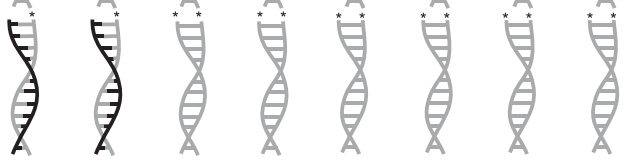
B



C



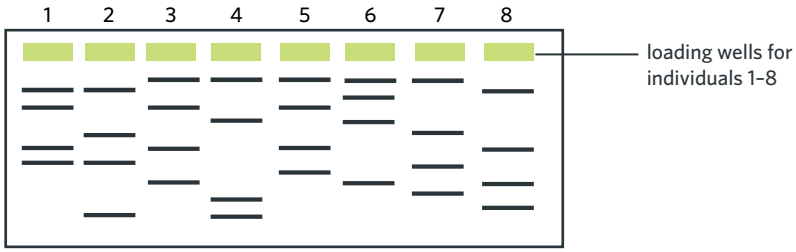
D



Adapted from VCAA 2011 Exam 2 Section A Q11

Question 6 (1 MARK)

Genetic testing can be used to test for the allele responsible for haemophilia, a genetic disorder that causes abnormal blood clotting, making it difficult to control bleeding. Eight individual family members were tested for the mutated haemophilia allele. The diagram shows the gel electrophoresis results of a test for the presence of the mutated allele. Individuals 1 and 3 have been diagnosed with the disease.



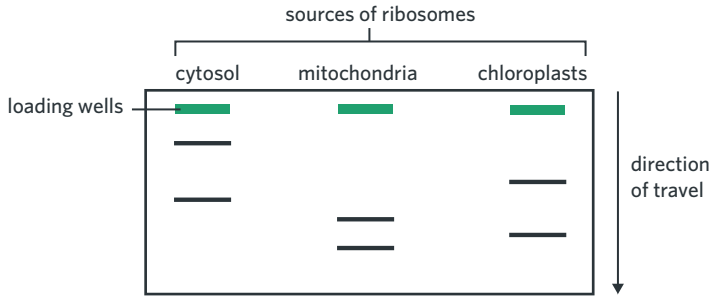
It was found that one other individual suffers from the disease. Which other individual is this most likely to be?

A 2
B 5
C 6
D 8

Adapted from VCAA 2018 Section A Q34

Question 7 (1 MARK)

A ribosome contains two distinct subunits: a large subunit and a small subunit. Ribosomes from different regions within plant cells were isolated and subjected to protein gel electrophoresis. The results are shown.



Which one of the following can be correctly concluded from the gel electrophoresis results?

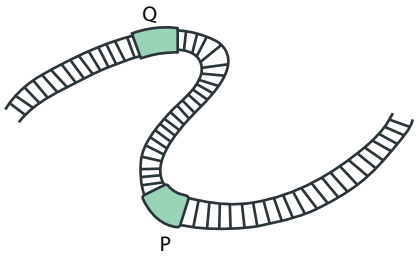
A Chloroplast ribosomal subunits have opposite charges.
B Mitochondria contain the smallest ribosomal subunits.
C Cytosolic ribosomal subunits travel at the greatest speeds.
D Cytosolic and mitochondrial ribosomes translate the same types of protein.

Adapted from VCAA 2015 Section A Q28

Question 8 (1 MARK)

The DNA of a small virus is depicted in the diagram, showing the positions of cutting sites (P and Q) for two restriction endonucleases.

The length of DNA fragments obtained when using these restriction endonucleases is shown in the table.



Restriction endonuclease used	Cutting site	Length of DNA fragments obtained (kbp)
AluI	Q	2, 8
HaeIII	P	6, 4

If both AluI and HaeIII are used together on this viral DNA, the length (in kbp) of the fragments obtained would be

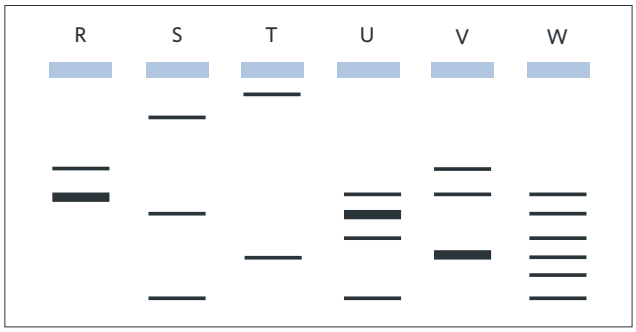
A 2, 8, 6, 4.
B 2, 14, 4.
C 2, 4, 4.
D 6, 4.

Adapted from VCAA 2008 Exam 2 Section A Q20

Use the following information to answer Questions 9 and 10.

The diagram represents a DNA molecule and the position of the recognition sites for the restriction endonucleases BamHI, EcoRI, and HindIII.

The following diagram shows an electrophoresis gel in which lanes R–W show the separation of DNA fragments resulting from digestion with one or more of the given restriction endonucleases.



Question 9 (1 MARK)

Which of the following shows the correct match between the lane and the restriction endonuclease(s) used to digest the DNA molecule?

	R	S	T	U
A	HindIII	EcoRI	BamHI	EcoRI, HindIII
B	BamHI	HindIII	BamHI	BamHI, HindIII
C	HindIII	BamHI	EcoRI	EcoRI
D	EcoRI	BamHI	HindIII	BamHI, EcoRI

Adapted from VCAA 2017 Section A Q38

Question 10 (1 MARK)

Which combination of restriction endonucleases were used to digest the DNA found in lane V?

A EcoRI, HindIII
B BamHI, EcoRI
C BamHI, HindIII
D BamHI, EcoRI, HindIII

Question 11 (1 MARK)

Scientists culture bacteria on four different nutrient agar plates. Two plates contain transformed bacteria which should have taken up a recombinant plasmid containing an antibiotic resistance gene. The results from this experiment are shown in the table.

Plate	**K** **untransformed bacteria only**	**L** **untransformed bacteria only**	**M** **transformed bacteria**	**N** **transformed bacteria**
Added to plate	nutrient agar only	nutrient agar and antibiotic	nutrient agar only	nutrient agar and antibiotic
Description of bacterial growth	lawn growth	no growth	lawn growth	colonies present

Which plate(s) act(s) as a control group in this experiment?

A plate K only
B plates K and L
C plates K and M
D plates K, L, and M

Use the following information to answer Questions 12 and 13.

Vitamin A deficiency is a major cause of preventable blindness in children, particularly in developing countries. This is due to insufficient vitamin A in their diet, which consists largely of rice. To fix this issue, scientists developed golden rice. Golden rice is a strain of rice that contains high levels of beta-carotene, an important precursor in the synthesis of vitamin A. The *PSY* gene from daffodils (*Narcissus pseudonarcissus*) and the *CRTI* gene from a soil bacterium (*Pantoea ananatis*) are inserted into a strain of rice (*Oryza sativa*), which alter the beta-carotene biosynthesis pathway. These genes cause beta-carotene to be stored in the rice grains which people eat, rather than in the leaves as would occur in regular rice.

Question 12 (1 MARK)

Which of the following statements indicates that golden rice is a transgenic organism?

A The golden rice genome contains segments of DNA that have been altered.
B The *CRTI* gene in golden rice comes from *P. ananatis*.
C *O. sativa* naturally stores beta-carotene in the leaves.
D Golden rice is a different colour from regular rice.

Question 13 (1 MARK)

One advantage of using a genetic engineering solution to increase beta-carotene content in golden rice is that it will

A give rice resistance to pests that suck vitamin A out of rice.
B remove the need to increase insecticide applications in rice crops.
C provide an easily accessible source of vitamin A in developing countries.
D avoid public concern about the possible risks of genetically modified food.

Use the following information to answer Questions 14 and 15.

Scientists have developed genetically modified salmon that are able to grow to market size in 16 months rather than three years. Raising GM salmon in aquaculture serves as a viable alternative to wild-caught salmon and may reduce overfishing of wild salmon populations.

Question 14 (1 MARK)

Reducing the impact on wild salmon populations would best be described as an example of

A a biological implication of GMOs.
B an ethical implication of GMOs.
C a social implication of GMOs.
D a legal implication of GMOs.

Question 15 (1 MARK)

Reducing the time and cost required to raise farmed salmon would be an example of

A a biological implication of GMOs.
B an ethical implication of GMOs.
C a social implication of GMOs.
D a legal implication of GMOs.

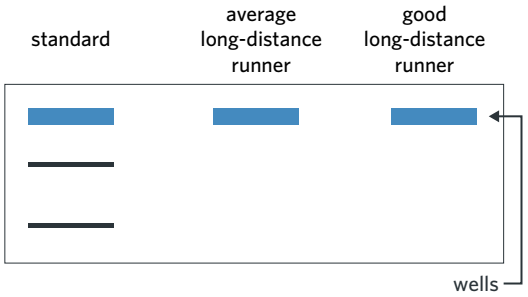
Section B (25 MARKS)

Question 16 (9 MARKS)

Scientists investigating the performance of athletes found that one gene contributing to the performance of sprinters is the *ACTN3* gene. There are two alleles of the gene, the *577R* allele and the *577X* allele. The *577X* allele codes for a very short protein fragment in muscle fibres due to a stop codon mutation. The table summarises the athletic potential for the three possible genotypes for the *ACTN3* gene.

***ACTN3* genotype**	**Athletic potential**
577R / 577R	good sprinter
577R / 577X	average sprinter or long-distance runner
577X / 577X	good long-distance runner

A scientist tested sprinters to see if they possessed the *577R* allele. Samples were obtained from athletes' muscle fibres and amplified using the polymerase chain reaction. A standard containing proteins of the same lengths as those coded for by both alleles *577X* and *577R* was used as a comparison. The standard and the samples were analysed using gel electrophoresis. The result for the standard is shown in the diagram.



a During the polymerase chain reaction, explain why the temperature is reduced in the annealing stage and then increased in the elongation stage. (3 MARKS)

b On a separate piece of paper, draw the above diagram and draw the bands expected for an average long-distance runner and for a good long-distance runner. (2 MARKS)

c Explain why these bands should be in these positions. (2 MARKS)

d Explain the purpose of the standard ladder in this experiment. (2 MARKS)

Adapted from VCAA 2012 Exam 2 Section B Q2b

Question 17 (6 MARKS)

Scientists are trying to develop a drought-tolerant strain of corn (*Zea mays*) for agricultural use in increasingly warmer climates. Drought-tolerant crops are able to produce yields in mild drought conditions, whereas regular crops produce little to none in the same conditions. The first approach the scientists considered was to breed the drought tolerance trait into *Z. mays* by crossing them with a wild corn species that had natural drought tolerance.

a Explain how developing drought-tolerant corn would increase crop productivity. (1 MARK)

b The scientists found that the cross between *Z. mays* and the wild corn species resulted in no viable offspring due to the two species being too distantly related. Scientists studied this wild corn species further and found that the drought tolerance trait was due to three genes called *NOT*, *TOO*, and *HOT*.

- **i** Other than undertaking selective breeding, outline how scientists could create drought-tolerant *Z. mays* crop. (1 MARK)
- **ii** Genetically engineered *Z. mays* is relatively expensive to obtain. Using a consequences-based approach to bioethics, explain why a farmer may choose not to use it. (1 MARK)

c When considering genetic engineering of the *Z. mays* crops, the scientists considered using CRISPR-Cas9 technology.

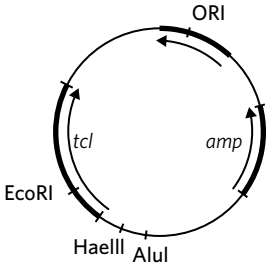
- **i** State one advantage of using CRISPR-Cas9 over restriction endonucleases. (1 MARK)
- **ii** Describe the changes CRISPR-Cas9 could make to the *Z. mays* genome. (2 MARKS)

Question 18 (10 MARKS)

A particular bacterial plasmid contains recognition sites for the restriction endonucleases EcoRI, AluI, and HaeIII, along with two antibiotic-resistant genes, ampicillin resistance (*amp*), tetracycline resistance (*tcl*), and an origin of replication (ORI). Scientists are attempting to insert the human gene for dystrophin into the plasmid for bacterial transformation.

a Explain how plasmids can be used to make bacteria produce human dystrophin. (3 MARKS)

b The diagram shows the positions of these recognition sites and antibiotic-resistant genes as well as the position of the origin of replication within this plasmid.



The restriction endonuclease EcoRI was used to insert the gene coding for human dystrophin into this plasmid.

i Draw and label a diagram to show the position of the human dystrophin gene in this plasmid when EcoRI is used. Include the position of the recognition sites for the restriction endonucleases AluI and HaeIII on the plasmid. (1 MARK)

ii After the scientists had carried out the steps required to make plasmids with the inserted human gene, the plasmids were mixed with a culture of bacteria. This mixture was treated so that the plasmids would move into the bacterial cells. Not all bacteria took up these plasmids, and some took up plasmids that had not successfully incorporated the dystrophin gene. Explain how scientists can select for transformed bacteria within the sample. (2 MARKS)

c The table outlines the recognition sequences of each of the restriction endonucleases that could cut the plasmid.

Restriction enzyme	Recognition sequence (read in 5′ to 3′ direction)
EcoRI	G* A A T T C C T T A A *G
AluI	A G* C T T C* G A
HaeIII	G G* C C C C* G G

i With reference to the recognition site, describe the difference between EcoRI and the other restriction endonucleases. (2 MARKS)

ii Explain the benefit of using EcoRI in this experiment instead of AluI or HaeIII to cut the plasmid. (2 MARKS)

UNIT 3

AOS2

How are biochemical pathways regulated?

In this area of study students focus on the structure and regulation of biochemical pathways. They examine how biochemical pathways, specifically photosynthesis and cellular respiration, involve many steps that are controlled by enzymes and assisted by coenzymes. Students investigate factors that affect the rate of cellular reactions and explore applications of biotechnology that focus on the regulation of biochemical pathways.

Outcome 1

On completion of this unit the student should be able to analyse the structure and regulation of biochemical pathways in photosynthesis and cellular respiration, and evaluate how biotechnology can be used to solve problems related to the regulation of biochemical pathways.

Reproduced from VCAA VCE Biology Study Design 2022-2026

CHAPTER

5

Photosynthesis

Key knowledge

- the general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product
- inputs, outputs, and locations of the light-dependent and light-independent stages of photosynthesis in C3 plants (details of biochemical pathway mechanisms are not required)
- the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration
- the role of Rubisco in photosynthesis, including adaptations of C3, C4, and CAM plants to maximise the efficiency of photosynthesis
- the factors that affect the rate of photosynthesis: light availability, water availability, temperature, and carbon dioxide concentration
- the general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors
- potential uses and applications of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields
- the role of the lymphatic system in the immune response as a transport network and the role of lymph nodes as sites for antigen recognition by T and B lymphocytes

5A THE PROCESS OF C3 PHOTOSYNTHESIS

A new era of the space race has begun. This time, people have their eyes set on Mars. 'How do we get to the Red Planet?' is the first question. 'Okay, now how do we survive?' will be the second. If this far-fetched dream to live on Mars is to become a reality one day in the future, we must be able to grow plants as a food source on Mars. Is this a possibility?

Image: Dotted Yeti/Shutterstock.com

Lesson 5A

In this lesson you will learn about the inputs, outputs, and locations of the light-dependent and light-independent stages of photosynthesis.

◀ *Prerequisite knowledge* | *Future applications* ▶

Years 7–10
Plants are autotrophs that convert light energy into chemical energy.

Year 11
Chloroplasts are the highly specialised plant organelles that enable photosynthesis.

Chapter 3
Enzymes and coenzymes are crucial for the reactions of photosynthesis.

Lesson 5B
Certain plants have evolved adaptations to maximise photosynthesis.

Lesson 5C
A number of factors can influence the rate of photosynthesis, including light, temperature, and carbon dioxide concentration.

Chapter 6
The processes of photosynthesis and cellular respiration share a number of similarities, and plants are capable of both.

Study design dot points

- the general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product
- inputs, outputs, and locations of the light-dependent and light-independent stages of photosynthesis in C3 plants (details of biochemical pathway mechanisms are not required)
- the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration

Key knowledge units

Overview of C3 photosynthesis	3.2.1.1
The light-dependent stage	3.2.4.1
The light-independent stage	3.2.4.2

Overview of C3 photosynthesis 3.2.1.1

OVERVIEW

Photosynthesis is the biological process where photoautotrophs capture light energy from the sun and convert it into chemical energy.

THEORY DETAILS

Overview of photosynthesis

Plants are **photoautotrophs**, meaning that they do not consume the food they need to survive as we humans and other animals do. Instead, they create their own energy via **photosynthesis**. Photosynthesis is the process in which light energy is harnessed to produce glucose – the energy source of plants. Algae and photosynthetic cyanobacteria are also photoautotrophs capable of undertaking photosynthesis.

photoautotroph an organism capable of undertaking photosynthesis

photosynthesis the process of capturing light energy to power the production of glucose and oxygen from carbon dioxide and water

In essence, photosynthesis uses two inputs – carbon dioxide and water – to produce the outputs – glucose, oxygen, and water (Figure 1). For this process to occur, sunlight is also required to energise the reaction. Glucose is the primary product of photosynthesis. It is either used immediately as a source of energy for cellular respiration, stored as starch, or used to form more complex molecules such as cellulose. Photosynthesis is not as simple as Figure 1 makes it look. Instead, it is a complex series of biochemical reactions (and their regulatory enzymes) that can be broken into two stages, which we will explore later in this lesson.

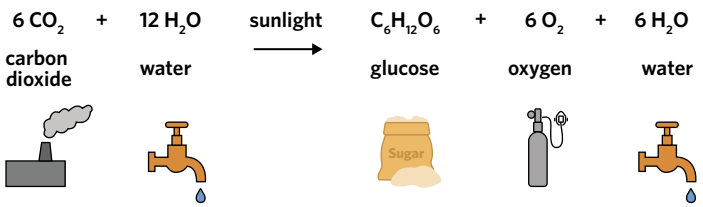


Figure 1 The chemical and word equations of photosynthesis. Note the inclusion of sunlight (above the arrow) to represent the need in the process.

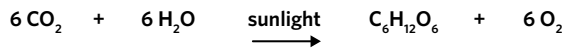


Figure 2 The simplified equation for photosynthesis. As water is both an input and output of the process, we can simplify the equation by subtracting 6 H_2O from each side.

Plant structures involved in photosynthesis

Leaves are the main site of photosynthesis in plants and typically have a large surface area to maximise the amount of light hitting the surface. Leaves contain many different cell types that perform varying functions, from structural support to channelling water and nutrients. The main cells in leaves that photosynthesise are called **mesophyll cells**. Inside mesophyll cells are large populations of **chloroplasts**, the organelle that is the site of both stages of photosynthesis. To zoom in even further, within chloroplasts is the photosynthetic pigment known as **chlorophyll**, which is directly responsible for initiating photosynthesis by capturing and being energised by light energy.

Tiny pores on the surface of leaves known as **stomata** open to allow carbon dioxide in the atmosphere to diffuse into the leaf. Stomata can also close to prevent water loss from the leaf in dry conditions. Water is absorbed by the root hair cells of plants from the soil and transported through the **xylem** to photosynthesising cells.

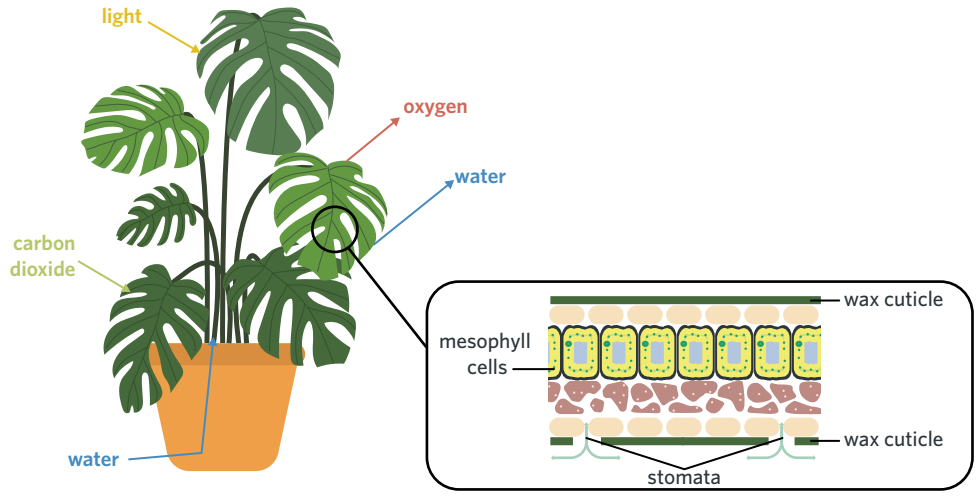


Figure 3 The inputs and outputs of photosynthesis and the structure of a leaf

mesophyll cell a plant cell type found in leaves that contain large amounts of chloroplasts

chloroplast a membrane-bound organelle only found in plant and photoautotroph cells that is the site of photosynthesis

chlorophyll a chemical found in the thylakoids of chloroplasts. It is responsible for absorbing light energy in photosynthesis

stoma (pl. stomata) a small pore on the leaf's surface that opens and closes to regulate gas exchange

xylem vascular tissue in plants responsible for transporting water and minerals from the roots to the leaves

Lesson link

In the next lesson, ***lesson 5B***, we will consider the difference between three types of plants – C3, C4, and CAM plants. These plants have evolved differences in their photosynthetic pathways. For the purposes of this lesson, however, we will focus solely on the process of C3 photosynthesis.

The light-dependent stage 3.2.4.1

OVERVIEW

In the first stage of photosynthesis, plants are dependent on light to split water into oxygen and hydrogen. This light-dependent stage occurs on the thylakoid membranes of chloroplasts.

THEORY DETAILS

As the name suggests, the **light-dependent stage** of photosynthesis only occurs when light is present. The light-dependent reactions occur on the chlorophyll-filled **thylakoid** membranes which make up the **grana** inside a chloroplast (Figure 4). The reactions in the pathway are catalysed by various enzymes and the purpose of this first stage is to generate the high energy coenzymes **NADPH** and **ATP** to power the second stage of photosynthesis.

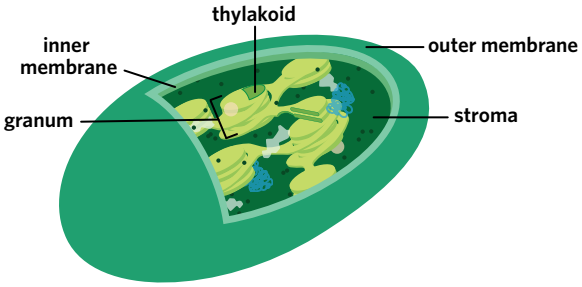


Figure 4 The key structures of a chloroplast. The light-dependent stage of photosynthesis occurs on the thylakoid membranes of the grana, whereas the light-independent stage occurs in the stroma.

The inputs of the light-dependent stage are:

- 12 water (H_2O) molecules
- 12 $NADP^+$
- 18 ADP + P_i.

The outputs of the light-dependent stage are:

- 6 oxygen (O_2) molecules
- 12 NADPH
- 18 ATP.

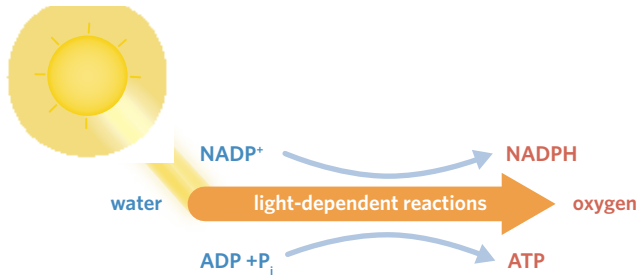


Figure 6 Summary of the light-dependent stage of photosynthesis

The steps in the light-dependent stage are:

1 Inside the thylakoid, light energy excites electrons in chlorophyll. The excited electrons (e^-) move along proteins in the thylakoid membrane. As they move, the energy in the electrons powers the pumping of H^+ into the thylakoid lumen. Water donates electrons to chlorophyll to replace the electrons that leave, which causes water to split into oxygen and two H^+. This process is known as **photolysis**.

2 The oxygen is released from the chloroplast. It will either diffuse out of stomata and into the environment or be used as an input for aerobic cellular respiration (you will learn more about this in Chapter 6).

3 The H^+ ions from water molecules are used to generate the high energy coenzyme NADPH ($NADP^+ + H^+ \rightarrow$ NADPH). The movement of H^+ down its concentration gradient (maintained by energy from excited electrons in step 1) generates the high energy coenzyme ATP (ADP + $P_i \rightarrow$ ATP). (Figure 7).

4 ATP and NADPH coenzymes then move on to the light-independent stage.

light-dependent stage the first stage of photosynthesis, where light energy splits water molecules into oxygen and hydrogen inside the thylakoid membranes. Also known as the **light-dependent reactions**

thylakoid a flattened sac-like structure housed inside the chloroplast. Each thylakoid is made up of a chlorophyll-containing membrane enclosing a lumen. Thylakoids are the location of the light-dependent stage of photosynthesis

granum (pl. grana) a stack of thylakoids

NADPH a coenzyme that is a proton (H^+) and electron carrier in photosynthesis

ATP adenosine triphosphate, a high energy molecule that, when broken down, provides energy for cellular processes

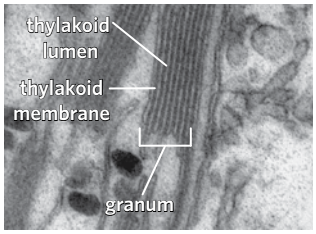
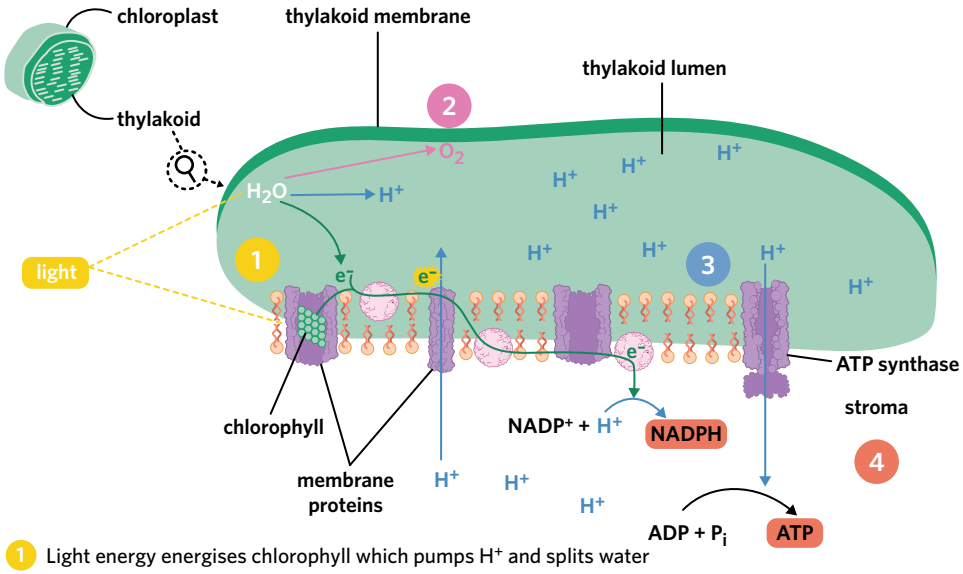


Figure 5 An electromicrograph of thylakoids in a chloroplast

photolysis the process in which molecules are broken down by the action of light



1 Light energy energises chlorophyll which pumps H^+ and splits water
2 Oxygen released
3 H^+ and e^- generate NADPH and ATP
4 NADPH and ATP are inputs for the light-independent stage

Figure 7 An overview of the light-dependent stage of photosynthesis. You do not need to know all the mechanisms, but an understanding can help you remember the inputs and outputs.

Ultimately, these steps demonstrate how the thylakoid turns:

(1) 12 H_2O, (2) 12 $NADP^+$, and (3) 12 ADP + P_i.

into:

(1) 6 O_2, (2) 12 NADPH, and (3) 12 ATP.

In summary, during the light-dependent stage of photosynthesis:

- Sunlight excites an electron within chlorophyll.
- Water absorbed by a plant's root hairs is split into O_2 and H^+ as it donates one electron to the chlorophyll.
- The excited electron and H^+ ion from water lead to the production of the coenzymes NADPH and ATP.
- The oxygen is released out of the chloroplast, and the coenzymes are ready for the second stage of photosynthesis.

Examiners' tip

The VCAA does not require you to know the details of the biochemical pathways in the light-dependent and independent stages of photosynthesis (i.e. Figure 7 is not directly examinable). However, the VCAA specifically states you must know the inputs, outputs, and locations of the stages of photosynthesis.

Examiners' tip

In the VCE Biology 2015 Exam Report, Question 3b, the VCAA stated the following:

- Light is not an input molecule of photosynthesis.
- NADP is an acceptable notation for $NADP^+$, however, NAD is not acceptable.
- ADP and P_i both can be acceptable inputs of the light-dependent stage (or outputs of the light-independent stage) as both are required to produce ATP. Although, listing both ADP + P_i as inputs will not negatively impact your answer.

Enzymes and coenzymes in photosynthesis

Enzymes catalyse most of the reactions in photosynthesis. For example, in the light-dependent stage, the enzyme ATP synthase catalyses the reaction ADP + P_i → ATP using energy from the flow of H^+ down its concentration gradient (Figure 7). Having enzymes regulate each step in photosynthesis ensures reactions are sped up and controlled, so plants can metabolise efficiently. We will focus on the function of another key enzyme in photosynthesis, called Rubisco, in the next lesson.

The coenzymes NADPH and ATP cycle through both stages of photosynthesis. In the light-dependent stage, the $NADP^+$ and ADP + P_i inputs are turned into high-energy NADPH and ATP outputs. These two high-energy molecules then go on to be inputs for the light-independent stage. As you will soon see, the coenzymes donate their energy in the light-independent reactions, forming the 'unloaded' $NADP^+$ and ADP + P_i. The unloaded coenzymes then return back to the light-dependent reactions and the cycling continues (Figure 8).

Examiners' tip

In the VCE Biology 2019 Exam Report, Question 2b, the VCAA summarised the role of both coenzymes in photosynthesis: NADPH transfers hydrogen ions while ATP transfers energy.

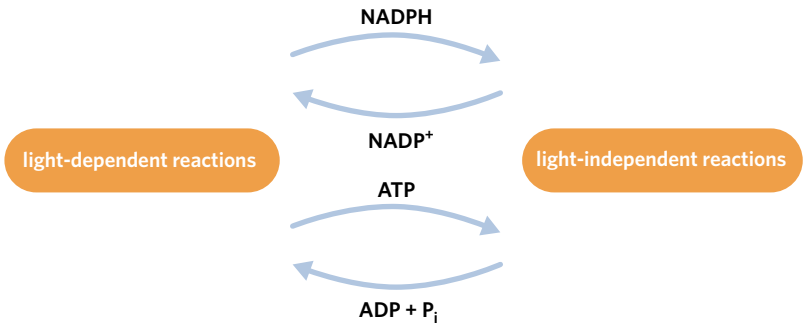


Figure 8 NADPH and ATP cycle between the stages of photosynthesis. This cycling of energy is necessary to turn carbon dioxide and water into glucose.

Lesson link

Cycle back to ***lesson 3B*** if you need to brush up on your coenzyme knowledge. For now, it is enough to remember that enzymes catalyse (speed up) reactions and coenzymes assist enzyme functioning.

The light-independent stage 3.2.4.2

OVERVIEW

During the second stage of photosynthesis, glucose is produced from carbon dioxide, NADPH, and ATP through a cycle of reactions occurring in the stroma of chloroplasts.

THEORY DETAILS

Unlike the light-dependent stage, the **light-independent stage** of photosynthesis does not require light to occur. Instead, the reactions are energised by the ATP and NADPH coenzymes produced in the light-dependent reactions. The light-independent stage occurs in the **stroma**, is facilitated by enzymes, and cycles through multiple reactions (which is why the stage is also referred to as the Calvin cycle).

light-independent stage the second stage of photosynthesis where carbon dioxide is used to form glucose in the stroma of a chloroplast. Also known as the **Calvin cycle**, the **dark stage**, or the **light-independent reactions**

stroma the fluid substance that makes up the interior of chloroplasts. It is the site of the light-independent stage of photosynthesis

The inputs of the light-independent stage are:

- 6 carbon dioxide (CO_2) molecules
- 12 NADPH
- 18 ATP.

The outputs of the light-independent stage are:

- glucose ($C_6H_{12}O_6$)
- 6 water (H_2O) molecules
- 12 $NADP^+$
- 18 ADP + P_i.

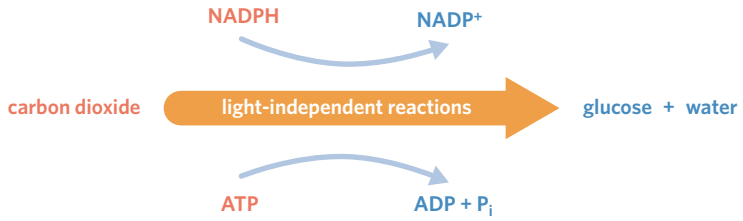


Figure 9 Summary of the light-independent stage of photosynthesis

The steps in the light-independent stage are:

1. Carbon dioxide molecules enter the Calvin cycle and undergo initial reactions. During these changes, the carbon from CO_2 combines with a five-carbon molecule, then splits into 2 x three-carbon molecules, which continue along the cycle.
2. NADPH molecules formed in the light-dependent reactions donate their hydrogen ions and electrons, and ATP molecules break into ADP and P_i to release energy to facilitate further changes to the carbon molecules.
3. Carbon molecules continue to change and rearrange as they move around the cycle. Eventually, one specific three-carbon molecule is created and leaves the cycle, going on to contribute to the formation of glucose. Overall, six CO_2 molecules must enter the cycle to produce glucose (carbon dioxide = one carbon; glucose = six carbons).
4. Some of the oxygen molecules leftover from the breaking of CO_2 at the beginning of the cycle combine with hydrogen ions from NADPH to create the output water.

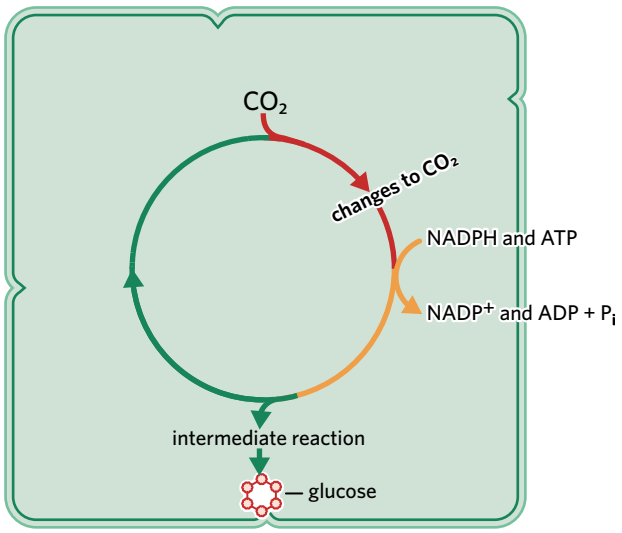


Figure 10 An overview of the light-independent stage of photosynthesis. You do not need to know all the mechanisms, but an understanding can help you remember the inputs and outputs.

Lesson link

In the next lesson, we will revisit Figure 10 and add a lot more detail to it.

Overall, within the stroma the reactions turn:

(1) 6 CO_2, (2) 12 NADPH, and (3) 12 ATP

into:

(1) $C_6H_{12}O_6$, (2) 12 $NADP^+$, (3) 12 ADP + P_i, and (4) 6 H_2O.

To summarise, in the light-independent stage of photosynthesis:

- CO_2 collected from the stomata in leaves enters a cyclic reaction.
- The carbon, from CO_2, undergoes reactions powered by ATP and NADPH to produce a series of carbon-based molecules.
- Eventually, a specific carbon molecule is reached that goes on to contribute to the formation of glucose, with water also being produced in this stage.

The production of glucose is the main outcome of photosynthesis. The plant, through a series of reactions over the two stages, has now converted sunlight energy into chemical energy stored within the bonds of a glucose molecule. The glucose is transported out of the chloroplast for cellular respiration or conversion into complex carbohydrates.

Theory summary

Photosynthesis is the process of turning light energy into chemical energy in the form of glucose. Photosynthesis occurs in two distinct stages, summarised in Figure 12 and Table 1.

$$6\,CO_2 + 6\,H_2O \xrightarrow{\text{sunlight}} C_6H_{12}O_6 + 6\,O_2$$

carbon dioxide + water $\xrightarrow{\text{sunlight}}$ **glucose + oxygen**

Figure 11 The simplified chemical and word equations for photosynthesis

Table 1 The inputs and outputs of the two stages of photosynthesis

	Location	Inputs	Outputs
Light-dependent stage	Grana/thylakoid membranes	12 H_2O 12 $NADP^+$ 12 ADP + P_i	6 O_2 12 NADPH 12 ATP
Light-independent stage	Stroma	6 CO_2 12 NADPH 12 ATP	$C_6H_{12}O_6$ 12 $NADP^+$ 12 ADP + P_i 6 H_2O

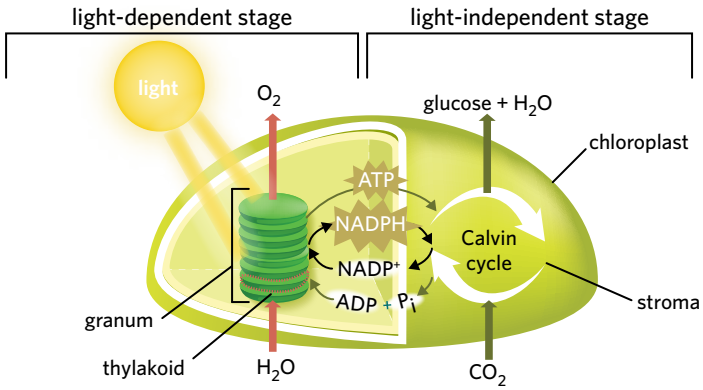


Image: Designua/Shutterstock.com

Figure 12 An overview of the stages of photosynthesis within the chloroplast

There are a number of factors that would make growing plants on Mars extremely difficult. Mars is about 80 million kilometres further away from the sun than Earth, so there is far less light energy reaching the surface of Mars. Additionally, Mars experiences frequent dust storms and strong winds and these further obscure the sun. Plants that we try to grow on Mars' surface would not receive enough sunlight to undertake the light-dependent stage of photosynthesis and, as a result, would die. For all of these reasons and more, scientists have been focused on researching artificial greenhouses that could be implemented on Mars where the light and climate of a plant's environment can be controlled.

5A QUESTIONS

Theory review questions

Question 1

The purpose of photosynthesis is to

A generate high-energy coenzymes to aid with cellular respiration.

B produce usable chemical energy in the form of glucose from light energy.

Question 2

Which of the following correctly expresses the simplified equation of photosynthesis?

A $6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$

B $6\ CO_2 + 6\ O_2 \rightarrow C_8H_{16}O_8 + 6\ H_2O$

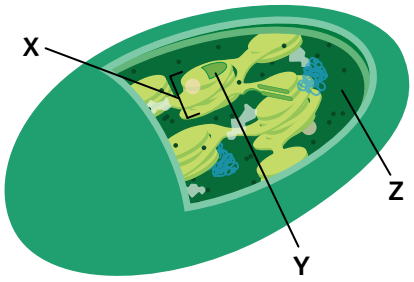
C $6\ CO_2 + 6\ O_2 \rightarrow C_6H_{12}O_6 + 6\ H_2O$

D $6\ CO_2 + 12\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$

Question 3

Label the parts of the chloroplast from the list of terms.

- stroma
- granum
- thylakoid



Question 4

Fill in the blanks in the following sentences.

The inputs of the light-dependent stage of photosynthesis are H_2O, _____, and ADP + P_i. On the other hand, the inputs of the light-independent stage are _____, _____, and ATP.

Question 5

Fill in the blanks in the following sentences.

The outputs of the light-dependent stage of photosynthesis are _____, NADPH, and ATP. Conversely, the outputs of the light-independent stage are _____, $NADP^+$, ADP + P_i, and _____.

Question 6

Categorise the following as relating to the **light-dependent** or **light-independent** stage of photosynthesis.

I Oxygen that is released into the environment exits through stomata. _____

II The thylakoid membrane/grana is the site of this stage. _____

III Carbon dioxide is used to produce glucose and water. _____

IV Light energy splits water and excites electrons. _____

V This process occurs in the stroma. _____

VI ADP + P_i are produced. _____

VII NADPH is produced. _____

Question 7

Order the steps to correctly describe the process of photosynthesis.

I Glucose is produced.

II Carbon dioxide enters the Calvin cycle.

III Light energy is absorbed by chlorophyll.

IV Hydrogen ions bind to $NADP^+$ to form NADPH molecules.

V Water molecules are split into H^+ and O_2 and an electron is donated to chlorophyll.

VI Oxygen from the splitting of water is then either released into the atmosphere or used later as an input in aerobic cellular respiration.

SAC skills questions

Case study analysis

Use the following information to answer Questions 8–12.

Life on land is dependent on plants as producers. As photosynthesising organisms, plants serve as the bottom of the food chain as they are consumed by herbivores, who in turn can be consumed by carnivores and so on. Plants gain their energy from the sun, and animals gain their energy from plants or other animals, making all of us dependent on photosynthesis. In the deep ocean, however, things are different. Sunlight only penetrates the uppermost layer of the ocean. There is a variety of sea plant life near coastal areas, as well as photosynthesising phytoplankton that can live further out to sea.

In the deep ocean, you can still find a variety of fish, octopuses, crustaceans, worms, and many tiny microbes and bacteria. Some of these organisms rely on dead carcasses floating down from the surface for food, but for an ecosystem to exist, there need to be producers. In the absence of light, specific bacteria have evolved adaptations to produce food from nutrient-rich hot springs on the ocean floor. These bacteria are chemoautotrophs, meaning that they are able to undertake chemosynthesis, a process that turns inorganic chemical compounds into usable energy to make food. These bacteria are fed upon by small crustaceans, who are then fed upon by small fish and the food chain continues.

Question 8

Plants are considered 'bottom of the food chain' as

A all animals consume plants to obtain the energy they need to survive.

B they produce their own food which allows for animals to eat plants and therefore animals to eat other animals.

Question 9

Phytoplankton

A turn sunlight into glucose.

B undertake chemosynthesis to produce food.

Question 10

Photosynthesis can't occur in the deep ocean because

A the input light is not readily available in this habitat.

B water cannot be split and electrons cannot be excited in the light-dependent stage.

Question 11

Chemosynthesis is similar to photosynthesis as both processes

A allow for the conversion of an otherwise unusable form of energy into a usable food source.

B contain a light-independent stage reliant upon the coenzymes ATP and NADPH.

Question 12

If a plant from the land was transported to the deep ocean, it would be safe to assume that it would

A be able to choose between photosynthesis and chemosynthesis, depending on the availability of their respective inputs.

B be forced to undertake chemosynthesis rather than photosynthesis to survive.

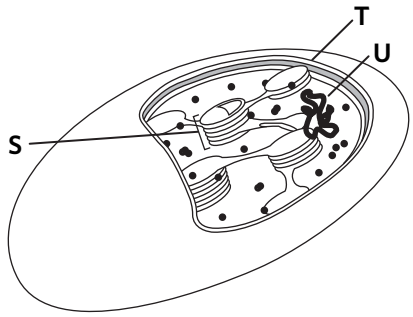
C not be able to undertake photosynthesis or chemosynthesis and would die.

Exam-style questions

Within lesson

Use the following information to answer Questions 13 and 14.

The following diagram shows a chloroplast.



Question 13 (1 MARK)

The region labelled U is called the

A thylakoid.
B stomata.
C stroma.
D grana.

Question 14 (1 MARK)

The light-dependent stage of photosynthesis occurs at

A S.
B T.
C U.
D both S and U.

Adapted from VCAA 2018 Section A Q14

Question 15 (1 MARK)

Which of the following correctly describes the primary function of a chloroplast?

A site of protein synthesis
B site where ATP for a cell is generated
C storage of wastes and other materials
D light energy is converted into chemical energy

Adapted from VCAA 2005 Exam 1 Section B Q1

Question 16 (1 MARK)

Which of the following statements about photosynthesis in chloroplasts is correct?

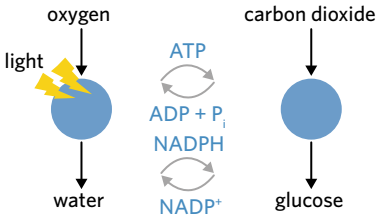
A The stroma is the site of the light-dependent stage.
B Chlorophyll in the grana traps light for use during the light-independent stage.
C The light-independent stage produces ATP for use during the light-dependent stage.
D The light-dependent stage forms NADPH for the light-independent stage to produce glucose.

Adapted from VCAA 2016 Section A Q11

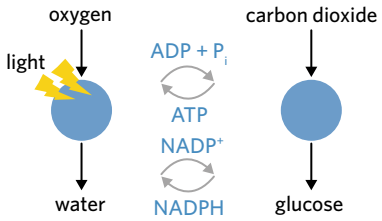
Question 17 (1 MARK)

Which of the following diagrams represents the inputs and outputs of photosynthesis?

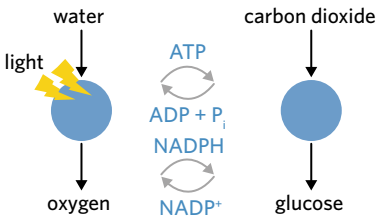
A



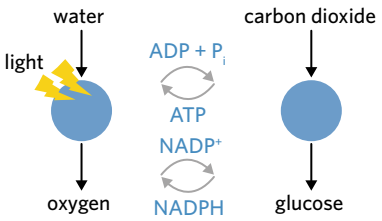
B



C



D



Adapted from VCAA 2018 Section A Q15

Question 18 (1 MARK)

The following image shows a portion of an electron photomicrograph of a chloroplast.

Region P and Q are both locations for stages of photosynthesis. By referring to your knowledge of photosynthesis, it is reasonable to conclude that

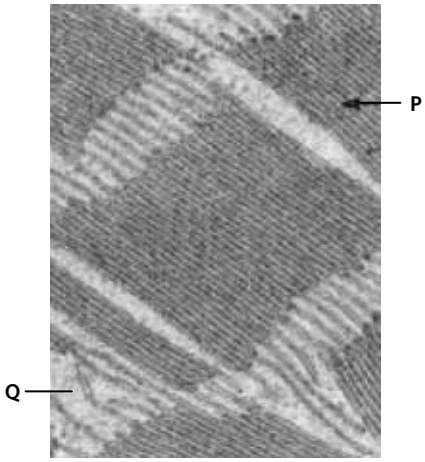
A carbon dioxide is an input at region P.

B sunlight is absorbed at region Q.

C oxygen is an output at region Q.

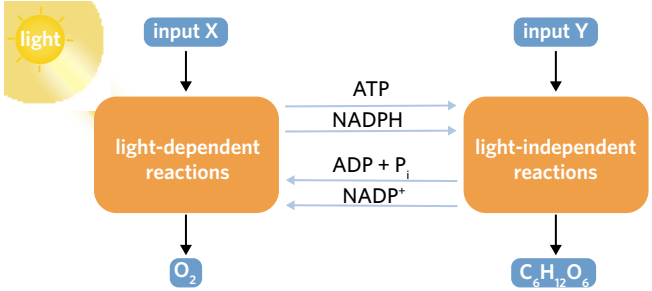
D water is split at region P.

Adapted from VCAA 2006 Exam 1 Section A Q18



Question 19 (2 MARKS)

Although photosynthesis is often summarised by a single equation, the process occurs in two distinct phases, the light-dependent stage and the light-independent stage. These two phases can be summarised in a diagrammatic form as follows, where input X and input Y can be seen.



a Identify input X. (1 MARK)

b Identify input Y. (1 MARK)

Adapted from VCAA 2005 Exam 1 Section B Q3

Question 20 (5 MARKS)

Complete the following tables by referring to your knowledge of photosynthesis.

Name of the stage of photosynthesis that occurs at the stroma	_____	
Two input molecules that are required for reactions at the stroma	**1** _____	**2** _____
Two output molecules from the reactions at the stroma	**1** _____	**2** _____

Name of the stage of photosynthesis that occurs at the grana	_____	
Two input molecules that are required for reactions at the grana	**1** _____	**2** _____
Two output molecules from the reactions at the grana	**1** _____	**2** _____

Adapted from VCAA 2015 Section B Q3

Multiple lessons

Question 21 (1 MARK)

A molecule that plays a role in many biochemical reactions is ATP.

It is correct to state that

A ADP becomes ATP when it is loaded with electrons.

B energy is released when ATP is converted to ADP.

C ADP has a higher energy content than ATP.

D ADP contains three phosphate molecules.

Adapted from VCAA 2017 Section A Q16

Question 22 (6 MARKS)

Elysia chlorotica is a bright green sea slug, with a soft leaf-shaped body. It has a lifespan of 9 to 10 months. This sea slug is extremely unique among sea slugs as it is able to survive on 'solar power'. *E. chlorotica* acquires chloroplasts from the algae it eats and stores them in its cells. Young *E. chlorotica* fed with algae for two weeks can seemingly survive on 'solar power' indefinitely.

a What is the product of photosynthesis that provides the energy which enables *E. chlorotica* to survive for so long without eating? (1 MARK)

b These slugs survive on 'solar power'. Name the organelle in the cells of *E. chlorotica* which allow them to survive on 'solar power'. (1 MARK)

c Sequencing of *E. chlorotica* DNA showed that certain genes essential for photosynthesis had been acquired by the slug from the nucleus of the algae. However, the majority of the mechanisms enabling *E. chlorotica* to harness 'solar power' remain unknown. Briefly contrast gene expression and gene regulation. (2 MARKS)

d Would *E. chlorotica* need to eat as much, more than, or less than a closely related black sea slug? Justify your response. (2 MARKS)

Adapted from VCAA 2010 Exam 1 Section B Q3a

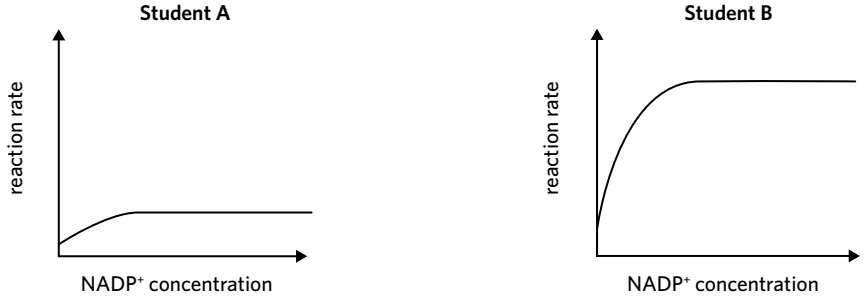
Key science skills and ethical understanding

Question 23 (7 MARKS)

Two students set up an experiment to measure the reaction rate of the light-dependent stage of photosynthesis. Grana were removed from chloroplasts and suspended in a solution that was sealed from the environment. The thylakoids were then exposed to different concentrations of $NADP^+$ and the rate of the reaction was measured by calculating the concentration of oxygen. Students A and B were both given grana from the same sample of chloroplasts and both followed the same method.

a Identify the independent and dependent variables. (2 MARKS)

b Identify a variable in this experiment that each student must control in order for their results to be comparable. (1 MARK)



c The results of both students can be seen in the graphs.

i Given that both students had the same experimental setup and method, describe a possible error that one of the students could have experienced and explain how it could have created the difference in results. (2 MARKS)

ii Student A wants to combine and average their results with Student B. Even though they plan to be honest and discuss this action in their report, Student B is still uncomfortable with the idea. Even after Student B expresses their concern, Student A combines their results anyway. Does this violate any bioethical concepts? Explain your response. (2 MARKS)

Adapted from VCAA 2017 Northern Hemisphere Exam Section B Q5

5B RUBISCO IN C3, C4, AND CAM PHOTOSYNTHESIS

Bad habits, we all have them. Biting your nails, procrastinating, eating junk food, phone overuse, or going to bed too late – all things we would love to kick. Just like us, a special little enzyme within plants has a bad habit too. It typically functions well, but when it acts out it can have severe impacts on the plant. Consequently, certain plants have evolved adaptations to combat this bad habit. What is this naughty little habit and how do plants combat it?

'Just one more episode.'
Image: lassedesignen/Shutterstock.com

Lesson 5B

In this lesson you will learn that the enzyme Rubisco is critical to photosynthesis, and requires adaptations in C4 and CAM plants to counter its key flaw.

◀ *Prerequisite knowledge*

Years 7–10
Plants undertake photosynthesis to create chemical energy.

Year 11
C4 and CAM plants have evolved adaptations suited to their environment.

Chapter 3
Rubisco is an enzyme that is pivotal in the light-independent stage of photosynthesis.

Future applications ▶

Lesson 5D
Scientists are experimenting with C4 and CAM plants to improve crop efficiencies of C3 plants in extreme conditions.

Chapter 6
Photosynthesis and cellular respiration processes share a number of similarities, and plants are capable of both.

Study design dot point

- the role of Rubisco in photosynthesis, including adaptations of C3, C4, and CAM plants to maximise the efficiency of photosynthesis

Key knowledge units

Rubisco	3.2.5.1
C3, C4, and CAM plants	3.2.5.2

Rubisco 3.2.5.1

OVERVIEW

Rubisco is a key enzyme of the light-independent stage of photosynthesis. Its action varies: sometimes it binds to carbon dioxide and facilitates further reactions in the photosynthesis process, whilst other times it binds to oxygen and initiates a wasteful process called photorespiration.

THEORY DETAILS

In the previous lesson, we learned about the basics of photosynthesis. Carbon dioxide (CO_2) and water (H_2O) are inputs, and with the help of sunlight, plants can produce glucose ($C_6H_{12}O_6$) along with some oxygen (O_2) and water (H_2O). Photosynthesis occurs over two stages:

- The **light-dependent stage** – where light energy splits water molecules to produce ATP, NADPH, and oxygen. This stage occurs in the thylakoid membranes of the grana.
- The **light-independent stage** – where carbon dioxide is converted into organic molecules using ATP and NADPH, and more water is produced. This stage occurs in the stroma, and is also known as the Calvin cycle.

In both stages, there are numerous micro-steps involved in the complex biochemical pathways that we typically don't display for simplicity. Virtually all of these small steps in the reactions are controlled by **enzymes**. In this lesson, we are going to focus on one crucial enzyme that is involved in the light-independent stage: ribulose bisphosphate carboxylase-oxygenase, also known as **Rubisco**.

light-dependent stage the first stage of photosynthesis, where light energy splits water molecules into oxygen and hydrogen inside the thylakoid membranes. Also known as the **light-dependent reactions**

light-independent stage the second stage of photosynthesis where carbon dioxide is used to form glucose in the stroma of a chloroplast. Also known as the **Calvin cycle**, **the dark stage**, or the **light-independent reactions**

enzyme an organic molecule, typically a protein, that catalyses (speeds up) specific reactions

Rubisco a pivotal enzyme involved in initial carbon fixation during the light-independent stage of photosynthesis

The role of Rubisco in photosynthesis

In most plants, Rubisco controls the first reaction in the light-independent stage of photosynthesis. Figure 1 should look similar to the shape of Figure 10 from lesson 5A, except for a key difference.

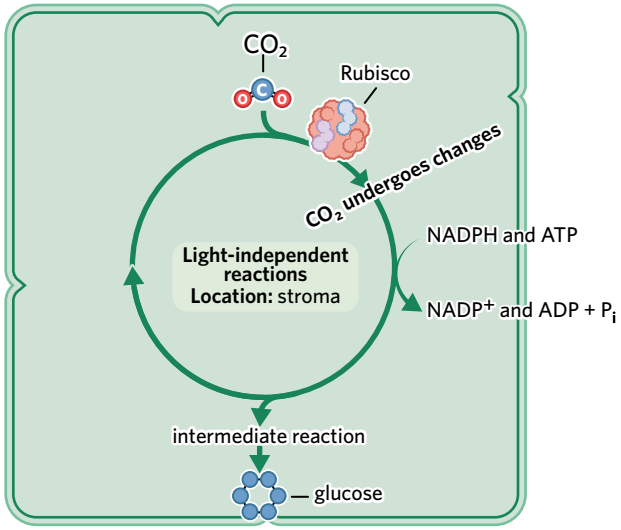


Figure 1 The enzyme Rubisco is responsible for the initial changes to CO_2 in the light-independent stage of photosynthesis.

Rubisco, the cheeky enzyme, was hiding last lesson, but now you can see that it is responsible for the initial changes to carbon dioxide at the beginning of the Calvin cycle. In the process:

- Rubisco uses 3 × CO_2 molecules and 3 × five-carbon molecules (called RuBP) to produce 6 × three-carbon molecules (called 3-PGA).
- The 6 × 3-PGA are then converted by ATP and NADPH from the light-dependent reactions to make different 6 x three-carbon molecules (called G3P). In other words, our 6 × 3-PGA molecules are changed into 6 × G3P molecules, both of which have three carbon atoms.
- One G3P molecule then leaves the cycle to undergo further reactions to contribute to making glucose. Note that 3 × CO_2 molecules must cycle in order for one G3P to leave, and two G3P (three-carbon) leaving are required to build one glucose (six-carbon), therefore 6 × CO_2 must enter to produce one glucose molecule.
- The remaining 5 × G3P are recycled with the help of ATP to regenerate the 3 × RuBP we had at the start of the cycle, and the cycle begins all over again (Figure 2). Overall, the cycle must turn twice to produce one glucose molecule (3 × CO_2 goes in twice, to contribute to six-carbon glucose).

Essentially, all of this cycling around and around between carbon molecules is to produce glucose from CO_2, NADPH, and ATP. Plants cannot convert CO_2 directly into glucose as it would waste too much energy. Instead, the cycle allows for the most effective way to use CO_2 to produce glucose.

To summarise, the previously mentioned steps demonstrate the chemical processes involved in the light-independent stage. Ultimately though, it can be helpful to think of the overall steps of the process as being part of three overarching stages (Figure 2):

1. **Carbon fixation** – which refers to the conversion of CO_2 and RuBP into 3-PGA. Here, we say that the carbon from the **inorganic** CO_2 is 'fixed' into an **organic** compound. Rubisco is responsible for taking carbon from an inorganic, gaseous form (CO_2) and incorporating it into an organic compound (3-PGA)
2. Reduction – NADPH donates electrons to (aka 'reduces') an intermediate three-carbon molecule in the cycle to produce G3P
3. Regeneration – the RuBP molecules needed to start the cycle again are reproduced.

carbon fixation the process in living organisms where inorganic carbon, typically within carbon dioxide, is converted into organic compounds such as glucose. Carbon fixation is a central part of the light-independent stage of photosynthesis

inorganic a compound that does not contain a carbon-hydrogen bond, e.g. carbon dioxide

organic a compound containing a carbon-hydrogen bond, e.g. glucose

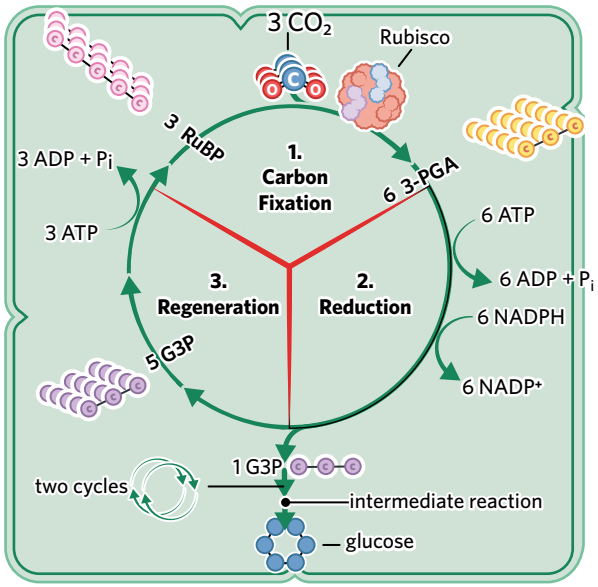


Figure 2 A more complex view of the steps of the Calvin cycle. Note the three stages: (1) carbon fixation, (2) reduction, and (3) regeneration.

Examiners' tip

The VCAA does not require you to know the specific details of the Calvin cycle mechanisms shown in Figure 2, they do, however, state that you must understand the role of Rubisco in photosynthesis. On a fundamental level, the role of Rubisco can be summarised as binding CO_2 and fixing the carbon into the organic 3-PGA, thus initiating the Calvin cycle.

The problem with Rubisco

Most of the time, Rubisco does its job well and fulfils its role of fixing carbon from CO_2 in the first step of the Calvin cycle. Rubisco, however, has a major flaw. Sometimes, rather than using CO_2 as a **substrate**, it uses O_2 instead. This may not sound like a big deal at first, but when you consider that Rubisco is responsible for catalysing the fixation of CO_2 as the first step of the Calvin cycle, you recognise that without this Rubisco-CO_2 pairing, photosynthesis cannot proceed. When Rubisco binds to O_2 instead, a different reaction called **photorespiration** occurs.

substrate the reactant of a reaction catalysed by an enzyme

photorespiration a wasteful process in plants initiated by Rubisco that limits photosynthesis

The process of photorespiration is wasteful and unwanted in plants. By binding O_2 and undertaking photorespiration, photosynthesis is disrupted as CO_2 loses an opportunity to bind with Rubisco. Less photosynthesis means less glucose is produced, which, combined with wasted energy used in the photorespiration pathway, negatively impacts a plant's ability to grow, survive, and reproduce. This is why we say photorespiration is an unwanted and wasteful process in plants. By initiating the photorespiration pathway, Rubisco can cause far more harm than good, so why would it ever bind to O_2?

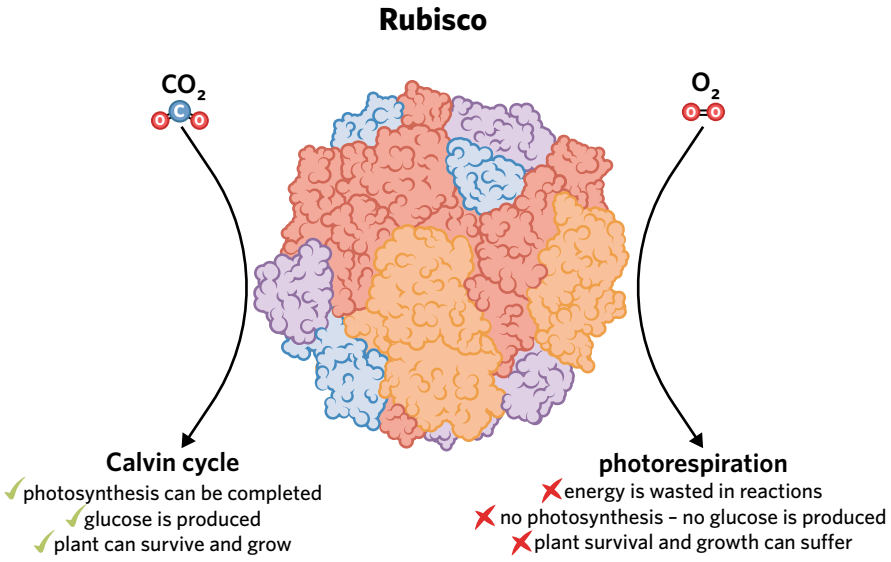


Figure 3 Rubisco can bind to CO_2, leading to further reactions of the Calvin cycle, or bind to O_2, leading to photorespiration.

The two key factors that influence whether Rubisco binds CO_2 or O_2 are temperature and substrate concentration. We have to remember that in the case of Rubisco, there are two different substrate concentrations to consider: CO_2 is the substrate of the Calvin cycle pathway and O_2 is the substrate of the photorespiration pathway.

- Substrate concentration – the more substrate is present, the greater chance it can bind to an enzyme and undergo a reaction. Because of this, plants 'want' to expose Rubisco to a high CO_2 concentration and a low O_2 concentration (so as to maximise photosynthesis). To facilitate this, the **stomata** of the plant leaves open to allow CO_2 to enter the plant, while O_2 and water vapour simultaneously diffuse out of the plant. However, when a plant needs to conserve water it will close its stomata, causing the O_2 produced during the light-dependent stage of photosynthesis to build up inside its cells. A greater concentration of oxygen in the cells leads to increased photorespiration.
- Temperature – at regular temperatures, Rubisco's **affinity** for CO_2 is far greater than that for O_2. At higher temperatures, the affinity for O_2 is higher, leading to Rubisco binding oxygen more often.

stoma (pl. stomata) a small pore on the leaf's surface that opens and closes to regulate gas exchange

affinity the tendency of a molecule/atom to bind or react with another molecule/atom

Overall, photorespiration is an unwanted process for plants. Photorespiration occurs more in hot and dry weather when Rubisco has a greater affinity for O_2 and the conditions have caused stomata to close. To counter photorespiration, certain plants have evolved adaptations to increase the likelihood of Rubisco binding to CO_2, thus increasing photosynthesis rates and improving plant health. The plant types with different strategies to counter photorespiration are C3, C4, and CAM plants.

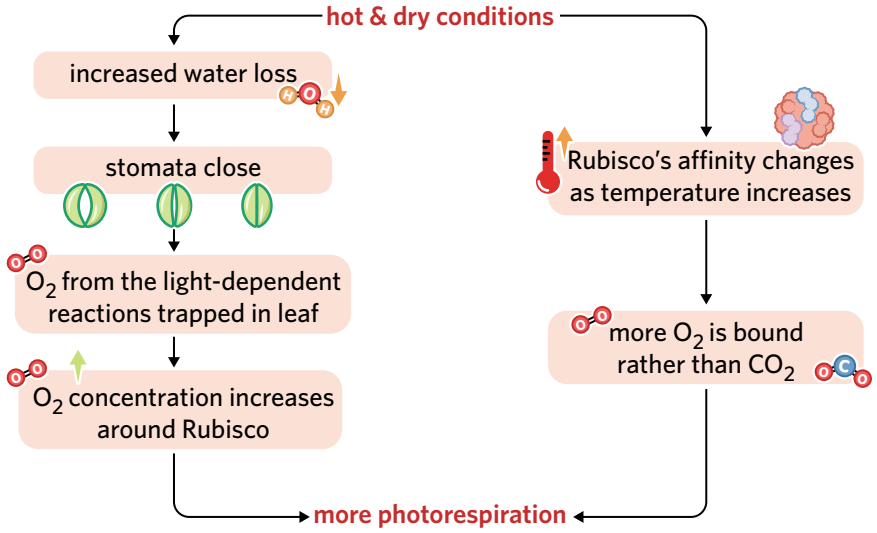


Figure 4 Hot and dry conditions lead to more photorespiration and less photosynthesis.

Lesson link

As temperature increases, the bonds holding Rubisco together 'loosen', which changes the 3D shape of the enzyme, resulting in a greater affinity towards oxygen. As the temperature continues to increase, the entire enzyme will denature and no reactions would take place at all. Flip back to ***lesson 3B*** if you need to revisit factors that influence enzymatic reactions.

C3, C4, and CAM plants 3.2.5.2

OVERVIEW

C3, C4, and CAM plants differ in their methods to restrict the wasteful photorespiration process. C3 plants have no features to fight photorespiration. C4 plants reduce photorespiration by separating initial carbon fixation from the remainder of the Calvin cycle spatially (over space), whilst CAM plants separate the two steps temporally (over time).

THEORY DETAILS

It is advantageous for plants to expose the enzyme Rubisco to high concentrations of CO_2 and low concentrations of O_2 in order to limit photorespiration and increase photosynthesis. Plants can be separated into three groups based on their adaptive mechanisms, or lack of mechanisms, to stop photorespiration from occurring.

C3 plants

C3 plants are what we consider to be 'normal' plants as they make up approximately 85% of plants on Earth. As such, C3 plants undertake the 'normal' photosynthesis that we explored last lesson, and these plants possess no adaptations to reduce photorespiration.

Figures 1 and 2 in this lesson represent the Calvin cycle in C3 photosynthesis. In this process, Rubisco is responsible for fixing carbon dioxide into three-carbon compounds (3-PGA) which then cycles through the pathway, and this all occurs within a single **mesophyll cell**. C3 photosynthesis gets its name from the three-carbon 3-PGA that the initial carbon fixation produces. Examples of C3 plants include all trees, cereals such as wheat and rice, and the majority of nuts, fruits, and vegetables.

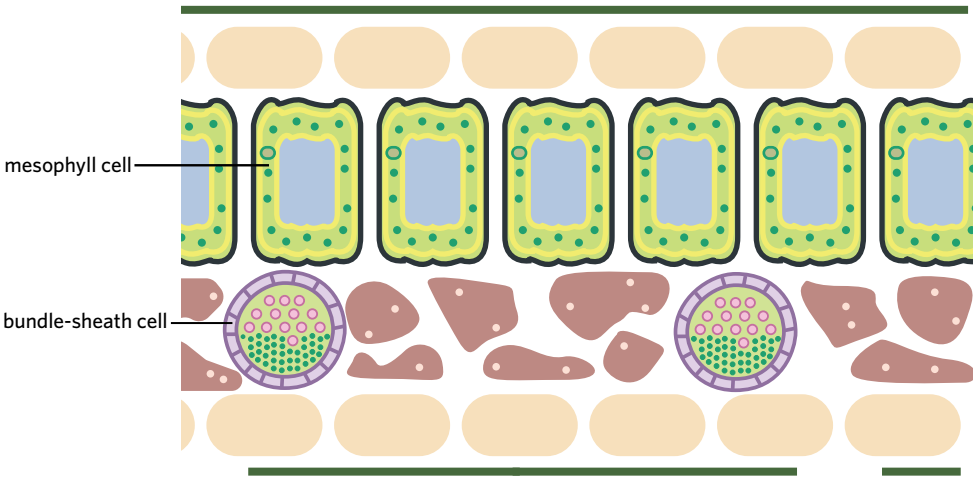


Figure 5 A simplified representation of the structure of a leaf

C4 plants

The light-dependent stage of photosynthesis in **C4 plants** is still exactly the same as C3 plants and what we learned in lesson 5A. The light-independent stage, however, has significant differences. In C4 plants, the initial carbon fixation and the remainder of the Calvin cycle are separated into two different cells rather than a single cell like in C3 plants. In C4 plants, initial carbon fixation occurs in a mesophyll cell, however, the remaining Calvin cycle occurs in specialised cells called **bundle-sheath cells**. C4 photosynthesis gets its name from the first four-carbon molecule produced in the initial carbon fixation.

C3 plants plants with no evolved adaptation to minimise photorespiration

mesophyll cell a plant cell type found in leaves that contain large amounts of chloroplasts

C4 plants plants that minimise photorespiration by separating initial carbon fixation and the remainder of the Calvin cycle over space

bundle-sheath cell a plant cell type that is the site of most of the Calvin cycle in C4 plants

Lesson link

The different types of photosynthesis found in C3, C4, and CAM plants are adaptations to the environment that arose from natural selection over hundreds of thousands of years. In short, it is advantageous for the survival and reproduction of certain plants to undertake certain photosynthesis pathways in certain climates. In the case of C3 plants, there has historically not been a strong selective advantage for ancient C3 plants to evolve adaptations to photosynthesis, therefore they possess none. You will learn more about natural selection in ***lesson 9B***.

Image: Leah-Anne Thompson /Shutterstock.com

Figure 6 All trees are C3 plants

Lesson link

In ***lesson 5A***, you explored photosynthesis in C3 plants. This is what we consider to be the 'regular' mode of photosynthesis. A small number of plants have adaptations to photosynthesis, but when 'photosynthesis' is mentioned without C3, C4, or CAM also being specified, you can usually assume that it refers to C3 photosynthesis.

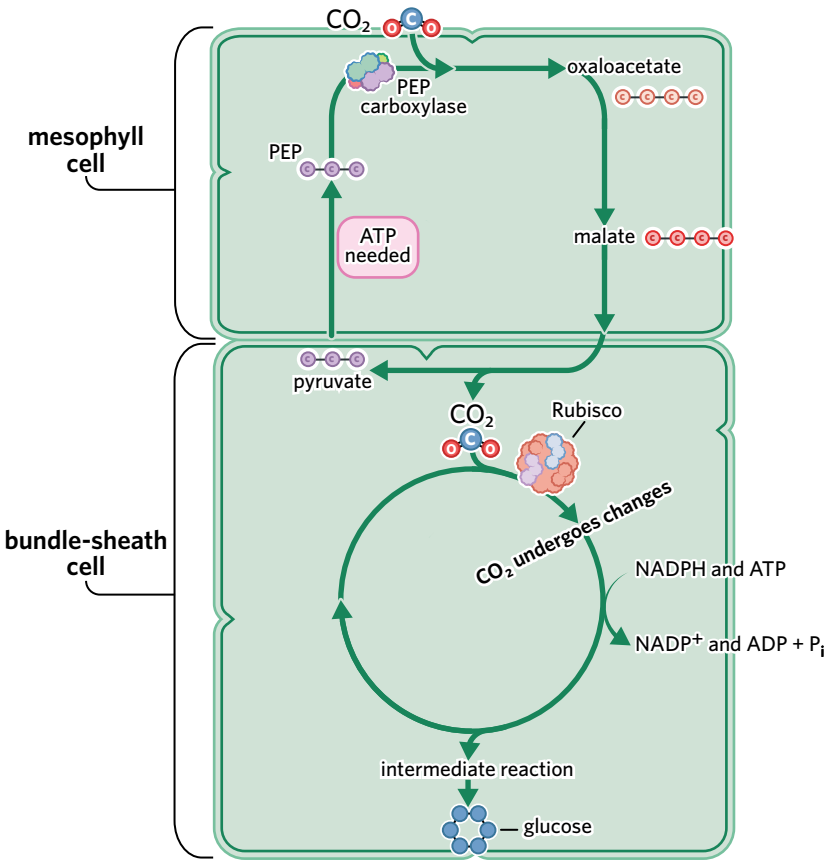


Figure 7 The light-independent stage of photosynthesis in C4 plants. Initial carbon fixation and the remainder of the Calvin cycle are separated between two cells to maximise photosynthesis.

Figure 7 shows the light-independent reactions in C4 plants. In this process:

1. Atmospheric CO_2 enters mesophyll cells and is fixed by the enzyme PEP carboxylase. The enzyme adds the carbon from CO_2 to a three-carbon molecule (PEP) to create a four-carbon molecule (oxaloacetate). Importantly, the enzyme responsible for the initial carbon fixation in C4 plants, PEP carboxylase, has no affinity to bind to O_2 (unlike Rubisco)
2. Oxaloacetate is converted to a different four-carbon molecule (malate) capable of being transported to bundle-sheath cells
3. Inside the bundle-sheath cell, malate breaks down and releases CO_2, which then enters the Calvin cycle in exactly the same way as C3 photosynthesis, leading to glucose production
4. Pyruvate formed from the breakdown of malate is transported back to the mesophyll cell and converted to another molecule, PEP, with the help of ATP
5. PEP is then ready to contribute to the fixation of CO_2 and production of oxaloacetate and the cycle continues all over again.

With the mesophyll cells constantly pumping a source of CO_2 (in the form of malate) into the bundle-sheath cells, there is always a higher concentration of CO_2 present for Rubisco rather than O_2. As a result, photorespiration is minimised and photosynthesis is maximised. There is a cost to undertaking C4 photosynthesis over C3 photosynthesis. Namely, ATP is required to convert pyruvate to PEP for the initial carbon fixation. Because of this, C4 plants use more energy to undertake photosynthesis than C3 plants. C4 photosynthesis is, however, advantageous in hot environments where C3 plants suffer from increased photorespiration. In such conditions, the benefits of reduced photorespiration typically outweigh the cost of using ATP in C4 photosynthesis. Examples of C4 plants include corn, sugarcane, switchgrass, and several weed species.

Examiners' tip

In wider literature, the term 'Calvin cycle' can be used to describe the entire light-independent stage of photosynthesis, or it can be used to describe only the cyclic reaction that starts with Rubisco (therefore excluding the initial reactions in C4 plants that occur in the mesophyll cell).
In the past, the VCAA has accepted 'Calvin cycle' as an acceptable alternative to the light-independent stage/reactions and for this reason we teach it as such.
It is important to remember, however, that other sources consider it a step within the light-independent stage, rather than the entire stage itself.

Examiners' tip

The VCAA does not require you to know the details of the biochemical pathway mechanisms in the light-independent stage of photosynthesis (i.e. Figures 7 and 9 are not directly examinable). However, the VCAA specifically states you must know the adaptations of C3, C4, and CAM plants to maximise photosynthesis.

Image: ANEK SANGKAMANE /Shutterstock.com

Figure 8 Corn (*Zea mays*) is a C4 plant

CAM plants

CAM plants plants that minimise photorespiration by separating initial carbon fixation and the remainder of the Calvin cycle over time

Like C4 plants, **CAM plants** have an adaptation to decrease photorespiration compared to C3 plants. CAM stands for crassulacean acid metabolism, named after the first family of plants the pathway was observed in. The light-dependent stage of photosynthesis in CAM plants is still identical to that of C3 and C4 plants, but the light-independent stage differs. This time, rather than separating the initial carbon fixation and the remainder of the Calvin cycle spatially over two cells like in C4 plants, CAM plants separate the steps over time.

At night, CAM plants open up their stomata to bring in CO_2. The CO_2 is fixed into a four-carbon molecule (oxaloacetate) by the enzyme PEP carboxylase, similarly to C4 plants. Oxaloacetate is then converted to a different four-carbon molecule (can be malate or another organic molecule). The malate (or other) molecule is stored inside vacuoles within the mesophyll cell until the daytime.

During the daytime, CAM plants do not open their stomata to prevent water loss. This makes them very resistant to water loss. They can still photosynthesise during the day as the malate (or other) molecule is transported out of the vacuole and broken down to release CO_2. The CO_2 is then free to enter the Calvin cycle in the same fashion as in C3 and C4 plants, leading to glucose production.

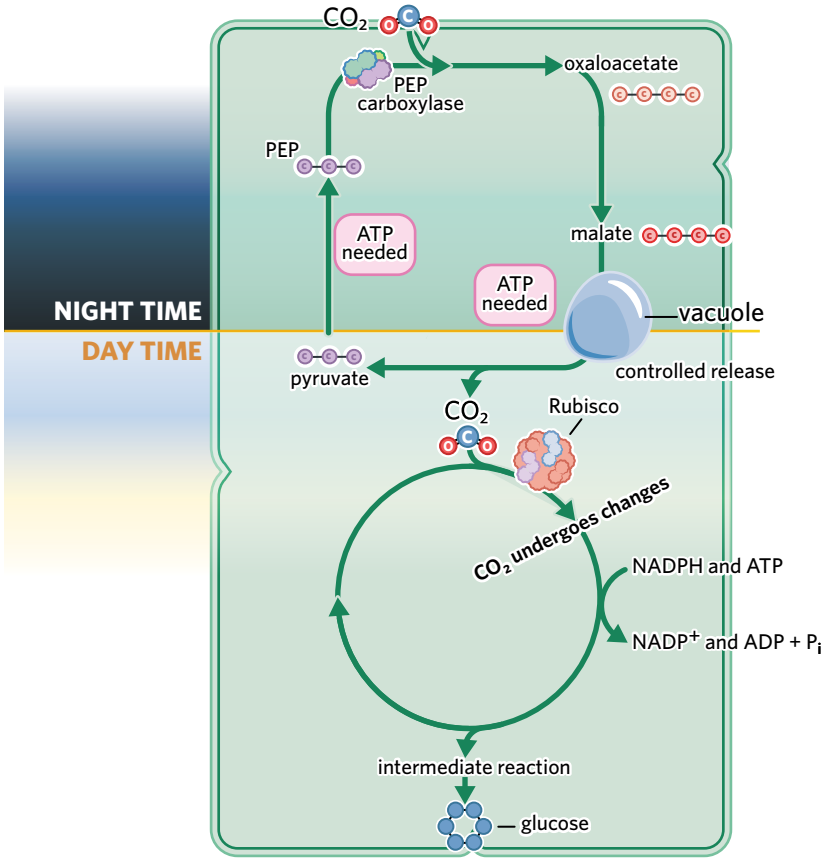


Figure 9 The light-independent stage of photosynthesis in CAM plants. Initial carbon fixation and the remainder of the Calvin cycle are separated between night and day to maximise photosynthesis.

The controlled release of molecules out of vacuoles ensures a high concentration of CO_2 is maintained near Rubisco, maximising photosynthesis and minimising photorespiration. Like C4 photosynthesis, the CAM pathway requires more ATP than C3 photosynthesis to cycle PEP. Water is also conserved in CAM plants as their stomata only open at night when it is typically cooler and more humid. Because of this, CAM plants are very prominent in very hot dry areas like deserts. CAM plants include almost all cacti, pineapples, vanilla, and orchids.

Lesson link

C4 and CAM plants possess desired traits for the agricultural industry. Such traits include greater resistance to drought or heat waves. As a result, biochemists are experimenting with applications of gene editing in crops and plants to improve crop yields in harsh conditions. You'll learn more about genetic modifications to improve photosynthetic efficiencies and crop yields in ***lesson 5D***.

Image: Johnny Coate/Shutterstock.com

Figure 10 Almost all cacti are CAM plants capable of surviving extremely hot and dry conditions.

Theory summary

Rubisco is a key enzyme involved in the light-independent stage of photosynthesis. It has an affinity to bind to either CO_2 (and initiate the Calvin cycle) or O_2 (initiating wasteful photorespiration). To minimise photorespiration and maximise photosynthesis, C4 and CAM plants have evolved adaptations to their photosynthesis process. A comparison between C3, C4, and CAM plants is found in Table 1.

Table 1 Comparison of C3, C4, and CAM plants

Type of photosynthesis	C3	C4	CAM
Limits photorespiration	No	Yes	Yes
Separation of initial CO_2 fixation and remainder of Calvin cycle	No separation	Between cells (over space)	Between night and day (over time)
Stomata open	Day	Day	Night
Advantages	Doesn't consume extra energy	Minimises photorespiration	Minimises photorespiration and reduces water loss
Disadvantages	Susceptible to photorespiration initiation	Consumes extra energy	Consumes extra energy
Best adapted to	Moderate, or cool and wet environments	Hot, sunny habitats	Very hot, dry habitats
Examples	Most plants, including wheat, rice, and all trees	Corn, sugarcane, and switchgrass	Cacti, pineapples, and orchids

C4 and CAM plants have mechanisms to limit Rubisco from its bad habit – binding oxygen and undertaking photorespiration. The process is energy-consuming, wasteful, and limits photosynthesis thereby impacting overall plant health. Perhaps like C4 and CAM plants, we should try to separate ourselves spatially and temporally from our bad habits. Maybe try throwing your phone in a river? Or time travel to the 1800s before they were invented?

Image: YAKOBCHUK VIACHESLAV /Shutterstock.com

5B QUESTIONS

Theory review questions

Question 1

Rubisco is an

A input of photosynthesis.

B enzyme required in the light-dependent stage of photosynthesis.

C enzyme required in the light-independent stage of photosynthesis.

Question 2

The difference between C4 and CAM plants is

A C4 plants undertake photosynthesis where the light-independent stage is separated over time whereas in CAM plants it is separated over space.

B CAM plants undertake photosynthesis where the light-independent stage is separated over time whereas in C4 plants it is separated over space.

Question 3

Rubisco has a lower affinity to bind to O_2 over CO_2 when

A there is high O_2 concentration or when temperatures are high.

B there is low O_2 concentration or when temperatures are normal or cool.

Question 4

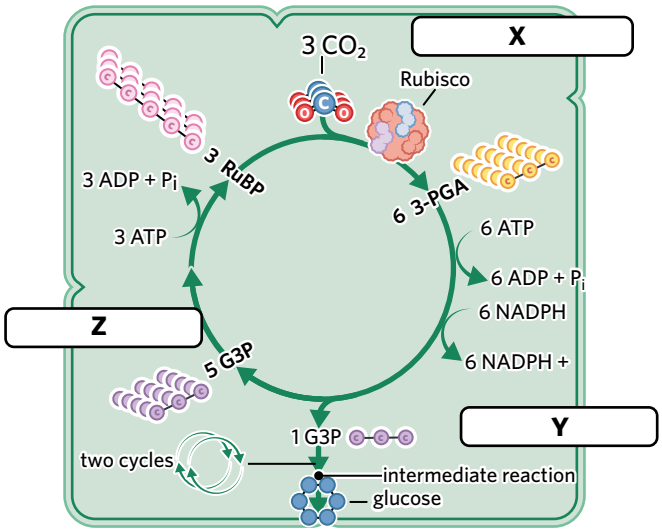
C3 plants

A contain no mechanism to minimise photorespiration.

B are capable of undertaking C4 photosynthesis when placed in extremely hot conditions.

Question 5

Label the steps of the Calvin cycle in C3 plants.



Question 6

Why is photorespiration harmful to plants?

A Photorespiration disrupts both stages of photosynthesis by interfering with coenzymes cycling between the stages.

B Undertaking photorespiration stops carbon dioxide from binding with Rubisco and fulfilling the light-independent stage of photosynthesis to produce glucose.

C The process inhibits the Calvin cycle of the light-independent stage of photosynthesis, effectively making the cycle consume glucose rather than produce it.

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 7–10.

Food insecurity is a current-day global problem, particularly in developing countries, and climate change is threatening to make it even worse. At present, we are heavily reliant on C3 plants as the main source of our diet. C3 plants include grain cereals such as rice and wheat, vegetables like tomatoes and potatoes, fruits such as apples and peaches, and most plant material that is fed to livestock.

C3 plants, however, are not well-equipped to handle hot environments. Global warming poses a threat to our reliance on C3 plant agriculture, as increasing temperatures will decrease agricultural yields. For this reason, biochemists have been trying to find ways to introduce traits found in C4 and CAM plants into C3 plants as a way to combat environmental changes. Such traits include high-temperature tolerance, resistance to drought and salinity, greater conservation of water, and overall higher yields in hot environments. Modifications to C3 plants are believed to be possible due to the similarity in genomes between C3 and C4 plants. However, attempts to breed hybrid organisms that combine C3 and C4 plants have been pursued for over fifty years with little to no success.

Question 7

Certain traits of C4 plants hold the potential to improve global agricultural yields, therefore improving the livelihood of many groups of people and individuals worldwide. C4 plants perform better in hot environments because they separate parts of the light-independent stage of photosynthesis

A over space, limiting wasteful photorespiration and maximising photosynthesis.

B over time, limiting wasteful photorespiration and maximising photosynthesis.

Question 8

Climate change poses a greater threat to the food stocks of

A developed countries as they consume a wider range of C3 and C4 plants.

B developing countries as food insecurity is already a pre-existing issue that will only worsen.

Question 9

The successful creation of a C3-C4 hybrid plant has seen little progress over five decades. If a scientist were to manipulate experimental data to make it seem like they were close to successfully making a C3-C4 hybrid, what bioethical concept would they not be adhering to?

A Beneficence, as such an action is not in the best interest of people in need of greater food yields.

B Integrity, as results need to be reported honestly, even if they are unfavourable towards people suffering from food insecurity.

C Justice, as the scientist has a moral obligation to ensure there is no unfair burden placed on people suffering from food insecurity.

Question 10

Some people believe that tampering with the DNA of organisms is unethical, arguing that it goes against the fundamental principles of good/righteous research. As such, they argue against the attempts to create C3-C4 hybrid plants, even though it could be a solution to food insecurity. Which approach to bioethics is framing this argument?

A Consequences-based approach, as they believe the outcome of combating food insecurity is the most important aspect of the topic.

B Duty/rule-based approach, as they believe that scientists have a duty to not experiment with DNA or solve food insecurity.

C Virtues-based approach, as they believe that tampering with DNA goes against core values of science, such as impartiality, unbiased inquiry, and due care.

Exam-style questions

Within lesson

Question 11 (1 MARK)

Which type of plant uses the least amount of energy to undertake photosynthesis?

A C3 plants.

B C4 plants.

C CAM plants.

D All three plants use the same amount of energy in photosynthesis.

Question 12 (7 MARKS)

Sugarcanes are a group of giant tropical grasses belonging to the *Saccharum* genus. Unlike other grain crops that store carbohydrates in their seeds, sugarcane stores carbohydrates in the form of liquid sucrose in its stalk. The liquid is extracted and then crystallised into raw sugar which goes on to become the energy-rich sugar we know and love.

Image: mailsonpignata/Shutterstock.com

The sugarcane farming industry is large in Australia. However, all of the farming occurs in Queensland and far north New South Wales. Sugarcane is a C4 plant.

a Explain how C4 plants have evolved adaptations to maximise photosynthesis. (2 MARKS)

b Pineapples (*Ananas comosus*) are tropical fruits that are grown on large farms in Queensland. Pineapples are CAM plants. Explain the differences between C4 and CAM photosynthesis. (2 MARKS)

c Cereal crops like wheat and barley grow well in Victoria. Explain why sugarcane would likely be outcompeted by other cereal crops if it were introduced into a similar Victorian environment. (3 MARKS)

Multiple lessons

Question 13 (1 MARK)

Which of the following statements about the stages of photosynthesis is correct?

A Both the light-dependent and light-independent stages of photosynthesis vary between C3, C4, and CAM plants.

B Both stages of photosynthesis are identical among C3, C4, and CAM, however, the role of Rubisco is altered in each plant type.

C The light-independent stage of photosynthesis is identical in C3, C4, and CAM plants but the light-dependent stage has significant differences.

D The light-dependent stage of photosynthesis is identical in C3, C4, and CAM plants but the light-independent stage has significant differences.

Question 14 (5 MARKS)

CAM plants live in extremely hot environments where dry conditions can cause water loss via the stomata.

a State the location of the light-dependent reactions. (1 MARK)

b Identify two outputs of the light-dependent reactions. (2 MARKS)

c CAM plants put energy into conserving water. Why don't CAM plants conserve water by reducing the number of water molecules used for an input of photosynthesis? (2 MARKS)

Key science skills and ethical understanding

Question 15 (13 MARKS)

LaMarcus is learning about photosynthesis and wants to compare the growth of a C3 plant and a CAM plant. He uses two species of grass for his experiment. The grass species are of similar size and shape, and both are native to Australia and have similar growing seasons. One species of grass, however, is a CAM plant and the other is a C3 plant.

LaMarcus plants a sample of each grass species in two separate pots. LaMarcus then records the height of each sample by selecting two blades of grass to measure, as measuring them all would take far too long. He then waters each pot with 10 mL of water and places them on the classroom bench with adequate sunlight. The classroom temperature is 20 °C.

After 24, 48, and 72 hours, LaMarcus waters each pot with another 10 mL of water. Once four days have passed, he re-measures each plant by selecting two blades of grass.

a State the independent and dependent variables in the experiment. (2 MARKS)

b State a reasonable hypothesis LaMarcus could be testing. (1 MARK)

c To save time in the experiment, LaMarcus often took the quickest option for his method. LaMarcus' results from the experiment are presented in the following table.

	Grass height before experiment (cm)		**Grass height after 4 days (cm)**		**Average growth (cm)**
	Blade 1	Blade 2	Blade 1	Blade 2	
C3 Grass	4	4	4	5	0.5
CAM Grass	3	4	6	5	2

i LaMarcus didn't use a measuring cylinder or syringe when adding the 10 mL of water to any of the pots. Instead, he simply added a small, unmeasured volume of water each time. Is this method repeatable and reproducible? Justify your response. (2 MARKS)

ii LaMarcus chose to measure the grass to the nearest centimetre on his ruler rather than to the nearest millimetre. Describe how this choice impacts the accuracy of LaMarcus' results. (1 MARK)

iii When measuring the height of each grass species, LaMarcus only sampled the height of two blades of grass to represent the height of the entire experimental population of each grass species. Why might this be a potential limitation to the experiment and how could it be overcome? (3 MARKS)

iv By referring to your knowledge of C3 and CAM plants, explain whether the results of LaMarcus' experiment are expected. In your response, refer to the environment of the classroom and the preferred conditions for C3 and CAM plants. (3 MARKS)

v LaMarcus noticed another student, Ursula, had different results in her investigation of C3 and CAM grass growth. LaMarcus was afraid he made a mistake, so he erased his results and copied Ursula's instead. Which bioethical concept is LaMarcus undermining? (1 MARK)

Adapted from VCAA 2018 Section B Q11

5C FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS

Plants can be difficult to maintain. At times, it feels like you are doing everything right, just for your overly dramatic autotroph friend to curl over and die. Most of the time, this has got something to do with how well the little guy is photosynthesising. There are several key factors that influence a plant's rate of photosynthesis, and therefore their ability to produce their own food, grow, and survive. Learn about them here and hopefully, we can help keep your plants happy and healthy together.

Image: Freer/Shutterstock.com

Lesson 5C

In this lesson you will learn that the rate of photosynthesis is dependent on many factors, including the amount of light available, the amount of water available, the temperature, and the concentration of carbon dioxide.

◀ *Prerequisite knowledge*

Lesson 3B
The optimal temperature of enzymes impacts the rate of photosynthesis.

Lesson 5B
The rate of photosynthesis in C3, C4, and CAM plants is affected by several conditions such as temperature and carbon dioxide concentration.

Future applications ▶

Lesson 6C
Many of the factors that affect photosynthesis also affect cellular respiration.

Study design dot points

- the factors that affect the rate of photosynthesis: light availability, water availability, temperature, and carbon dioxide concentration
- the general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors

Key knowledge units

Light	3.2.6.1
Temperature and pH	3.2.6.2
Carbon dioxide	3.2.6.3
Water	3.2.6.4
Enzyme inhibition	3.2.3.6

Light 3.2.6.1

OVERVIEW

Light is required for the light-dependent stage of photosynthesis to occur. Without it, the reaction rate is limited. As light increases, the photosynthesis rate increases – until a certain point.

THEORY DETAILS

You are always told to give your plants plenty of sunlight, and there is a reason why they always die when you inevitably don't. Plants need light to photosynthesise, so the amount or intensity of light can affect the **rate** of photosynthesis. Recall from lesson 5A that light is not considered an input in the reactions, but it is responsible for exciting electrons in chlorophyll, kick-starting the entire light-dependent stage. As such, the amount of light available determines the rate of photosynthesis.

rate the speed at which a chemical reaction proceeds

Theory in action

Check out scientific investigations 5.1 and 5.2 to put this into action!

Consider Figure 1. This graph shows the relationship between the availability of light (also known as light intensity) and the rate of photosynthesis in a given plant. Notice that the rate of photosynthesis increases as light intensity increases, but only up until point X. The increase in the rate of photosynthesis towards point X is because the plant is exposed to greater light energy, which can energise the chlorophyll within many more plant cells, thus increasing overall photosynthesis.

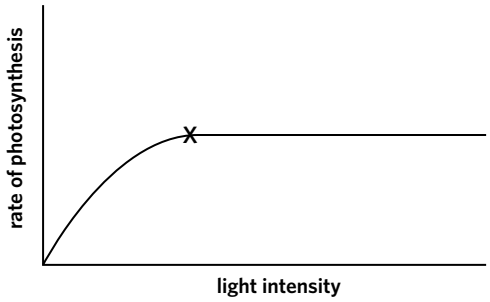


Figure 1 Photosynthesis rate increases with light intensity until plateauing.

After point X, something quite strange happens. The rate of photosynthesis does not increase as light continues to increase. Instead, the rate of photosynthesis remains high, yet constant. We say that after point X on the graph, the rate of photosynthesis **plateaus**. The point at which the reaction plateaus is the flattening of the curve to a level that will remain the same indefinitely without external changes. It is important to remember that after point X, photosynthesis is still occurring at a high rate. The plateau just means that the rate of photosynthesis is no longer increasing.

plateau to reach a state where no further change occurs

Memory device

After the plateau on the light intensity vs photosynthesis rate graph, photosynthesis is still occurring at a high rate. You can think of reaching the plateau on the graph as like reaching the speed limit on a highway – your car is still moving at a fast rate, but it is no longer speeding up.

There are two things that can cause the plateau on the graph, either (1) you've reached the maximum possible rate of photosynthesis, or (2) one of the other inputs or requirements for photosynthesis is limiting the rate.

In terms of Figure 1, the two reasons are described as follows:

1. The maximum rate of photosynthesis can be reached when increasing light intensity, assuming the other factors of photosynthesis are unlimited. This maximum possible rate cannot increase as the **enzymes** within chloroplasts are operating at their full capacity. In this case, it is known as a light-saturation curve, as the plant is saturated with light, and point X is known as the **saturation point**.
2. The **limiting factor** is one of the reactants needed for photosynthesis which there isn't enough of compared to other factors. You can think of the limiting factor as the 'weakest link' that is restricting the reaction from reaching a higher rate. When it is increased, the overall reaction rate will increase. In this instance, before point X, light is the limiting factor as increasing light while keeping all other factors constant increases the reaction rate. After point X, another factor (such as temperature or carbon dioxide) has become the limiting factor.

enzyme an organic molecule, typically a protein, that catalyses (speeds up) specific reactions

saturation point the point at which a substance (e.g. an enzyme) cannot receive more of another substance (e.g. a substrate)

limiting factor a factor that prevents the rate of reaction from increasing

In reality, there is usually a limited supply of inputs for photosynthesis, making the maximum rate of photosynthesis more theoretical rather than practical (in other words, we are unlikely to ever reach a 'maximum'). Therefore the plateau on the graph is typically caused by a limiting factor.

In general, most plants are exposed to large amounts of light and therefore light is not typically a limiting factor, unless the plant's habitat is unusually dark, such as a cave or in the ocean.

Comparing the effect on C3, C4, and CAM plants

Last lesson, we learned about the differences between **C3**, **C4**, and **CAM plants** and their modes of photosynthesis. Due to their differences, certain factors affecting photosynthesis may influence the plant types differently. Light, however, influences the plant types in the same manner. This is because the plant types have the same light-dependent reactions.

C3 plants plants with no evolved adaptation to minimise photorespiration

C4 plants plants that minimise photorespiration by separating initial carbon fixation and the remainder of the Calvin cycle over space

CAM plants plants that minimise photorespiration by separating initial carbon fixation and the remainder of the Calvin cycle over time

Theory in context

TOO MUCH OF A GOOD THING

Figure 1 suggests that the photosynthetic rate will plateau once light intensity reaches a certain level, and that increasing light exposure beyond this point will make no further difference. However, this relationship is more theoretical rather than practical. In reality, exposing plants to intense light can have negative effects on the plant.

High-intensity light will lead to temperature increases in the plant as some of the light energy is converted to heat (we will explore temperature impacts on photosynthesis rate next). High-intensity light can also disrupt water balances in plants as they attempt to cope. This means that it is possible to have too much of a good thing – light can be too intense. Nevertheless, Figure 1 shows how we can visualise the relationship between light intensity and photosynthesis rate as that is how the VCAA have handled it in the past.

Some of the other factors we will consider in this lesson fall into the same category. For example, CO_2 concentration is another factor where too much can be damaging due to the acidic results of certain CO_2 reactions. A plant can also be exposed to too much water and struggle to cope or 'drown'. For the purposes of VCE Biology, however, it is best to think of these relationships in a more theoretical manner as we have demonstrated in Figure 1.

Theory in context

LIGHT COLOUR AND PHOTOSYNTHESIS

Companies that grow plants commercially know that it is not just the intensity of light that influences photosynthesis rate – the wavelength (and therefore colour) of light also impacts this process. Figure 2 shows that the greatest rate of photosynthesis occurs when a plant is exposed to violet or red light and that the rate of photosynthesis is relatively low under green light (most green light is reflected, which is why we see leaves as green). The VCAA have not tested photosynthesis rates under differing wavelengths in the past, however, you may come across SACs that investigate this and it is important to understand that the rate of photosynthesis depends on a variety of factors.

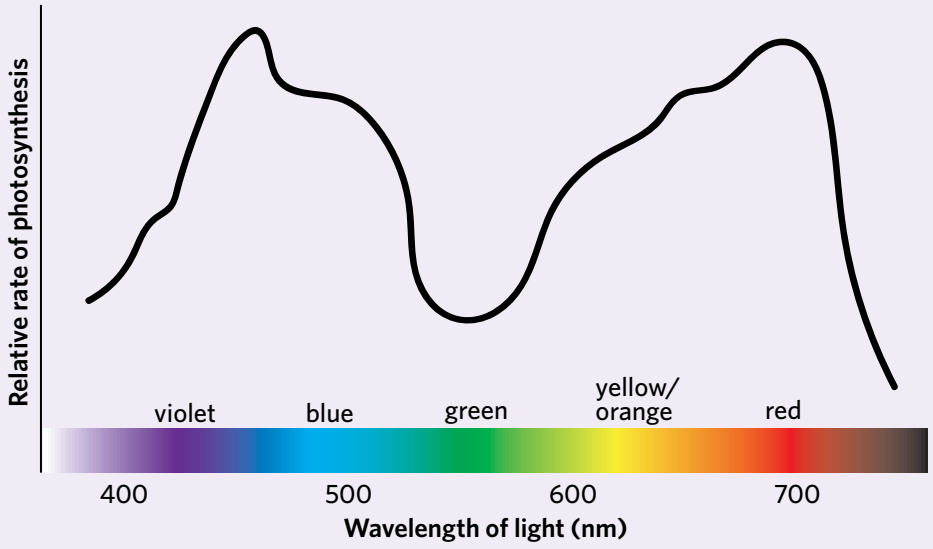


Figure 2 Photosynthesis rate is also dependent on the wavelength of light.

Examiners' tip

It is important to note that in the past the VCAA have frequently tested the topic of factors affecting photosynthesis using graphs. The rate of photosynthesis can be measured in a number of ways, meaning that when presenting these graphs, the y-axis may be labelled with variables other than 'rate of photosynthesis', which you might have otherwise expected. Such labels could be: 'uptake of CO_2', 'CO_2 consumed', 'O_2 produced', 'O_2 output', 'relative rate of photosynthesis', or simply 'reaction rate'. As you should be able to recognise, all these labels equate to the same thing – they are all measures of the rate of photosynthesis.

Similarly, you may come across slightly different x-axis labels that represent factors affecting the rate of photosynthesis. For example, light could be represented as 'light intensity', 'availability of light', 'absorbed light', or simply 'light'. Again, you should be able to recognise that in the context of photosynthesis, these all mean the same thing.

Take a look at the following questions used in recent exams. These are just two of many examples where the VCAA have required students to understand photosynthesis rate in graphical form.

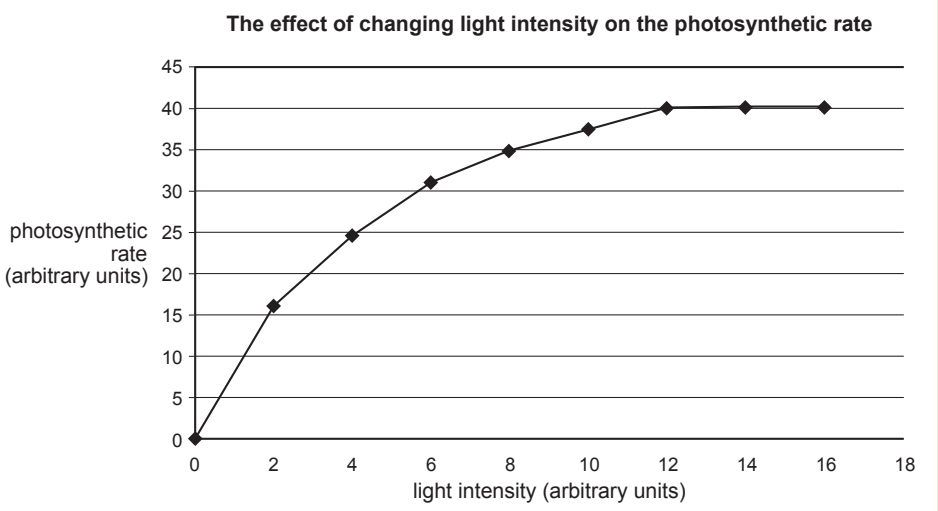


Figure 3 Question 15 in Section B of the 2019 VCE Biology Exam required students to understand the relationship between light intensity and the rate of photosynthesis.

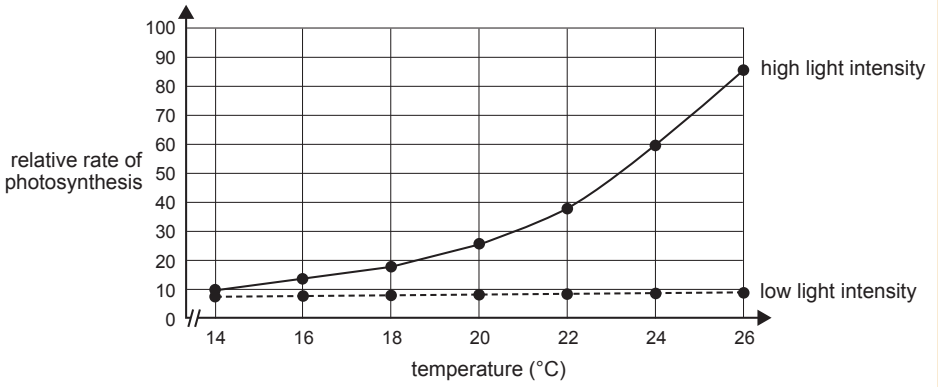


Figure 4 Question 1b in Section B of the 2018 VCE Biology Northern Hemisphere Exam required students to understand how both temperature and light affect the rate of photosynthesis, demonstrating the need for a deep understanding of all the factors that affect photosynthesis.

Temperature and pH 3.2.6.2

OVERVIEW

Photosynthesis requires many enzymes which function best at their optimal temperature and pH.

THEORY DETAILS

Enzymes catalyse various reactions in both stages of photosynthesis. Given enzymes are affected by temperature, so too is the rate of photosynthesis. The rate of photosynthesis is greatest when the temperature matches the enzyme's **optimal** temperature. Every enzyme is unique, but the optimal temperatures of enzymes within a plant is likely to be similar as they have evolved to be suited to the plant's environment.

optimal the point at which for a given condition (e.g. temperature), the maximum function of an enzyme occurs. Also known as **optimum**

Consider Figure 5. The shape of the graph represents the relationship between temperature and enzyme activity, and therefore photosynthetic rate. Notice that the rate of photosynthesis increases toward the enzyme's optimal temperature due to more frequent enzyme-substrate collisions. However, above the optimal temperature, the enzymes begin to **denature** and are unable to function, causing a steep drop-off in photosynthesis rate.

denature the disruption of a molecule's structure by an external factor such as heat

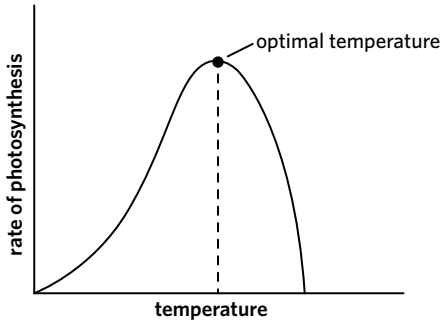


Figure 5 The rate of photosynthesis is greatest at the plant enzyme's optimal temperature.

Similarly, enzymes function best at their optimal pH and photosynthesis occurs fastest under these conditions. Above and below the optimal pH, enzymes denature. This means that a graph of pH against the rate of photosynthesis (Figure 6) is more symmetrical than the graph of temperature against the rate of photosynthesis (Figure 5). Interestingly, the enzymes in the thylakoid lumen must be able to function well at a pH as low as 4! This is because protons are pumped into this space during photosynthesis, which leads to high acidity.

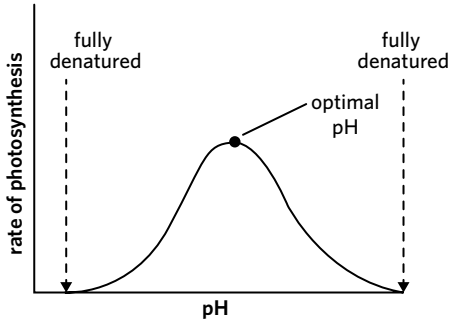


Figure 6 Photosynthesis rate is greatest at the plant enzyme's optimal pH.

Comparing the effect on C3, C4, and CAM plants

C3, C4, and CAM plants are all impacted by temperature and pH as each pathway of photosynthesis is heavily reliant on enzymes. Each plant type has evolved to be suited to their respective environments, meaning that while the general shape of the graph remains the same, the optimal temperature and pH values and ranges may differ between them. In general, C4 and CAM plants are better adapted to hot and dry environments, whereas C3 plants are better suited to cooler temperatures (Figure 7).

Lesson link

Enzymes and factors affecting enzymes are covered in ***chapter 3***.

Theory in context

PHOTOSYNTHESIS IN C3 AND C4 PLANTS UNDER TEMPERATURE CHANGES

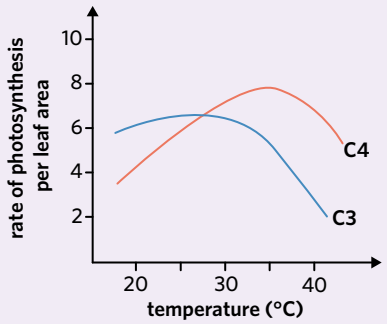


Figure 7 Example of temperature response curves in a C3 and C4 plant.

Figure 7 is an example of what experimental data could look like for C3 and C4 plants when exploring the relationship between rate of photosynthesis and temperature. Although the shape of the lines are not identical to the shape discussed in Figure 5, the concept remains the same. Both plants have an optimal temperature that aligns with their enzymes, and rate of photosynthesis begins to drop above this optimum due to denaturation. You can see that this specific C4 plant has a higher optimal temperature than the C3 plant, as well as a higher maximum rate of photosynthesis per leaf area than the C3 plant.

Carbon dioxide 3.2.6.3

OVERVIEW

As carbon dioxide is an input in the light-independent stage of photosynthesis, its concentration impacts the rate of photosynthesis. As carbon dioxide concentration increases, the rate of photosynthesis increases, up until a certain point. Due to their photosynthetic adaptations, C4 and CAM plants are less affected by carbon dioxide concentration reductions than C3 plants are.

THEORY DETAILS

Plants need carbon dioxide to photosynthesise. Plants take in CO_2 from the atmosphere via open **stomata** on their leaves. If stomata are closed or there are low CO_2 levels in the atmosphere, however, CO_2 may limit the rate of photosynthesis.

stoma (pl. stomata) a small pore on the leaf's surface that opens and closes to regulate gas exchange

Consider Figure 8 showing carbon dioxide concentration against the rate of photosynthesis of a given plant. The rate of photosynthesis increases as CO_2 concentration increases, but only up until point X where it starts to plateau. The initial increase is due to chloroplasts having a higher concentration of CO_2 molecules for the light-independent stage. Thus, the relationship is just like the relationship between substrate concentration and enzyme activity, which we learned about in lesson 3B.

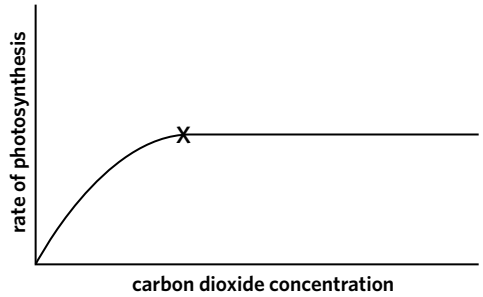


Figure 8 Photosynthesis rate increases with CO_2 concentration until plateauing.

Just like the graph of light availability, two things can cause the plateau on the CO_2 graph:

1. The theoretical maximum rate of photosynthesis can be reached with increasing CO_2, assuming light and water are unlimited and temperature is optimal. This is because the enzyme-catalysed systems within the chloroplast are fully saturated and operating as fast as possible.
2. Another requirement has become the limiting factor. We can say that CO_2 was the limiting factor before the plateau but now it could be light, water, or temperature.

Memory device

You can think of the relationship between CO_2 concentration and photosynthesis rate like sitting down to a plate of cupcakes with your friends. If you increase the number of cupcakes, the rate of cupcake consumption will increase. But each person can only eat one cupcake at a time, so adding more cupcakes once everyone is eating will make no difference to the rate of cupcake consumption (which will remain at its maximum – the plateau).

As we learned in lessons 5A and 5B, when CO_2 concentrations within plant cells are low, plants are susceptible to undertaking the wasteful process of **photorespiration**. The process is initiated by the enzyme **Rubisco**, which has an **affinity** for both CO_2 and O_2. Binding CO_2 initiates photosynthesis, whereas binding O_2 starts photorespiration. Low CO_2 levels make it more likely that O_2 is bound and photorespiration occurs, thus removing an opportunity for photosynthesis and decreasing the overall photosynthetic rate. As such, low CO_2 concentrations can be debilitating to plants.

photorespiration a wasteful process in plants initiated by Rubisco that limits photosynthesis

Rubisco a pivotal enzyme involved in initial carbon fixation during the light-independent stage of photosynthesis

affinity the tendency of a molecule/atom to bind or react with another molecule/atom

Comparing the effect on C3, C4, and CAM plants

C4 and CAM plants have evolved adaptations to counter photorespiration and expose Rubisco to greater levels of CO_2. Because of this, C4 and CAM plants are less susceptible to the impacts of low CO_2 concentration on the rate of photosynthesis compared to C3 plants, which have no strategy to combat photorespiration.

Water 3.2.6.4

OVERVIEW

Water can also influence the rate of photosynthesis given it is an input in the light-dependent stage of photosynthesis and influences the opening and closing of stomata.

THEORY DETAILS

In general, plants have an adequate supply of water to photosynthesise. This means that water is not typically considered a limiting factor in photosynthesis. In certain cases, however, plants may experience a lack of water. This water stress can be caused by droughts or periods of hot weather or any changes to the plant's external environment. To prevent mass water loss, plants close their stomata on their leaves so that water does not evaporate out of the plant.

Recall from lessons 5A and 5B that when plants close their stomata to conserve water they also limit the gaseous exchange of CO_2 and O_2 with the environment. CO_2 can no longer enter the leaves through open stomata to be an input of the light-independent stage and O_2 produced in the light-dependent stage can no longer be released. This can have detrimental effects on the plant, given what we already know about the enzyme Rubisco, which is crucial to the light-independent stage of photosynthesis and has an affinity for both CO_2 and O_2. With stomata closed, O_2 is most likely more abundant than CO_2, meaning that Rubisco is more likely to bind O_2 and initiate the wasteful photorespiration pathway, rather than photosynthesis. This means that the plant wastes energy and loses an opportunity to photosynthesise, which decreases the overall rate of photosynthesis (Figure 9).

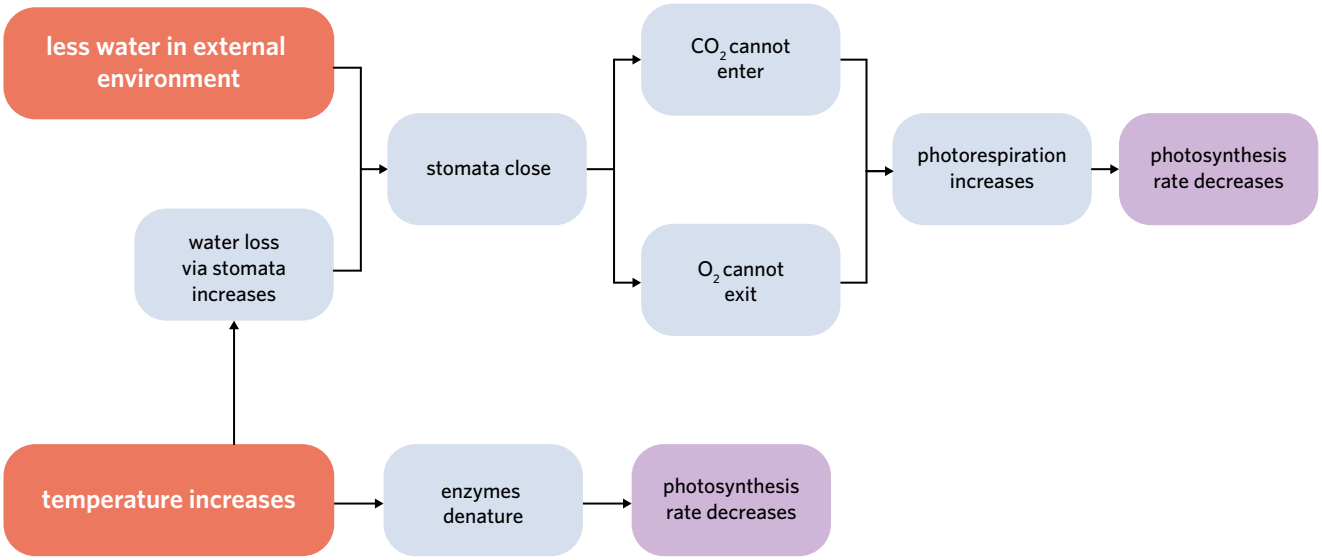


Figure 9 Summary of how water loss impacts photosynthesis rate

Comparing the effect on C3, C4, and CAM plants

Figure 9 demonstrates how water availability influences the rate of photosynthesis. In summary, decreased amounts of water lead to closed stomata, and in turn, decreased CO_2 and increased O_2 concentrations. This decreases the overall rate of photosynthesis. Therefore, due to their evolved adaptations C4 and CAM plants are not affected by water availability unless it is extreme, whereas C3 plants are more susceptible to water loss and the impacts of reduced water on photosynthesis rate.

Enzyme inhibition 3.2.3.6

OVERVIEW

Competitive and non-competitive inhibitors can act on enzymes to reduce the rate of photosynthesis.

THEORY DETAILS

Enzyme inhibitors influence the function of enzymes and, as a result, the rate of photosynthesis. **Competitive inhibitors** bind to the active sites of enzymes to prevent the catalysis of substrates. Meanwhile, **non-competitive inhibitors** bind to an **allosteric site** of an enzyme causing a conformational change to the active site meaning the substrate can no longer bind.

Many herbicide chemicals are inhibitors of enzymes involved in photosynthesis. For example, the light-dependent reactions can be inhibited by triazines, uracils, and benzothiadiazoles. Each of these herbicides can bind to the D1 quinone-binding protein in the electron transport chain, severely disrupting photosynthesis and having potentially fatal consequences for the plant. These are just a few examples of inhibitors that can influence photosynthesis, you don't need to know any of the specifics, just understand that enzyme inhibition is relevant in the photosynthesis context.

In general, the presence of inhibitors lowers the rate of photosynthesis. However, the effect of competitive inhibitors can be gradually overcome if the substrate concentration is continually increased (Figure 10). On the other hand, increasing substrate concentration does not reduce the effect of non-competitive inhibitors. This means that the maximum possible rate of reaction is reduced in the presence of non-competitive inhibitors.

enzyme inhibitor a molecule that binds to and prevents an enzyme from functioning

competitive inhibitor a molecule that hinders an enzyme by blocking the active site and preventing the substrate from binding

non-competitive inhibitor a molecule that hinders an enzyme by binding to an allosteric site and changing the shape of the active site to prevent the substrate from binding

allosteric site a region on an enzyme that is not the active site

Comparing the effect on C3, C4, and CAM plants

As enzyme inhibitors can target enzymes within any part of both stages of photosynthesis, all three types of plants are susceptible to the negative impact of inhibitors. As such, we think about the effect of inhibitors on all plant types as represented by Figure 10.

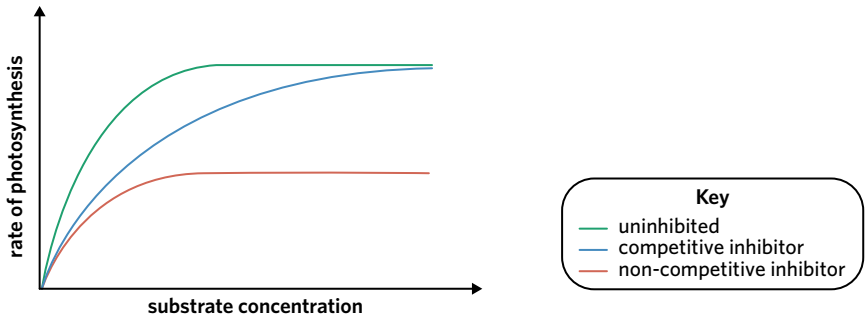


Figure 10 The effect of different inhibitors on the rate of reaction as substrate concentration is increased.

Theory summary

The rate at which a plant undergoes photosynthesis can vary depending on a few key factors.

Table 1 Summary of the factors affecting the rate of photosynthesis

	What happens to photosynthesis if you increase the factor?	What happens to photosynthesis if you decrease the factor?	Differences in C3, C4, and CAM plants
Light	Increases photosynthesis rate until a plateau is reached	Decreases photosynthesis rate	Same among all plants
Temperature	Increases rate when below the optimal, decreases rate when above optimal	Decreases rate due to fewer enzyme-substrate collisions	Affected in a similar pattern, with C4 and CAM plants better suited to hotter environments and C3 plants better suited to cooler environments
pH	Increases rate when below the optimal, decreases rate when above optimal	Increases rate when above the optimal, decreases rate when below optimal	All can be affected
Carbon dioxide concentration	Increases photosynthesis rate until a plateau is reached	Decreases photosynthesis rate	C4 and CAM plants are less impacted by changes than C3 plants, due to their respective abilities to consistently expose Rubisco to CO_2
Water	Typically in excess in a plant. Still, as an input, we can say that increasing it will increase photosynthesis by avoiding the closing of stomata	Can result in closed stomata and a lower CO_2 concentration, decreasing photosynthesis rate	C4 and CAM plants are less impacted by changes than C3 plants, due to their respective abilities to consistently expose Rubisco to CO_2
Enzyme inhibition	Greater inhibitors decrease photosynthesis rate	Fewer inhibitors increases photosynthesis rate	All can be affected

If your plants have struggled in the past, consider if they are supplied with suitable conditions.

Light – is there enough light reaching the plant? For some plants, too much light can be damaging and cause them to heat up and use water.

Temperature – is the temperature suitable for the plant species? The optimal temperature will differ greatly between plant species.

CO_2 concentration – can the plant exchange gases with the atmosphere? Typically this will be fine but access to fresh air can be beneficial.

Water – does the plant receive appropriate quantities of water regularly? If it is inside, this means watering when required depending on the plant species.

5C QUESTIONS

Theory review questions

Question 1

The rate of photosynthesis is

A influenced by key factors including light intensity and temperature.

B constant within an individual plant species.

Question 2

Which of the following are all true about the rate of photosynthesis?

A	can reach a maximum rate despite increases in light intensity	increases with increasing enzyme inhibitors	always increases with temperature	CO_2 concentration impacts reaction rate
B	is slowed in the presence of bright light	decreases with increasing enzyme inhibitors	always increases with temperature	CO_2 concentration impacts reaction rate
C	can reach a maximum rate despite increases in light intensity	decreases with increasing enzyme inhibitors	increases with temperature until the optimal is reached	CO_2 concentration impacts reaction rate
D	can reach a maximum rate despite increases in light intensity	increases with increasing enzyme inhibitors	increases with temperature until the optimal is reached	CO_2 concentration does not impact reaction rate at all

Question 3

Fill in the blanks in the following sentences.

As carbon dioxide levels increase, the rate of photosynthesis generally _____. Changes in carbon dioxide levels are more influential in _____ plants that lack mechanisms to combat the wasteful process of _____. In CAM and _____ plants, changes to carbon dioxide are less influential on photosynthesis rate due to their evolved adaptations.

Question 4

How does water availability affect the rate of photosynthesis?

A Water is often a limiting factor, as it is an input of the light-dependent reactions and an increase in water leads to an increase in the rate of photosynthesis.

B Water is typically in excess but is still required as an input. Low water levels cause a plant to close its stomata, leading to decreased CO_2 and increased O_2 within plant cells, which has impacts on the rate of photosynthesis.

Question 5

Categorise the following as relating to **light**, **temperature**, **CO_2 concentration**, or **water** in the context of photosynthesis.

I C3 plants are more susceptible to changes as this molecule is the key input in the light-independent stage of photosynthesis. _____

II Can reach a theoretical maximum rate with increases, when a light-saturation point is reached and cannot be increased. _____

III Affects C3, C4, and CAM plants in the same manner as it relates to the light-dependent stage of photosynthesis. _____

IV Decreases cause stomata to close which can lead to several issues within the plant. _____

V Has an optimum level where the maximum photosynthesis rate is seen. _____

VI If levels decrease, the plant will open its stomata. _____

VII Involves denaturation at high levels. _____

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 6–10.

Climate change poses a number of serious challenges for plants in the future. Carbon dioxide continues to be emitted into the atmosphere at high levels, leading to more solar radiation being trapped within the atmosphere and the Earth warming as a whole. This process is known as the greenhouse effect, as the Earth and its atmosphere are mimicking greenhouses – buildings constructed to maximise the light energy of the sun, keeping plants warm, and avoiding cold temperatures.

Predicted future impacts of the greenhouse effect include increasing temperatures, more frequent hot days and heat waves, increased drought prevalence, more frequent flooding, more frequent storms, and changes to precipitation. Of course, the predictions vary by location and intensity. There is extreme uncertainty involved in predicting future climatic and weather events. Nevertheless, the predictions are alarming, particularly for the agricultural industry that relies on plants to produce the food we consume on a daily basis.

Question 6

Increasing atmospheric CO_2 levels are predicted to cause extensive damage to ecosystems around the world. However, plant species in certain locations are predicted to benefit from the increased exposure to atmospheric CO_2. This is because

A increasing CO_2 increases the rate of photosynthesis up to a point, allowing the plants to produce more energy, grow, and survive more efficiently.

B CO_2 leads to the greenhouse effect, causing rising temperatures that benefit many plants as elevated temperatures increase photosynthesis rate.

Question 7

There is a large amount of uncertainty surrounding climate science predictions. Which bioethical concept should be considered to ensure there is fair consideration of competing claims on the topic?

A respect

B integrity

C justice

Question 8

Uncertainty often affects climate change research, as most research is novel and previously unexplored. Uncertainty can be thought of as

A the doubt surrounding measurements due to a lack of exact knowledge from previous work.

B measurements that deviate far from the other measurements, making the initial results questionable.

C errors within the experimental design that impact the relevance of the method to measure what is meant to be measuring.

Question 9

Climate extremes such as droughts, floods, and storms will threaten the lives of many people in the near future.
Which bioethical concept should be applied when considering the minimisation of damage or degradation to peoples livelihoods and wellbeing?

A non-maleficence

B beneficence

C respect

Question 10

Agricultural productivity may increase in certain locations that have historically been too cold for certain fruit, vegetable, and crop species. Despite this, many governments in these countries agree that strict anti-global warming measures must be put in place despite the apparent benefits that could arise for their own agricultural sectors.

How might a duty/rules-based approach to bioethics be used to explain this position?

A In this scenario, the negative outcomes outweigh the potential gains. It is detrimental for the Earth to warm any further, as the effects that this would have globally exceed the individual benefits for some historically colder countries.

B In this scenario, politicians and leaders are responsible for protecting the environment and our long-term ecosystems. This is a moral obligation that goes beyond regional interests and the individual situations of specific countries.

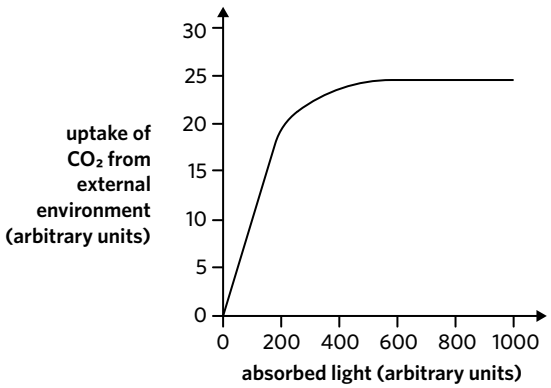
C In this scenario, each individual politician and leader should be left to act according to their own beliefs, given that they are elected as good and honest representatives of the interests of their communities. Many of these leaders believe in fairness, equity, and environmental protection above financial measures like GDP and agricultural output.

Exam-style questions

Within lesson

Use the following information to answer Questions 11 and 12.

The graph shows the uptake of carbon dioxide by a leaf from its external environment as light availability is altered. All other variables are kept constant throughout the experiment.



Question 11 (1 MARK)

The plateau in the graph can be explained by

A the plant being a C4 plant that cannot photosynthesise at a faster rate.

B the given leaf being unable to take up more than this level of CO_2.

C light availability becoming the limiting factor.

D the uptake of carbon dioxide increasing.

Question 12 (1 MARK)

The greatest rate of photosynthesis is seen

A at 200 arbitrary units of light.

B only at 800 arbitrary units of light.

C when the graph touches the x-axis.

D from approximately 500 arbitrary units of light onwards.

Question 13 (1 MARK)

A decrease in temperature away from a plant's optimal range decreases the rate of photosynthesis.

The rate of photosynthesis decreases because

A O_2 cannot be released from the leaves.

B there is less atmospheric CO_2 surrounding the plant.

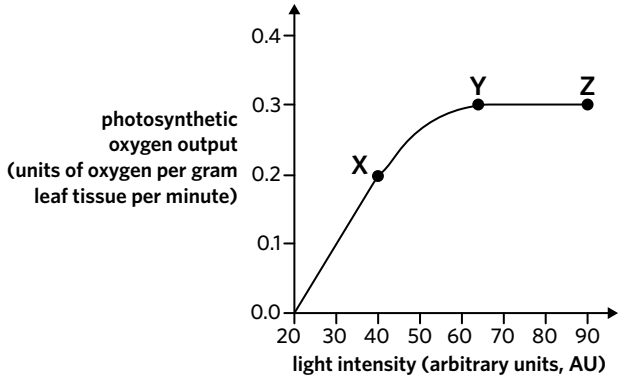
C high temperatures are required to form the bonds within glucose.

D the enzyme and substrate molecules within the chloroplast collide less frequently.

Adapted from VCAA 2014 Section A Q8

Use the following information to answer Questions 14 and 15.

The graph shows the photosynthetic output of oxygen in spinach leaves as light intensity is increased. Temperature is kept constant during the experiment.



Question 14 (1 MARK)

Which one of the following conclusions can be made based on the graph?

A Spinach leaves undertake C3 photosynthesis.

B Photosynthesis at point X is limited by the temperature.

C At point Z the light-independent stage of photosynthesis is not occurring.

D Above 70 AU of light intensity there is no increase in photosynthesis rate.

Adapted from VCAA 2017 Section A Q13

Question 15 (1 MARK)

At point Y

A an increase in light will increase the rate of glucose production.

B carbon dioxide is being consumed at a faster rate than at point Z.

C light intensity is no longer the limiting factor of photosynthesis rate.

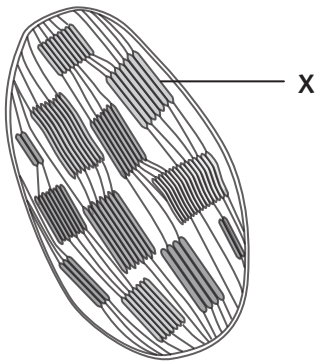
D light intensity has become the limiting factor of photosynthesis rate.

Adapted from VCAA 2017 Section A Q13

Multiple lessons

Question 16 (9 MARKS)

The diagram shows an organelle found in plant cells.



a Structure X is involved in converting light energy into glucose.

i Identify structure X. (1 MARK)

ii Which stage of photosynthesis occurs here? (1 MARK)

b All plants undergo photosynthesis to generate energy. Write the simplified chemical equation for photosynthesis. (1 MARK)

c Describe how an increase in light intensity, CO_2 concentration, and pH each affect the rate of photosynthesis in a C3 plant. Assume the conditions begin at the optimal and the other factors are unlimited. (3 MARKS)

d Briefly explain the key differences between C3, C4, and CAM plants, with regard to changes in water availability. (3 MARKS)

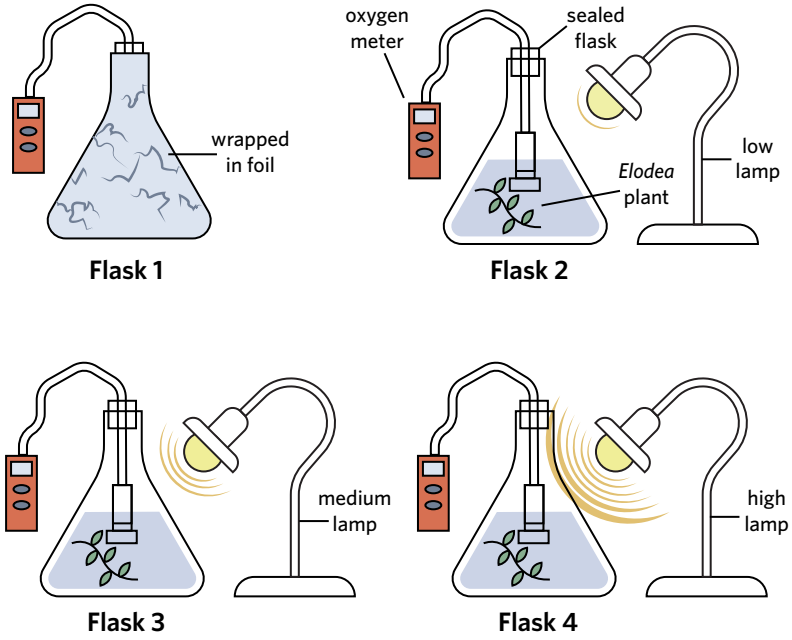
Adapted from VCAA 2011 Exam 1 Section B Q7

Key science skills and ethical understanding

Question 17 (11 MARKS)

Three students, Jerry, Elaine, and George, wanted to test the rate of photosynthesis of a green leafy plant as the intensity of light changed. They set up four identical sealed flasks containing a healthy sample of an aquatic *Elodea* plant known to undertake C3 photosynthesis.

The flasks were labelled 1–4 and set up on a bench in a classroom. Each flask was then exposed to a different intensity of light. Three lamps were used that contained a 'low', 'medium', and 'high' setting. Flask 2 was placed next to a lamp on low, Flask 3 was placed next to a lamp on medium, and Flask 4 was placed next to a lamp on high. Flask 1 was not exposed to any light, as the flask was wrapped in aluminium foil. Each beaker contained an oxygen meter that recorded the levels of dissolved oxygen over time. The flasks were left to sit for only half an hour, as the oxygen meter continuously recorded the rate of oxygen production. A summary of the experimental setup is shown.



a Identify the purpose of the oxygen meter. (1 MARK)

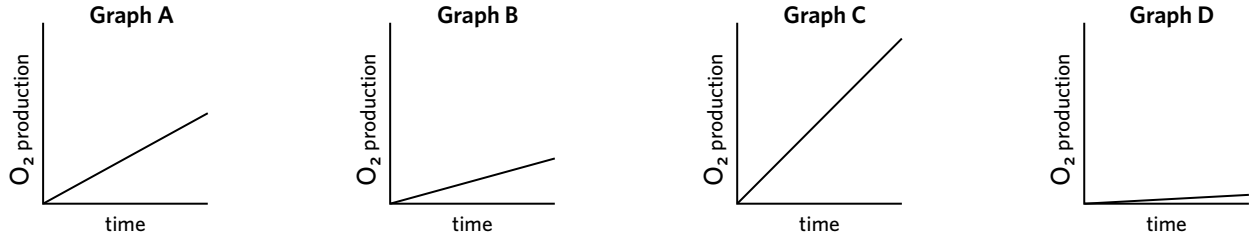
b What was the purpose of Flask 1? (1 MARK)

c Identify why the flasks were sealed in the experiment. (1 MARK)

d In which flask is the fastest rate of photosynthesis expected to be seen? Justify your response. (2 MARKS)

e Identify three variables that need to be controlled for all flasks in this experiment. (3 MARKS)

f Foolishly, the students forgot to return to their experiment and observe the results. When they returned the following period, the flasks had been packed away and all that remained from the experiment was the data from the oxygen meters. Luckily, the students were able to access the rate of oxygen production over the first half an hour of their experiment and produced the following graphs.



i Due to their mistakes, there was confusion as to which graph corresponded to which flask. The three students made the following suggestions.

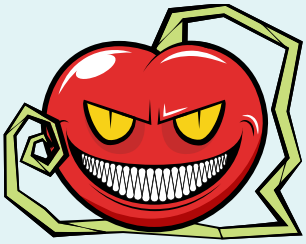
Student	Graph A	Graph B	Graph C	Graph D
Jerry	Flask 2	Flask 4	Flask 1	Flask 3
Elaine	Flask 3	Flask 1	Flask 4	Flask 2
George	Flask 3	Flask 2	Flask 4	Flask 1

Which student is most likely correct? Justify your response. (2 MARKS)

ii When completing their practical report, the students had an obligation to honestly report their results and undertake proper evaluation of any sources of error including the mistake of forgetting to return to their experiment. Which bioethical principle guides the obligation to report honestly? (1 MARK)

5D AGRICULTURAL APPLICATIONS OF CRISPR-CAS9

Oh no! Excessive and repeated tampering with the genome of tomatoes over many years has provided them with some kind of malevolent sentience. One day in the future when all this becomes too real, we'll be able to look back and say it all started with CRISPR-Cas9.



Lesson 5D

In this lesson you will learn how CRISPR-Cas9 applications can improve the efficiency of photosynthesis in crops.

◀ *Prerequisite knowledge*

Lesson 4B
CRISPR-Cas9 is a system found within bacteria that can be utilised as a genetic editing tool to alter an organism's DNA.

Lesson 4F
GMOs have a number of agricultural uses including increasing crop productivity and disease resistance.

Lesson 5B
Rubisco, a key enzyme in the light-independent stage of photosynthesis, can initiate photorespiration thereby impacting photosynthesis.

Future applications ▶

Lesson 6D
CRISPR-Cas9 agricultural applications could play a role in plant matter destined for biofuels.

Study design dot point

- potential uses and applications of CRISPR-Cas9 technologies to improve photosynthetic efficiencies and crop yields

Key knowledge unit

CRISPR-Cas9 in agriculture	3.2.10.1

CRISPR-Cas9 in agriculture 3.2.10.1

OVERVIEW

CRISPR-Cas9 technologies can be used to edit the genome of agricultural crops, potentially improving photosynthesis and crop yields in a variety of ways.

THEORY DETAILS

What are CRISPR-Cas9 technologies?

In lesson 4B, you learned all about the basics of CRISPR-Cas9 technologies. **CRISPR** are detectable sequences of DNA that act as an adaptive immune system within prokaryotes such as bacteria. **Cas9** is an endonuclease that can cut and glue DNA back together. Importantly, Cas9 can be instructed by CRISPR to target specific DNA recognition sites to cut and join, altering the DNA.

CRISPR short, clustered repeats of DNA found in prokaryotes which protects them against viral invasion

CRISPR-associated protein 9 (Cas9) an endonuclease that creates a blunt end cut at a site specified by guide RNA (gRNA)

This makes the CRISPR-Cas9 pairing one of the most iconic duos since Bert and Ernie, Han and Chewie, or the Veronicas. CRISPR-Cas9 technology can therefore be utilised to **genetically modify** an organism's genome.

An important application of CRISPR-Cas9 is removing unwanted or disadvantageous alleles within an organism's genome. This can result in an improved or desired phenotype. The new and improved **genetically modified organism** (GMO) can then adapt to and survive its environment more effectively.

Why do we need to improve photosynthetic efficiencies and crop yields?

In lesson 5B, you learned about the function of **Rubisco** and how it can initiate **photorespiration** which decreases the efficiency of photosynthesis. Recall that **C3 plants** have no adaptations to limit photorespiration, whilst **C4** and **CAM plants** do. The most abundant plants on Earth – C3 plants – also encompass most agricultural crop species that we rely upon for food. By 2050, it is predicted that agricultural productivity will need to almost double to cope with the demands of the rising global population. Climate change, however, has the potential to significantly reduce crop yields globally and **arable land** is already largely exhausted. Furthermore, clearing more land releases greenhouse gases into the atmosphere. Therefore, the situation is as follows: we need to get more out of the land we use. That is, agricultural **yields** need to increase while using the same amount of agricultural land. This is where gene editing comes in. Editing crop genomes could enable farmers to maximise crop productivity without clearing any additional and (Figure 1).

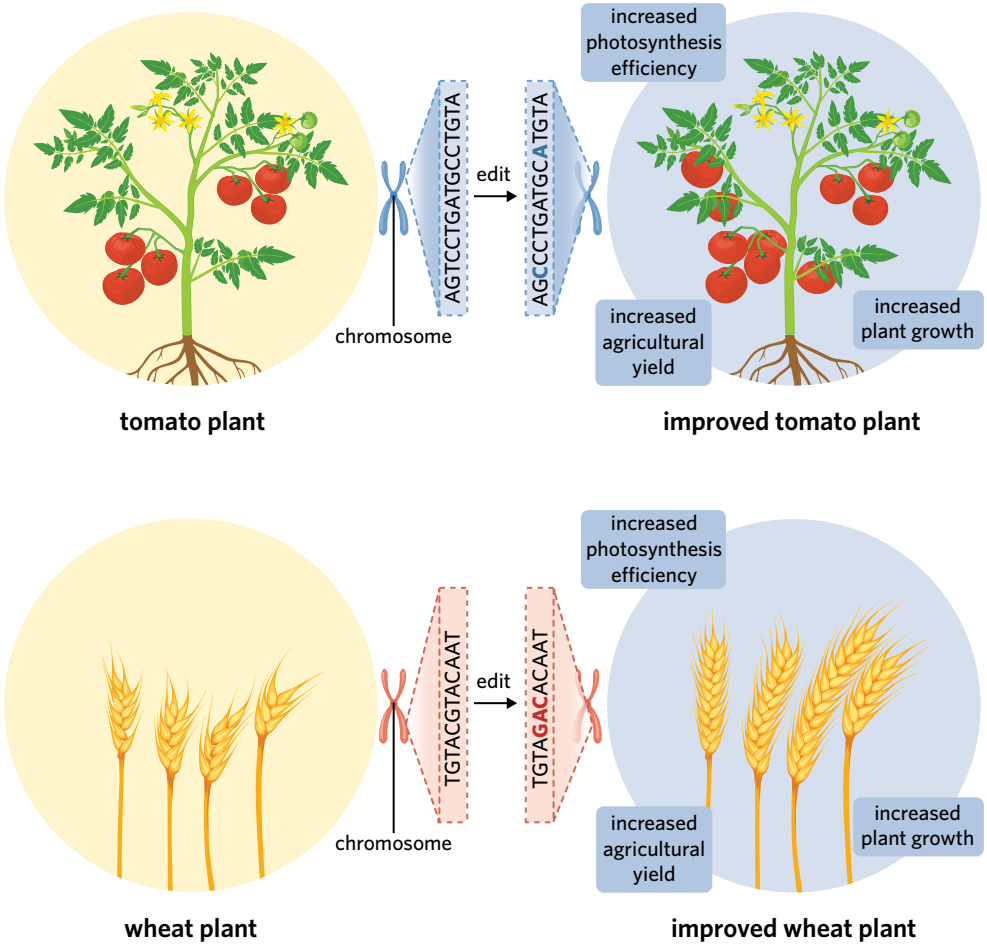


Image: Kazakova Maryia, Alfmaler/Shutterstock.com

Figure 1 Genetic engineering could be used to increase the productivity of crops such as tomato or wheat.

How can CRISPR-Cas9 technologies improve photosynthetic efficiencies and crop yields?

Thanks to the discovery of CRISPR-Cas9, we now possess a novel gene editing tool that is more precise and affordable than its alternatives and could aid with our global agricultural productivity needs. As such, researchers are busy with CRISPR-Cas9 studies and experiments with the aim of engineering more productive crop species. Ideally, this will increase the yield of crops that can be grown and harvested on a large scale like cereal grains such as wheat or barley, vegetables such as potatoes or tomatoes, or fruits such as apples and bananas.

genetic modification the manipulation of an organism's genetic material using biotechnology

genetically modified organism (GMO) an organism with genetic material that has been altered using genetic engineering technology

Lesson link

In ***lesson 4F*** you learned about some of the agricultural applications of GMOs and the associated ethical considerations. CRISPR-Cas9 technology is frequently used to genetically modify a crop's DNA, making their products GMOs. These GMOs must be given appropriate ethical considerations before commercialisation.

Rubisco a pivotal enzyme involved in initial carbon fixation during the light-independent stage of photosynthesis

photorespiration a wasteful process in plants initiated by Rubisco that limits photosynthesis

C3 plants plants with no evolved adaptation to minimise photorespiration

C4 plant plants that minimise photorespiration by separating initial carbon fixation and the remainder of the Calvin cycle over space

CAM plant plants that minimise photorespiration by separating initial carbon fixation and the remainder of the Calvin cycle over time

arable land land that is suitable for growing crops

yield the amount of agricultural product harvested per area of land

One method to increase the photosynthesis efficiency in agricultural plants using CRISPR-Cas9 is to engineer crops that bypass photorespiration, somewhat mimicking the function of C4 and CAM plants. Other possible changes to maximise photosynthesis efficiency could be to target Rubisco's function directly, or edit the function of chloroplasts to make them more efficient, or target stomata to reduce the impacts of water stress. These, however, are not the only potential applications. Given CRISPR-Cas9 can edit a plant's DNA, it can, theoretically, change almost anything about the plant. The potential of CRISPR-Cas9 technologies to improve the agricultural industry is huge. Figure 2 summarises some of the potential applications.

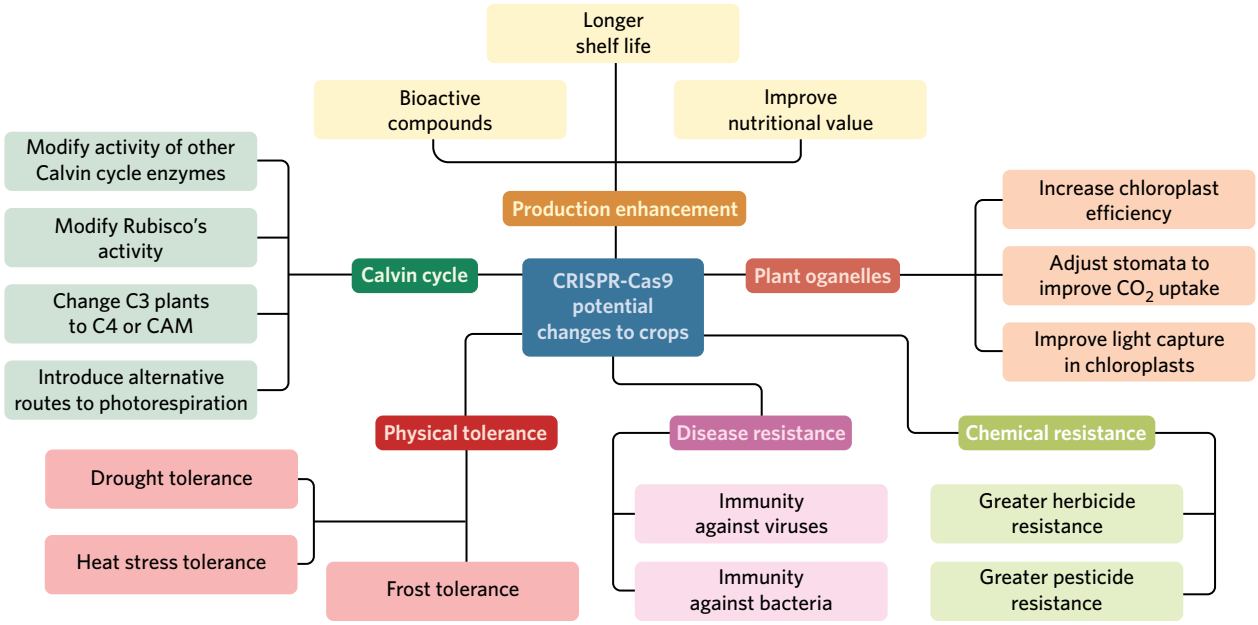


Figure 2 Some of the potential areas that CRISPR-Cas9 could be used to target in order to increase agricultural crop productivity.

Research into the appropriateness of CRISPR-Cas9 applications to improve the photosynthesis of a given crop species involves the following steps:

- Understanding the entire photosynthetic process of the target crop species, in order to gain a solid understanding of the genome of the target crop and how specific genes contribute to photosynthesis and growth. This also includes understanding the regulation of the photorespiration pathway.
- Utilising high-level computers to model the photosynthetic pathway and identify inefficiencies.
- Using CRISPR-Cas9 technologies to target and edit the genes responsible for the identified inefficiencies.

Overall, it is important to recognise that CRISPR-Cas9 technology is still relatively new. The potential for CRISPR-Cas9 applications in the agriculture sector is enormous, as established in Figure 2. Currently, CRISPR-Cas9 applications mostly apply to research and development. These applications must undergo rigorous checks to determine whether they adhere to Australian GMO standards and regulations before being made available for public consumption. Still, recent CRISPR-Cas9 research has produced some extremely promising results. See the Theory in Context boxes for examples of recent agricultural applications of CRISPR-Cas9, but remember that specific details of such studies are beyond the scope of VCE Biology.

Theory in context

HORMONES AND MORE PRODUCTIVE RICE

Rice (*Oryza sativa*) is a staple food for a large proportion of the world's population, thus making it an ideal candidate for CRISPR-Cas9 applications. In China, researchers have been exploring mutations in a family of genes within rice relating to responses to the plant hormone abscisic acid. Tests showed manipulating this family of genes resulted in a 25-30% increase in grain yield.

Image: FeniloQ/Shutterstock.com

Figure 3 The alteration of genes relating to hormones in rice may result in more productive crops.

Theory in context

ALTERING RUBISCO IN TOBACCO PLANTS

Researchers in the United Kingdom have been targeting the function of Rubisco in tobacco (*Nicotiana tobacaum*) plants. The aim was to use CRISPR-Cas9 to better understand the many genes relating to Rubisco's complex function. Research showed that editing certain genes resulted in lower Rubisco content and subsequently lower photosynthesis rates. Now the race is on to uncover how the genes can be edited to improve Rubisco and overall photosynthesis efficiency.

Image: Piyawat Nandeenopparit/Shutterstock.com

Figure 4 Tobacco plants are an area of substantial CRISPR-Cas9 research.

Theory in context

LONGER SHELF LIFE OF MUSHROOMS

In the United States, CRISPR-Cas9 has successfully been used to delete an unwanted gene in white button mushrooms (*Agaricus bisporus*). The gene contributes to faster browning of mushrooms, hence lowering their shelf life. Deletion of the gene was shown to extend the shelf life of mushrooms by 30%.

Image: Stephen Gibson/Shutterstock.com

Figure 5 The white button mushroom suffers from fast browning and aging, which CRISPR-Cas9 gene editing can alleviate.

Theory in context

ENGINEERING GLUTEN-FREE WHEAT

CRISPR-Cas9 applications are even being explored in the production of gluten-free wheat (*Triticum aestivum*). Gluten-free wheat would allow those with coeliac disease to consume wheat without suffering any adverse effects. Researchers in Spain have reported successful editing of wheat DNA to produce low-gluten wheat that resulted in an 85% reduction in 'immunoreactivity' to gluten, providing hope for gluten-free wheat in the future.

Image: Igor Meshkov/Shutterstock.com

Figure 6 Wheat and all wheat products contain gluten. CRISPR-Cas9, however, can be used to create low-gluten wheat that is less likely to trigger an immune response in affected individuals.

Theory summary

CRISPR-Cas9 technologies have the potential to improve the agricultural industry in a number of ways. By editing a crop's genome, the efficiency of photosynthesis can be improved by adjusting key factors in the process such as Rubisco or the photorespiration pathway. Other potential applications include increasing crop tolerance for harsh physical conditions such as drought, frost, disease, or the chemicals used in farming. The potential of CRISPR-Cas9 technologies is huge, however, due to its recency, most applications are still in the early stages of research and development.

It looks as though scientists took it too far when editing tomatoes. At first, they used CRISPR-Cas9 to produce bigger tomatoes, then longer-lasting tomatoes, then disease-resistant tomatoes. But the scientists got too cocky and wanted to push the limits of genetic engineering! The scientists decided to produce square-shaped tomatoes, tomatoes that play music when you open them, and finally tomatoes with consciousness. Luckily for us, this far-fetched scenario is nothing but hogwash and we still reign supreme over tomatoes ... for now.

5D QUESTIONS

Theory review questions

Question 1

How can CRISPR-Cas9 technologies be used to advance the agricultural industry?

A CRISPR-Cas9 applications can produce GMO crops by causing mutations in a crop's DNA.

B CRISPR-Cas9 applications can be used to target and alter certain unwanted genes within a crop's genome.

Question 2

CRISPR-Cas9 could improve the efficiency of a plant's photosynthesis process by

A providing the plant with greater resistance to known diseases.

B altering the function of Rubisco to make the enzyme more efficient.

Question 3

Fill in the blanks with the following terms.

- genes
- genome
- drought tolerance
- disease resistance
- herbicide resistance

CRISPR-Cas9 applications have the potential to improve crops by editing _____ within the crop's _____. Common objectives of CRISPR-Cas9 applications to crops include providing greater _____ to handle a lack of rainfall, _____ to better survive the use of agricultural chemicals, and _____ to better survive known pathogens.

Question 4

Order the steps to correctly describe the process of utilising CRISPR-Cas9 to improve the efficiency of photosynthesis within a given fruit, from research to consumption.

I Ensure the GMO fruit meets all required standards and regulations and is approved.

II Use high-level computing software to model the process in order to identify inefficiencies in the process.

III Study the photosynthetic pathways of the fruit to attempt to understand the role of genes involved in the process.

IV Utilise CRISPR-Cas9 technologies to target and edit the genes responsible for the inefficiencies in photosynthesis.

V After the GMO fruit is approved, provide appropriate labelling and communication of the product to the public for consumption.

VI Determine whether gene editing was successful by experimenting on the new GMO fruit and comparing it to the non-GMO fruit.

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 5–8.

Food security means that all people have physical, social, and economic access to sufficient and safe food at all times. Many countries today already suffer from low food security (or food insecurity), as people are consistently going hungry and experiencing the effects of malnutrition (a lack of sufficient nutrients in the body). The main driver of low food security is man-made conflict. Some of the worst-affected countries today in terms of food insecurity include Yemen, South Sudan, Syria, Afghanistan, and the Democratic Republic of Congo.

In war-torn countries, ending conflicts would enable agricultural practices to safely resume on a large scale and provide sufficient food for populations. Still, CRISPR-Cas9 research conducted in developed countries could play a role in assisting agriculture in war-torn developing countries down the track. For instance, CRISPR-Cas9 could be used to increase crop yields in developed countries which could provide a greater amount of food products for foreign aid. Additionally, CRISPR-Cas9 could be used to engineer seeds that are better suited for the environment where the aid is needed.

Image: akramalrasny/Shutterstock.con

Question 5

War-torn countries typically have

A high food security as the conflict provides food resources for the population to use.

B low food security as conflict disrupts or completely stops local agricultural processes.

Question 6

Which of the following factors would be consistent with an area of low food insecurity?

A Poor growing weather, insufficient agricultural equipment, droughts, and ongoing conflict.

B Safe access to food, affordable products, sufficient agricultural land and equipment, and suitable weather.

Question 7

Providing foreign aid in the form of CRISPR-Cas9 genetically modified organisms (GMOs) requires the consideration of a number of ethical concepts. Which of the following is a correct consideration regarding the concept of integrity?

A No undue risk or harm should be placed on the populations and individuals receiving the GMO aid.

B Fair consideration must be provided to the beliefs and customs of recipients in regard to genetic engineering.

C All results regarding CRISPR-Cas9 applications in organisms should be reported in a clear and honest manner.

D Disputing claims regarding the debate surrounding GMOs and CRISPR-Cas9 applications should be given fair consideration.

Question 8

CRISPR-Cas9 applications in developed countries could produce more productive crops that could then increase foreign aid to countries in need. However, research and applications of such gene-editing technologies are extremely expensive and demanding compared to traditional methods of agriculture and foreign food aid. There is, therefore, a decision to be made as to what is more beneficial to international food aid: invest more in CRISPR-Cas9 as it may increase crop yields in the future thus helping aid, or invest less in CRISPR-Cas9 and more in traditional food production and transport methods to contribute to aid. How would a virtues-based approach to bioethics correctly frame this decision?

A More money should be invested in agricultural CRISPR-Cas9 applications as developed countries have a responsibility to provide the maximum benefits to developing countries in the long term.

B Less money should be invested in agricultural CRISPR-Cas9 applications as developed countries should act in a moral manner and provide as much foreign aid to the people in need right away.

Exam-style questions

Within lesson

Question 9 (1 MARK)

CRISPR are

A gene-editing technology capable of targeting and cutting specific genes.
B traits in agricultural crops that are capable of being edited.
C short detectable sequences of repeating DNA.
D endonucleases that cut and join DNA strands.

Question 10 (1 MARK)

Which of the following would be a desired trait that scientists could edit into the genome of a crop using CRISPR-Cas9 technology if the crop was grown in Victoria?

A reduced CO_2 intake
B shorter shelf life of seeds
C increased heat stress tolerance
D increased sensitivity to herbicide

Question 11 (1 MARK)

Which of the following would be a desired trait that scientists could edit into the genome of a crop using CRISPR-Cas9 technology if the crop was grown in tropical far-north Queensland?

A increased immunity against disease
B decreased pesticide resistance
C decreased drought tolerance
D increased frost tolerance

Multiple lessons

Question 12 (1 MARK)

Which of the following is not an example of genetic modification?

A Selecting the seeds from the tomato plant that stayed ripe the longest in a plantation to grow future generations of tomatoes.
B Choosing the largest bunch of bananas from a banana tree to take home and consume.
C CRISPR-Cas9 cutting a sequence of DNA at a specific site guided by gRNA.
D Using an endonuclease to cut and glue DNA back together.

Question 13 (7 MARKS)

Pineapples (*Ananas comosus*) are CAM plants grown in tropical locations including Queensland, Australia. Contrary to popular belief, pineapples do not grow on trees. Rather, they grow from the centre of a short leafy plant. The fruit itself is formed from a cluster of 100–200 flowers that have been fused together. Like all plants, the pineapple plant has evolved and adapted to be well-suited to its environment over many generations.

Image: Noppadon stocker/Shutterstock.com

a Briefly discuss how pineapples are well-suited to warm tropical locations. (2 MARKS)
b What is the difference between C4 and CAM plants? (2 MARKS)
c One potential use of CRISPR-Cas9 technologies in the agricultural industry is to engineer C3 plants to be more like C4 or CAM plants. This could be achieved by modifying the function of Rubisco or by introducing a pathway that acts as an alternative to photorespiration.
  i What would be the intended change in Rubisco's activity as a product of CRISPR-Cas9 intervention? (1 MARK)
  ii CRISPR-Cas9 could also be used to improve a plant's CO_2 uptake. How does the concentration of CO_2 relate to photorespiration in C3 plants? (2 MARKS)

Question 14 (3 MARKS)

A number of factors influence the rate of photosynthesis including light, CO_2 concentration, water concentration, and temperature. Theoretically, CRISPR-Cas9 technologies hold the power to edit any genes within a plant's genome given a sufficient understanding of the genes and their action.

a Suggest how CRISPR-Cas9 could be used to improve a plant's rate of photosynthesis with regard to light availability. (1 MARK)

b Suggest how CRISPR-Cas9 could be used to improve a plant's rate of photosynthesis with regard to CO_2 and water concentration. (2 MARKS)

Key science skills and ethical understanding

Question 15 (6 MARKS)

CRISPR-Cas9 research and experimentation are currently being performed on a variety of potatoes to investigate whether the genome of the potatoes can be altered to improve potato weight, size, and shelf life. In order to achieve this, researchers studied the genome of the potatoes extensively before using CRISPR-Cas9 technology.

An experiment was carried out in order to edit the genomes of potato seeds in the hope of increasing their size and shelf life after growing. Three samples of CRISPR-Cas9 edited potato seeds were used in the experiment, each with a sample size of one hundred seeds. One hundred potato seeds that did not undergo any gene editing were also monitored. Seeds were grown for three months and were subsequently picked and monitored at room temperature.

The researchers assumed that the density of the grown potatoes would remain the same and therefore weight would indicate size. To determine shelf life, the potatoes were checked for dark spots, bad odours, or a soft or mushy texture daily. If these signs were detected, the potato was marked as bad and the shelf life was noted. The experiment went on until all potatoes went off. The results are shown in the table.

	Unedited sample	**Edited sample 1**	**Edited sample 2**	**Edited sample 3**
Number of seeds that successfully grew to adulthood	97	96	94	98
Average weight (g)	173.4	179.7	171.0	178.4
Average shelf life (days)	38.4	36.1	34.1	36.7

a Summarise the potato weight and shelf life results seen in the table. (2 MARKS)

b The daily shelf-life checks to determine whether the potatoes had gone off were carried out by the same researcher each day. Is this method repeatable? (1 MARK)

c Discuss whether the experiment was a success in terms of increasing the size and shelf life of the potatoes. (2 MARKS)

d Although certain results were unexpected, the researchers are required to openly communicate their findings to the scientific community. Which bioethical concept stipulates this requirement? (1 MARK)

CHAPTER 5 SUMMARY

Photosynthesis equations

$$\underset{\text{carbon dioxide}}{6\,CO_2} + \underset{\text{water}}{12\,H_2O} \xrightarrow{\text{sunlight}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{oxygen}}{6\,O_2} + \underset{\text{water}}{6\,H_2O}$$

Two stages of photosynthesis

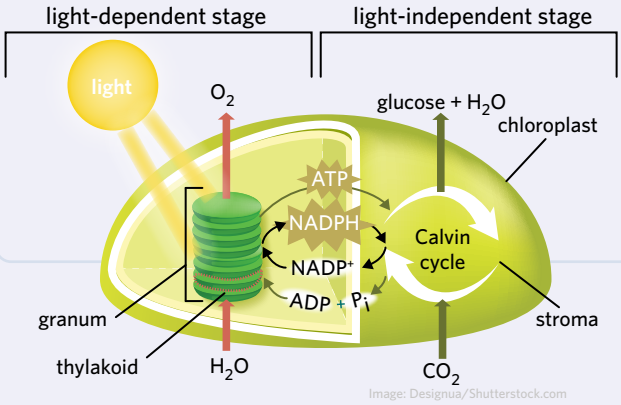


Image: Designua/Shutterstock.com

Plant cells harness light energy to produce glucose via the following steps:

1 Sunlight excites an electron within chlorophyll in the grana, causing water from the roots to split into oxygen and hydrogen.
2 The excited electron and the hydrogen ion from the split water facilitate the production of ATP and NADPH. These molecules are essential for the Calvin cycle.
3 The oxygen from the split water is released out of the chloroplast as a by-product.
4 Carbon dioxide enters via the stomata. With the help of ATP and NADPH, the carbon from carbon dioxide undergoes a series of reactions in the Calvin cycle.
5 Eventually, a molecule is produced that contributes to the formation of glucose and the cyclic reaction continues. Some water is also formed in this stage.

Inputs, outputs, and location of photosynthesis stages

	Location	Inputs	Outputs
Light-dependent stage	Grana/thylakoid membranes	12 H_2O 12 $NADP^+$ 12 ADP + P_i	6 O_2 12 NADPH 12 ATP
Light-independent stage	Stroma	6 CO_2 12 NADPH 12 ATP	$C_6H_{12}O_6$ 12 $NADP^+$ 12 ADP + P_i 6 H_2O

Comparison of C3, C4, and CAM plants

Type of plant	C3	C4	CAM
Limits photorespiration	No	Yes	Yes
Separation of initial CO_2 fixation and remainder of Calvin cycle	No separation	Between cells (over space)	Between night and day (over time)
Stomata open	Day	Day	Night
Advantages	Doesn't consume extra energy	Minimises photo respiration	Minimises photo-respiration and reduces water loss
Disadvantages	Susceptible to photorespiration initiation	Consumes extra energy	Consumes extra energy
Best adapted to	Moderate, or cool and wet environments	Hot, sunny habitats	Very hot, dry habitats
Examples	Most plants, including wheat, rice, and all trees	Corn, sugarcane, and switchgrass	Cacti, pineapples, and orchids

CRISPR-Cas9 technologies to improve agricultural productivity

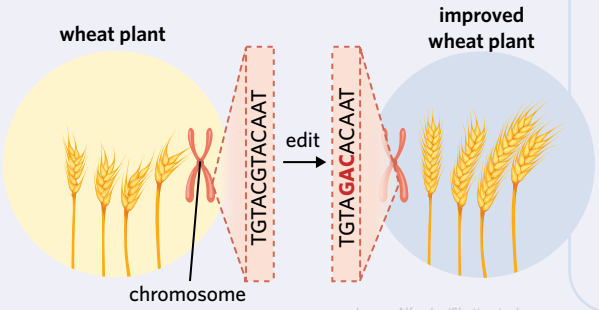
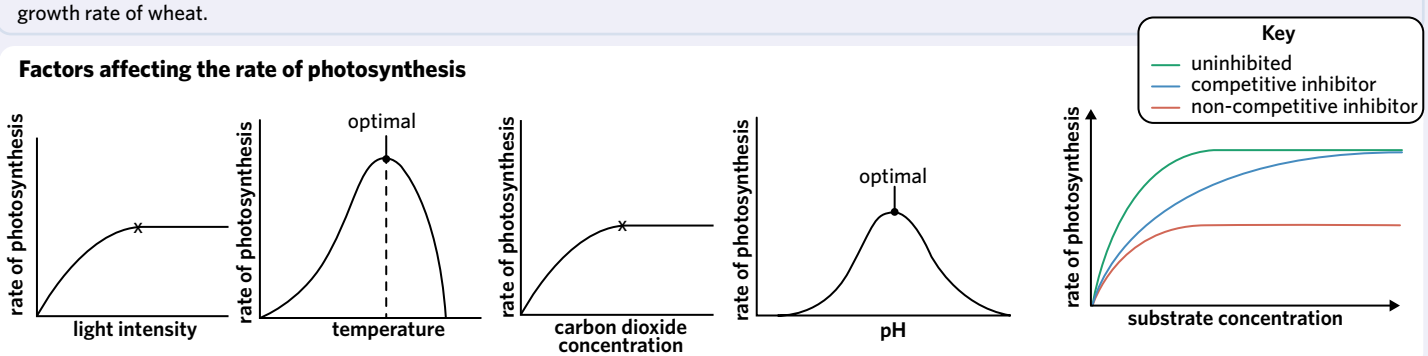


Image: Alfmaler/Shutterstock.com

CRISPR-Cas9 technologies have the potential to improve the agricultural industry by influencing the efficiency of how plants photosynthesise, live, and grow. By targeting and editing a plant's genome, virtually any characteristic of the plant can be improved if the plant's genome is well understood. Examples of agricultural applications of CRISPR-Cas9 include increasing a crop's photosynthesis efficiency, shelf life, disease resistance, or tolerance to weather extremes such as droughts or frost. This example is hypothetical, and represents the alteration of a specific sequence of DNA to increase the growth rate of wheat.

Factors affecting the rate of photosynthesis



CHAPTER 5 SAC PRACTICE

SAC skills covered in this section:

✓ Case study analysis ✓ Data analysis

PLANT GROWTH RACE (21 MARKS)

Image: bonandbon/Shutterstock.com

Two siblings, Sammy and Lara, are visiting a plant nursery. Lucky for them, their grandmother has just sent them a Christmas gift. Unlucky for them, the gift is a voucher for a small plant each that they will have to look after. Sammy decides on a small leafy plant and Lara picks a small succulent. When they get home, they research the two species of plants online. They find that the expected size of the leafy plant, when fully grown, is 20 cm tall and 10 cm wide, while the expected size of the succulent when fully grown is 20 cm tall and 30 cm wide. Both plants require plenty of access to sunlight in order to achieve this growth. The leafy plant requires water every week, regardless of the temperature, while the succulent requires water every week when it's hot but only every two weeks when it's cold. Sammy and Lara decided to have a race to see who could grow their plant to reach 20 cm in height first. They recorded the height and width of each plant using a ruler, ensuring that the other watched to limit any bending of the truth. Measurements were taken weekly and are shown in the table along with growth rate calculations compared to the previous week.

	Sammy					
	Height			Width		
Week	Measure (cm)	Growth (cm)	Growth (%)	Measure (cm)	Growth (cm)	Growth (%)
0	8.1	-	-	4.2	-	-
1	8.4	0.3	3.7	4.2	0	0
2	8.8	0.4	4.76	4.3	0.1	2.38
3	9.6	0.8	9.09	4.7	0.4	9.3
4	10.8	1.2	12.5	5.4	0.7	14.89
5	11.4	0.6	5.56	5.7	0.3	5.56
6	13.1	1.7	14.91	6.0	0.3	5.26
7	15.0	1.9	14.5	6.5	0.5	8.33
8	15.9	0.9	6	7.1	0.6	9.23
9	19.1	3.2	20.13	7.8	0.7	9.86
10	21.1	2.0		9.0	1.2	

Week	Lara					
	Height			Width		
	Measure (cm)	Growth (cm)	Growth (%)	Measure (cm)	Growth (cm)	Growth (%)
0	8.5	-	-	12.9	-	-
1	8.9	0.4	4.71	13.5	0.6	4.65
2	9.4	0.5	5.62	13.9	0.4	2.96
3	10.0	0.6	6.38	14.7	0.8	5.76
4	10.7	0.7	7	15.9	1.2	8.16
5	11.4	0.7	6.54	17.4	1.5	9.43
6	12.3	0.9	7.89	18.8	1.4	8.05
7	14.5	2.2	17.89	20.6	1.8	9.57
8	15.8	1.3	8.97	22.9	2.3	11.16
9	17.1	1.3	8.23	24.5	1.6	6.99
10	18.5	1.4		25.9	1.4	

1 In which week did Sammy's plant experience the largest growth (in cm) in terms of height? (1 MARK)

2 In which week did Sammy observe the smallest percentage growth of her plant in terms of either height or width? (1 MARK)

3 In which week did Lara observe the largest percentage growth of her plant in terms of either height or width? (1 MARK)

4 In which pair of consecutive weeks did Lara's plant experience the largest growth (in cm) in terms of height? (1 MARK)

5 One of the siblings had a week that saw identical growth (%) in both the width and height of their plant. Identify both the sibling and the week that is being referred to. (1 MARK)

6 Which plant experienced a more consistent growth rate in height over the first 9 weeks? Use data to support your response. (2 MARKS)

7 After 10 weeks, how did the height and width of each plant compare to the expected sizes of the two species? (2 MARKS)

8 Sammy's plant won the race to 20 cm after 10 weeks. After the race was over, both siblings lost interest in recording the percentage growth. When determining the percentage increase, the following formula can be used:

$$\text{percentage increase} = \frac{\text{final value} - \text{starting value}}{\text{starting value}} \times 100$$

In our example, given that the final value is equal to our measure for a new week (e.g. Week 10), and the starting value is equal to the measure of the week that came before (e.g. Week 9), we can write the formula like this:

$$\text{growth (\%)} = \frac{\text{weekly growth}}{\text{last week's measure}} \times 100$$

For the percentage growth of Sammy's plant in terms of height in Week 10 the calculation is as follows:

$$\text{growth (\%)} = \frac{2.0}{19.1} \times 100 = 10.47\%$$

Complete the table by calculating the remaining percentage growth values for Week 10. (3 MARKS)

9 Is it likely that using a straight ruler to measure plant height and width is reproducible? Why/why not? (2 MARKS)

The siblings did their best to ensure that both plants were exposed to the same conditions throughout the experiment. The two plants sat side-by-side and were exposed to the same light and fresh air from a nearby window. It was assumed that the temperature they were exposed to was the same given their proximity. Both plants were watered weekly, given that it was a hot period. The only factor that differed between the two plants was the volume of water provided, as well as when and for how long they were watered, given that watering was left to the discretion of each sibling.

10 How does water concentration affect the rate of photosynthesis and what happens when a plant experiences water stress? (2 MARKS)

11 Many succulents are CAM plants that undertake a different mode of photosynthesis to regular C3 plants, such as the leafy plant used in the experiment. Briefly describe the difference in the Calvin cycle between C3, C4, and CAM plants. (3 MARKS)

12 Furious about losing the race to her sibling, Lara undertook research to find out how she could make her plant grow faster next time. She came across research into CRISPR-Cas9 applications to increase growing rates in various agricultural crops. How can CRISPR-Cas9 technology be used to improve the efficiency of photosynthesis in a given C3 plant? (2 MARKS)

CHAPTER 5 EXAM PRACTICE

60 min

Section A (13 MARKS)

Question 1 (1 MARK)

An increase in temperature towards a plant's optimal increases the rate of photosynthesis. The rate of photosynthesis increases because

A more enzymes within the plant begin to denature and speed up the overall reaction rate.

B enzymes and their substrates have greater kinetic energy and collide and react more frequently.

C higher temperatures lead to more energised chlorophyll within the light-dependent stage of photosynthesis.

D the elevated temperature encourages greater uptake of water and carbon dioxide which improves the rate of photosynthesis.

Adapted from VCAA 2014 Section A Q8

Use the following information to answer Questions 2 and 3.

Although photosynthesis is often summarised by a single equation, the process occurs in two distinct phases: the light-dependent stage and the light-independent stage.

Question 2 (1 MARK)

Which one of the following correctly describes one input and one output of the light-dependent reactions?

	Input	Output
A	Light	O_2
B	H_2O	O_2
C	CO_2	H_2O
D	O_2	H_2O

Question 3 (1 MARK)

The enzymes required for the light-independent stage of photosynthesis are found in the

A stroma.

B cytosol.

C stomata.

D thylakoid membrane.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q4

Question 4 (1 MARK)

A by-product to a chemical reaction can be thought of as a secondary product that is different from the intended product of the reaction. Which of the following can be considered a by-product of photosynthesis?

A ATP

B oxygen

C glucose

D carbon dioxide

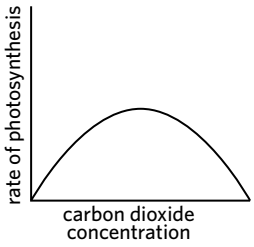
Adapted from VCAA 2004 Exam 1 Section B Q3e

Question 5 (1 MARK)

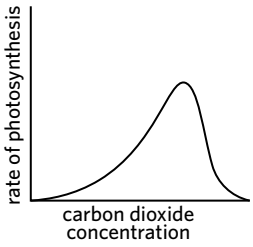
Josh set up an experiment to measure the rate of photosynthesis in a sample of plants. Each plant was exposed to differing concentrations of carbon dioxide and the rate of reaction was measured.

Which of the following graphs correctly represents the expected relationship between carbon dioxide concentration and the rate of photosynthesis?

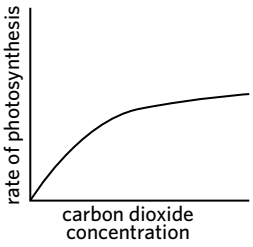
A



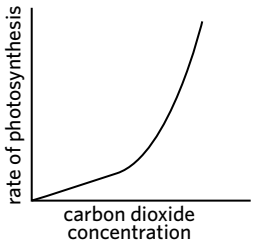
B



C



D



Question 6 (1 MARK)

An increase in available light typically increases the rate of photosynthesis. The rate of photosynthesis increases because

A the rate of the light-dependent reactions on the thylakoid membranes of the chloroplasts increases.

B water loss from the leaf decreases, resulting in the availability of water for photosynthesis increasing.

C the rate of the light-independent reactions in the stroma increases with the increase in available light.

D the increased CO_2 level lowers the pH inside the chloroplasts and increases the rate of enzyme-catalysed reactions.

Adapted from VCAA 2014 Section A Q8

Question 7 (1 MARK)

If a plant was exposed to a very limited supply of CO_2, which stage of photosynthesis would be primarily affected?

A neither stage as CO_2 is an output

B light-independent stage

C light-dependent stage

D both stages equally

Question 8 (1 MARK)

During photosynthesis in chloroplasts, energy is used to split water, forming oxygen and hydrogen ions.

The hydrogen produced

A is released into the atmosphere.

B binds to ADP to form ATP and is used in the light-dependent stage.

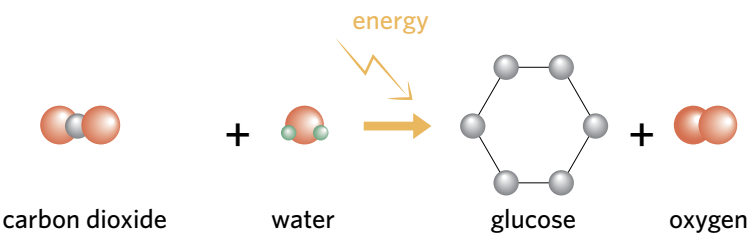
C binds to NAD^+ to form NADH and is used in the light-independent stage.

D binds to $NADP^+$ to form NADPH and is used in the light-independent stage.

Adapted from VCAA 2014 Section A Q7

Question 9 (1 MARK)

The diagram shows the reaction which causes the formation of glucose.



The source of energy for this reaction is

A sunlight.

B glucose.

C NADPH.

D carbon dioxide.

Adapted from VCAA 2012 Exam 1 Section A Q21

Question 10 (1 MARK)

How does the influence of carbon dioxide concentration on the rate of photosynthesis differ between C3, C4, and CAM plants?

A All three types of plants are affected in the same manner.

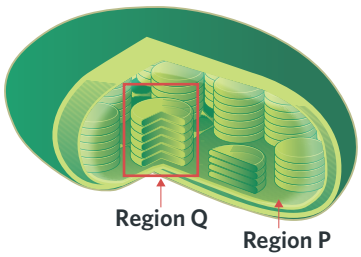
B CAM plants are the most susceptible to changes in carbon dioxide, followed by C4 and C3 plants which are less susceptible due to the absence of mechanisms to combat photorespiration.

C C3 and C4 plants are the most affected by changes in carbon dioxide concentration as their stomata are typically open during the day and are therefore vulnerable to losing carbon dioxide to the atmosphere.

D CAM and C4 plants are far less impacted by changes in carbon dioxide concentration compared to C3 plants, as they possess mechanisms to alter internal carbon dioxide concentration in order to combat photorespiration.

Question 11 (1 MARK)

The diagram shows a chloroplast.



The region labelled P is the

A stroma.

B granum.

C thylakoid.

D chlorophyll.

Adapted from VCAA 2017 Northern Hemisphere Exam Section B Q5a

Question 12 (1 MARK)

Plants grown in direct sunlight were supplied with air containing radioactive carbon dioxide. After four hours, an analysis of the chemicals in and around the plant was undertaken.

Which one of the following would contain radioactive carbon atoms after four hours?

a oxygen gas

b glucose

c protein

d water

Adapted from VCAA 2016 Section A Q10

Question 13 (1 MARK)

CRISPR-Cas9 could improve the agricultural industry in a number of ways. Possible reasons for editing a crop's genome using CRISPR-Cas9 technologies could include

A larger size of seeds, increased heat stress tolerance, lower yields, and modified activity of Calvin cycle enzymes.

B improved CO_2 uptake, modifying Rubisco's activity, decreased frost tolerance, and increased pesticide resistance.

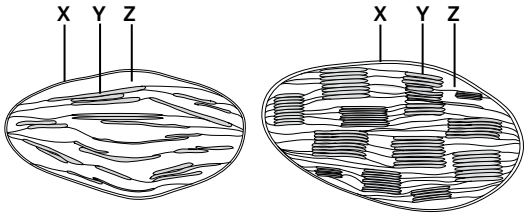
C longer shelf life of seeds, increased drought tolerance, greater herbicide resistance, and decreased viral susceptibility.

D increased disease immunity, decreased nutritional value, greater heat stress susceptibility, and improved light capture.

Section B (31 MARKS)

Question 14 (5 MARKS)

The bird's-nest fern, *Asplenium nidus*, usually grows in deeply shaded rainforests and has dark green fronds. Sometimes it is found in open, sunny locations by roadsides where it tends to have lighter coloured fronds. Two bird's-nest ferns, one from each of these two habitats, were examined. A sample of cells from a frond of each of the ferns was collected. These cells were examined under an electron microscope and a typical chloroplast from each habitat was drawn.



from plant A from plant B

a Which of the labelled parts (X, Y, or Z) are the locations of the light-independent stage? Name the part. (1 MARK)

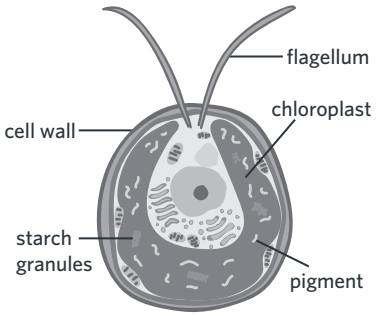
b Which plant, A or B, shows a chloroplast from the roadside habitat? Explain the reason for your choice in terms of the relationship between structure and function. (2 MARKS)

c Other than light, identify a factor that can affect the rate of photosynthesis and explain how the factor affects the rate of photosynthesis. (2 MARKS)

Adapted from VCAA 2007 Exam 1 Section B Q4

Question 15 (3 MARKS)

A *Chlamydomonas* cell has a single large chloroplast containing a green pigment.



Photosynthesis and cellular respiration are two biochemical processes that are crucial for the maintenance of life on Earth. Photosynthesis takes place in two stages. One of the products of the light-independent reactions is used in cellular respiration to provide energy for an organism.

During the light-dependent stage of photosynthesis, water is split into hydrogen ions and oxygen gas. These hydrogen ions are required in the light-independent stage.

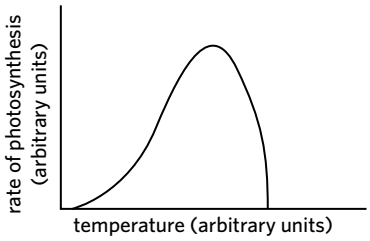
a Name the loaded form of the proton carrier used during photosynthesis. (1 MARK)

b State the net production of the proton carrier for the full photosynthesis reaction. Justify your response. (2 MARKS)

Adapted from VCAA 2004 Exam 1 Section B Q3e

Question 16 (6 MARKS)

A group of scientists wanted to test the effect of temperature on the rate of photosynthesis. They conducted an experiment on isolated chloroplasts, where they kept the light intensity and the concentrations of carbon dioxide and water constant. After the experiment, the following graph was produced.



a Explain why this trend was seen. (2 MARKS)

b Different plants are better adapted to suit different temperatures and other environmental conditions. Describe the preferred environmental conditions of C3 and CAM plants. (2 MARKS)

c The relationship between the rate of photosynthesis and temperature is dependent on a plant's enzymes. Name the two high-energy coenzymes that are critical to photosynthesis. (2 MARKS)

Question 17 (6 MARKS)

The herbicide propanil is used in the agricultural industry to kill weeds. Propanil's mode of action involves inhibiting photosynthesis by binding to proteins in the thylakoid membranes. As a result, the Calvin cycle cannot function given that it cannot be energised.

a For which stage of photosynthesis is carbon dioxide an input, and where in the chloroplast does this stage occur? (2 MARKS)

b Identify the key product of photosynthesis that would not be produced when a plant is inhibited by propanil. (1 MARK)

c CRISPR-Cas9 technologies have the potential to genetically engineer agricultural species in order to be more productive. One method of improving agricultural species is to increase their resistance to herbicides and other chemicals.

i Briefly explain how improving a crop's herbicide resistance would impact the crop's overall production. (2 MARKS)

ii Genetically engineering crops to have greater resistance to herbicides raises an ethical dilemma. Crops with greater resistance to herbicides could encourage an overall increased use of herbicides which, due to their toxic nature, can have unwanted side effects. For example, spraying herbicide onto a paddock of wheat can impact the trees and other plants surrounding the paddock area. State the bioethical concept that is concerned with ensuring minimal harm is caused to all organisms involved in an action. (1 MARK)

Question 18 (11 MARKS)

Patrick and Atong notice bubbles forming on the submerged leaves of an *Elodea* plant growing in an aquarium. The bubbles seen on the leaves are the result of a gas formed in the leaf cells. The temperature and pH are optimal for the *Elodea* plant.

There is a bright light shining on the aquarium. The bright light does not affect the temperature of the water. Patrick and Atong's teacher instructs them that the bubbles they see are caused by the *Elodea* plant photosynthesising.

Image: QuinxGhoul/Shutterstock.com

a State two inputs and two outputs of photosynthesis. (2 MARKS)

b Identify the gas formed on the leaves. (1 MARK)

c Identify where in the chloroplast this gas was produced. (1 MARK)

d Light is captured by a chemical in the chloroplast. Name the pigment that captures light. (1 MARK)

e The light is consistently shining on the aquarium.

i Explain what is expected to occur if the light source is switched off. (2 MARKS)

ii The *Elodea* plant is known to undertake C3 photosynthesis. Would the expected result identified in part i differ if the plant undertook C4 photosynthesis instead? Why/why not? (2 MARKS)

f Explain why it is important that the light source does not affect the temperature of the water. (2 MARKS)

CHAPTER

6

Cellular respiration

Key knowledge

- the general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product
- the main inputs, outputs, and locations of glycolysis, Krebs Cycle, and electron transport chain including ATP yield (details of biochemical pathway mechanisms are not required)
- the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration
- the location, inputs, and the difference in outputs of anaerobic fermentation in animals and yeasts
- the factors that affect the rate of cellular respiration: temperature, glucose availability, and oxygen concentration
- the general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitor
- uses and applications of anaerobic fermentation of biomass for biofuel production
- the role of the lymphatic system in the immune response as a transport network and the role of lymph nodes as sites for antigen recognition by T and B lymphocytes

6A AEROBIC CELLULAR RESPIRATION

Over 2 billion years ago, the Earth faced one of its largest ever extinction events that would permanently alter the course of evolution. But what was the cause? An asteroid? Supervolcanoes? Both?

The real culprit: oxygen. In what was hypothesised as the Great Oxygenation Event, cyanobacteria like blue-green algae dominated the oceans and produced enough oxygen through photosynthesis to completely transform the Earth's atmosphere. But, not only did this possibly lead to the extinction of vast numbers of bacterial species, it may have also paved the way for multicellular lifeforms to grow rapidly in size and complexity.

So what's so special (or poisonous) about oxygen? Take a deep breath, and let's find out...

Blooming cyanobacteria in the Baltic Sea
Image: Lukasz Barzowski/Shutterstock.com

Lesson 6A

In this lesson you will learn about the location, inputs, and outputs of the three main stages of aerobic cellular respiration: glycolysis, the Krebs cycle, and the electron transport chain.

◀ *Prerequisite knowledge*

Year 11
The mitochondrion is a specialised cell organelle and is the site of various stages of aerobic cellular respiration.

Chapter 3
Enzymes are crucial for catalysing the reactions in each step of aerobic cellular respiration.

Future applications ▶

Chapter 6
The biochemical pathways of aerobic cellular respiration and anaerobic fermentation are interconnected and will each be favoured under different environmental conditions.

Study design dot points

- the general structure of the biochemical pathways in photosynthesis and cellular respiration from initial reactant to final product
- the main inputs, outputs, and locations of glycolysis, Krebs Cycle, and electron transport chain including ATP yield (details of biochemical pathway mechanisms are not required)
- the general role of enzymes and coenzymes in facilitating steps in photosynthesis and cellular respiration

Key knowledge units

Overview of cellular respiration	3.2.1.1
Glycolysis	3.2.7.1
The Krebs cycle	3.2.7.2
The electron transport chain	3.2.7.3
Enzymes and coenzymes in cellular respiration	3.2.2.3

Overview of cellular respiration 3.2.1.1

OVERVIEW

Cellular respiration allows cells to break down large molecules and produce substantial amounts of the high-energy molecule ATP. Cellular respiration is vital to all living organisms and occurs via two distinct biochemical pathways: aerobic cellular respiration and anaerobic fermentation.

THEORY DETAILS

What is cellular respiration and why is it important?

All of the cells in the human body require a constant supply of energy in order to perform their crucial, life-sustaining functions. But where does this energy come from? One of the primary methods for producing energy in the human body is the process of **cellular respiration**, which primarily involves the breakdown of **glucose** ($C_6H_{12}O_6$). Glucose is a sugar molecule commonly found within carbohydrates in the food we eat such as bread, honey, or potatoes. However, glucose carries too much energy to be useful in most biochemical reactions in cells. Cellular respiration breaks the energy stored in glucose into smaller packages stored in **ATP**.

cellular respiration the process by which cells create usable energy in the form of ATP from a series of biochemical reactions, involving the breakdown of glucose

glucose a simple 6-carbon sugar molecule with the formula $C_6H_{12}O_6$

ATP adenosine triphosphate, a high energy molecule that, when broken down, provides energy for cellular processes

Glucose can be broken down to produce ATP via two different pathways: **aerobic cellular respiration** or **anaerobic fermentation**. The main difference between the pathways is the presence or absence of oxygen. Aerobic cellular respiration requires oxygen, whereas anaerobic fermentation does not.

aerobic cellular respiration cellular respiration that occurs in the presence of oxygen. Involves three stages, during which glucose and O_2 are converted into ATP, CO_2, and water

anaerobic fermentation a metabolic pathway that occurs in the absence of oxygen. Involves glycolysis, followed by further reactions that convert pyruvate into lactic acid in animals, or ethanol and CO_2 in yeast

In Figure 1, we can see an overview of the aerobic cellular respiration reaction in which glucose reacts with oxygen in the body's cells to produce carbon dioxide, water, and ATP. Aerobic cellular respiration produces 36 or 38 ATP molecules per glucose molecule. This is one reason why it's important for humans to breathe in oxygen – it allows us to produce lots of energy. The process of aerobic cellular respiration is similar in all aerobically respiring organisms.

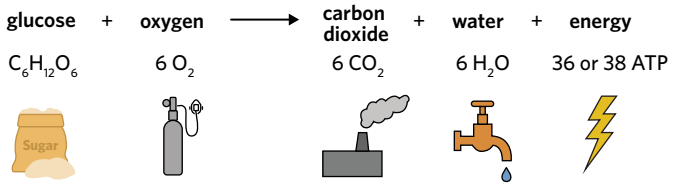


Figure 1 The overall equation for aerobic cellular respiration

Without oxygen, an organism is unable to aerobically respire. They can, however, undertake the alternate metabolic pathway to produce usable energy – anaerobic fermentation. The downside of this? Anaerobic fermentation produces only 2 ATP. Another drawback of the anaerobic pathway is that this process produces a harmful by-product (lactic acid or ethanol) that cells must promptly dispose of before it accumulates. Also, note in Figure 2 that the anaerobic fermentation pathway is different in yeast and plants compared to animals, whereas the aerobic cellular respiration pathway remains the same in both. These differences will be explored more in later lessons.

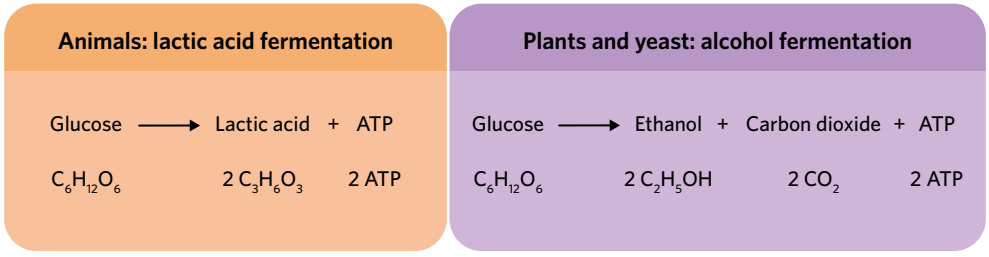


Figure 2 Anaerobic fermentation in animals compared to plants and yeast. Note that only the word equations are required knowledge for VCE Biology.

The production of energy from glucose in aerobic cellular respiration is not as simple as purely breaking the bonds in glucose and oxygen and immediately forming ATP, H_2O, and CO_2 within our cells. Instead, there are many enzyme-controlled reactions in between these inputs and outputs that have to occur so that our cells can safely harness the energy from a honey sandwich.

Aerobic cellular respiration occurs in three distinct stages:

1. **Glycolysis**
2. **The Krebs cycle**
3. **The electron transport chain**.

Memory device

A full molecule of glucose is like a $1000 bill. It's awesome to have that much money, but think of how annoyed your local cafe would be if they had to give you change for that amount of cash. ATP is like a $10 bill – way more useful for the day-to-day transactions that get you your coffee, and keep your cells running smoothly!

Lesson link

The key stages and processes within anaerobic fermentation are explored in greater depth in ***lesson 6B*** and ***lesson 6D***.

glycolysis the first stage of aerobic cellular respiration in which glucose is converted to two pyruvate molecules

Krebs cycle the second stage of aerobic cellular respiration, where multiple reactions occur to create ATP, NADH, $FADH_2$, and the waste product CO_2. Also known as the **citric acid cycle** or **TCA cycle**

We will investigate these three stages in this lesson, and in the next lesson we will explore the stages of anaerobic fermentation in animals and yeast.

The role of mitochondria in aerobic cellular respiration

Mitochondria are crucial to aerobic cellular respiration as they are the site of the second and third stages, with the first stage (glycolysis) occurring in the **cytosol** of the cell.

Mitochondria are complex organelles made up of many different structures (Figure 3). This includes an inner and outer membrane each composed of a phospholipid bilayer. The space inside the inner membrane is the **mitochondrial matrix** and is filled with a dense fluid containing many enzymes and solutes. The mitochondrial matrix is the site of the second stage of aerobic cellular respiration (the Krebs cycle). The inner membrane folds into peaks and ridges called **cristae**, which facilitate the function of the third stage of aerobic cellular respiration (the electron transport chain). The intermembrane space between the inner and outer membranes is narrow and has a small volume compared to the matrix. As we will discover, it also plays an important role in the electron transport chain.

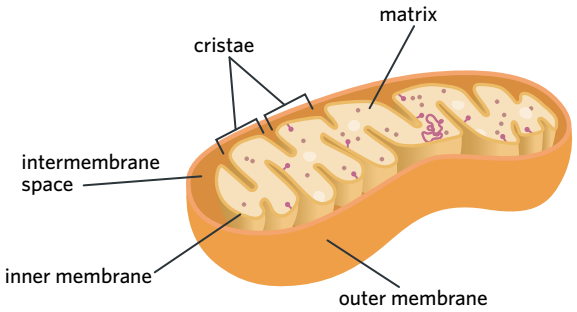


Figure 3 A labelled diagram of the structures of a mitochondrion

Aerobic cellular respiration vs photosynthesis

Many people assume that the equation for aerobic cellular respiration is simply the reverse of the photosynthesis equation. This is not true. The processes share a number of similarities, however the individual reactions are not the opposite of one another, and very different structures and enzymes are involved. For example, photosynthesis requires water as an input and output whilst water is only an output of aerobic cellular respiration.

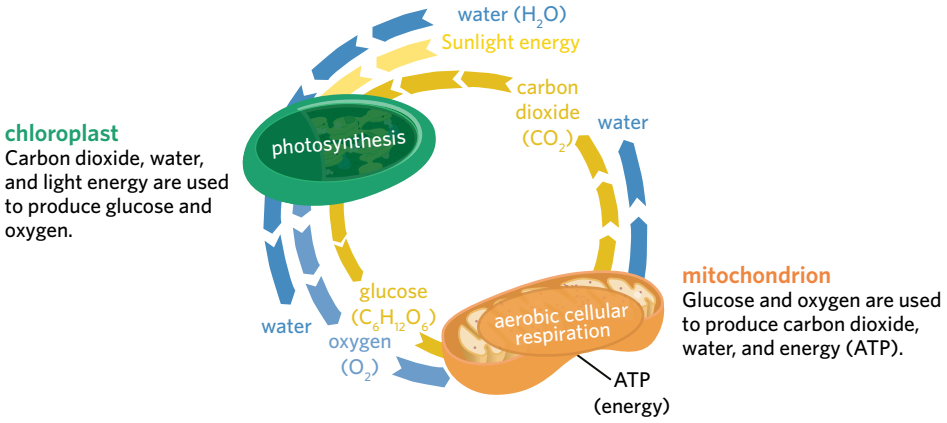


Figure 4 Photosynthesis and aerobic cellular respiration are related processes.

Nevertheless, Figure 4 shows that photosynthesis and cellular respiration are related, as each process can 'recycle' the outputs of the other reaction, using them as inputs. For plants or algae (organisms that contain both chloroplasts and mitochondria and are therefore capable of both processes), this means that they don't need to source all their photosynthesis and cellular respiration inputs from the environment.

electron transport chain the third stage of aerobic cellular respiration, in which a series of protein complexes embedded in the inner membrane of a mitochondrion harness the stored energy in NADH and $FADH_2$ to generate large amounts of ATP

mitochondrion (pl. mitochondria) a double-membrane-bound organelle that is the site of the second and third stages of aerobic cellular respiration

cytosol the aqueous fluid that surrounds a cell's organelles inside the plasma membrane

mitochondrial matrix the space inside the inner membrane of a mitochondrion. The site of the Krebs cycle

crista (pl. cristae) the folds of the inner membrane of a mitochondrion. The site of the electron transport chain

Lesson link

The processes of photosynthesis (***lesson 5A***) and cellular respiration are related in a cyclic fashion, as each pathway can integrate certain input and output molecules from the other (Figure 4). However, while the processes are related, it is important to understand the differences between them, including being aware of their inputs, outputs, and overall purposes.

Glycolysis 3.2.7.1

OVERVIEW

Glycolysis is the first of the three stages of aerobic cellular respiration. It occurs in the cytosol and is where glucose breaks down into two pyruvate molecules, creating two ATP and two NADH molecules in the process.

pyruvate a three-carbon molecule that can be formed from the breakdown of glucose via glycolysis

nicotinamide adenine dinucleotide (NAD) a coenzyme that acts as a proton (H^+) and electron carrier in cellular respiration. NAD can cycle between its NAD^+ and NADH forms, depending on the reaction it takes part in

THEORY DETAILS

Role of glycolysis

Glycolysis is the first stage in the whole process of cellular respiration. It involves the breakdown of 6-carbon glucose into two 3-carbon pyruvate molecules. A small amount of ATP is made in glycolysis, and this can be used to power cellular reactions. Importantly, the **pyruvate** and **NADH** that are produced will go on to help make even more ATP in the next two stages of aerobic cellular respiration.

How glycolysis works

Glycolysis occurs in the cytosol of the cell (Figure 5). Glucose (a six-carbon molecule) is broken down via a sequence of ten enzyme-regulated reactions to form two pyruvate molecules (two three-carbon molecules). However, the VCAA only requires you to know the overall inputs, outputs, and location of glycolysis, as summarised in Table 1.

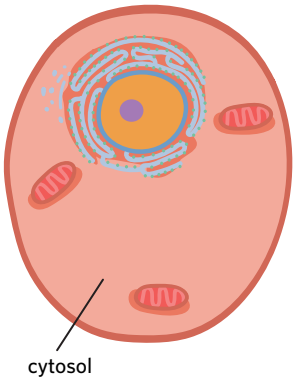


Image: Achiichiii/Shutterstock.com

Figure 5 The cytosol: the site of glycolysis

Table 1 The inputs, outputs, and location of glycolysis

Glycolysis	
Location: cytosol	
Inputs	Outputs
1 glucose ($C_6H_{12}O_6$)	2 pyruvate
2 ADP + 2 P_i	2 ATP
2 NAD^+ + 2 H^+	2 NADH

As glucose is broken down into pyruvate, energy is released that can be harnessed by the cell. As shown in Figure 6, this energy powers two key reactions:

- 2 ADP + 2 P_i → 2 ATP
  - The ATP is now free to power cellular reactions.
- 2 NAD^+ + 2 H^+ + 4 e^- → 2 NADH
  - The electrons (two per NAD^+) are often not written in this equation, but they are necessary for the reaction to proceed.
  - The H^+ and electrons come from the breakdown of glucose.
  - The two NADH molecules will be transported to the mitochondria, where each molecule will deliver protons and electrons to the electron transport chain, to help make more ATP. For this reason, we call NADH an 'electron and proton carrier'.

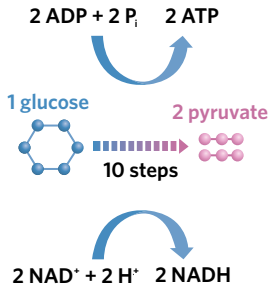


Figure 6 Summary of glycolysis, the first stage of both aerobic cellular respiration and anaerobic fermentation

The two pyruvate molecules will be transported to the mitochondria, where they will then be modified and broken down further in stage two of aerobic cellular respiration: the Krebs cycle.

The Krebs cycle 3.2.7.2

OVERVIEW

The second stage of aerobic cellular respiration is the Krebs cycle. It occurs in the matrix of mitochondria (Figure 7), and it produces four CO_2, two $FADH_2$, six NADH, and two ATP for every two pyruvate molecules created via glycolysis.

THEORY DETAILS

Role of the Krebs cycle

The Krebs cycle generates lots of high-energy electron and proton carriers, NADH and **$FADH_2$**, which can be used in the electron transport chain. Carbon dioxide is released, and small amounts of ATP are also produced.

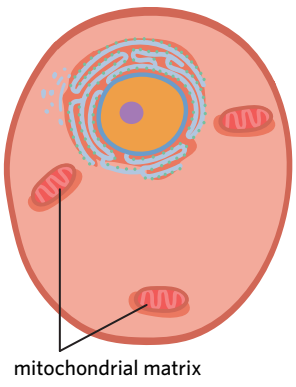


Image: Achiichiii/Shutterstock.com

Figure 7 The mitochondrial matrix: the site of the Krebs cycle

The link reaction

To link glycolysis and the Krebs cycle, pyruvate is transported to the matrix of the mitochondria and combines with **coenzyme A (CoA)** to form **acetyl-CoA**. The link reaction also releases carbon dioxide, a waste product that is exhaled, and produces NADH that can be used later at the electron transport chain.

> ***Examiners' tip***
>
> The VCAA excludes the link reaction from exams and the study design, but in order to remember that acetyl-CoA (not pyruvate) is an input for the Krebs cycle, you need to remember this small connecting step.

How the Krebs cycle works

After the link reaction, the breakdown products of glycolysis are now ready to undertake the Krebs cycle.

Table 2 The inputs, outputs, and location of the Krebs cycle

The Krebs cycle	
Location: the mitochondrial matrix	
Inputs	**Outputs**
2 acetyl-CoA (derived from 2 pyruvate)	4 carbon dioxide (CO_2)
2 ADP + 2 P_i	2 ATP
6 NAD^+ + 6 H^+	6 NADH
2 FAD + 4 H^+	2 $FADH_2$

The Krebs cycle is a series of eight reactions that extract the energy from the 'acetyl-' (two-carbon) component of acetyl-CoA, breaking it down and allowing the coenzyme A molecule to be recycled back for use in the link reaction. Although the specifics of each of these reactions are beyond the scope of VCE Biology, it is important to note the following overall results from this key metabolic stage:

- By breaking down acetyl-CoA, protons and high-energy electrons are released. These protons and electrons are loaded onto NAD^+ and FAD molecules to generate high-energy coenzymes NADH and $FADH_2$.
- The Krebs cycle produces two CO_2 molecules for every one acetyl-CoA molecule. When added to the single CO_2 molecule produced from each of the two pyruvates undergoing the link reaction, this means a total of six CO_2 molecules are produced for every original glucose molecule.
- The Krebs cycle produces a small amount of energy in the form of two ATP (one per acetyl-CoA molecule).

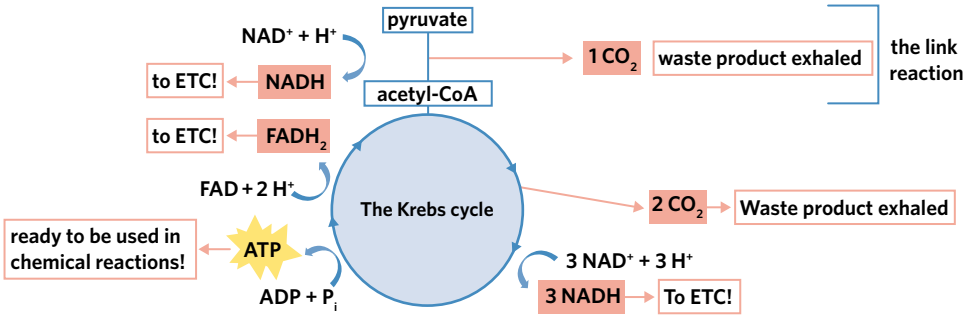


Figure 9 A summary of the Krebs cycle. Note that the Krebs cycle occurs twice per glucose molecule because each glucose molecule is broken down into two pyruvates then two acetyl-CoA molecules.

flavin adenine dinucleotide (FAD) a coenzyme that acts as a proton (H^+) and electron carrier in cellular respiration. FAD can cycle between its FAD and $FADH_2$ forms, depending on the reaction it takes part in

coenzyme A a large organic non-protein molecule that plays a key role in the modification of pyruvate to allow it to enter the Krebs cycle. Also known as **CoA**

acetyl-CoA the product of the link reaction where pyruvate is conjugated to coenzyme A, creating the primary input into the Krebs cycle

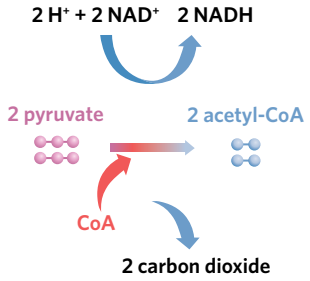


Figure 8 A summary of the link reaction

> ***Memory device***
>
> You can think of the coenzymes NAD and FAD as waiters who run food and drinks at a restaurant. At the kitchen (glycolysis, the link reaction, and the Krebs cycle) they fill their trays up with food (high energy electrons and protons) to become NADH and $FADH_2$, then deliver it to tables (the electron transport chain). Then the empty plates (unloaded NAD^+ and FAD) are taken back to the kitchen to reuse!

The electron transport chain 3.2.7.3

OVERVIEW

The electron transport chain, the third step of aerobic cellular respiration, occurs on the inner membrane (or 'cristae') of the mitochondria (Figure 10). During this step, energy from the electrons unloaded by NADH and $FADH_2$ generates a proton gradient that drives significant ATP production.

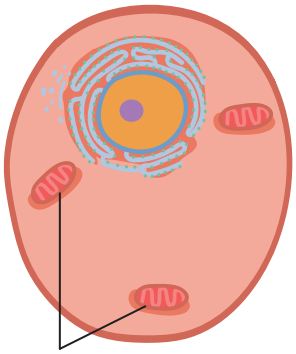


Image: Achiichiii/Shutterstock.com

Figure 10 The inner membrane of the mitochondria: the site of the electron transport chain

THEORY DETAILS

Role of the electron transport chain

The electron transport chain is where the majority of ATP is produced in the process of aerobic cellular respiration. In doing so, it also converts the high-energy coenzymes NADH and $FADH_2$ back to their NAD^+ and FAD forms, which are then recycled for continued use in glycolysis and the Krebs cycle.

How the electron transport chain works

Table 3 The inputs, outputs, and location of the electron transport chain

The electron transport chain Location: the cristae of the mitochondria	
Inputs	**Outputs**
6 oxygen (O_2) + 12 H^+	6 water (H_2O)
32 or 34 ADP + 32 or 34 P_i	32 or 34 ATP
10 NADH	10 NAD^+ + 10 H^+
2 $FADH_2$	2 FAD + 4 H^+

For VCE Biology, only the inputs, outputs, and the location of the electron transport chain are required knowledge. However, remembering them will be easier if you understand how the electron transport chain works. Here is a brief outline of the steps involved in making ATP at the electron transport chain, included for conceptual understanding:

1. NADH and $FADH_2$ unload electrons and protons at the first and second protein complexes of the electron transport chain that reside in the inner mitochondrial membrane. The following reactions take place:

   (1) NADH → $NAD^+ + H^+ + 2\ e^-$

   (2) $FADH_2$ → $FAD + 2\ H^+ + 2\ e^-$.

2. The excited electrons (from NADH and $FADH_2$) are transferred through a number of different protein complexes embedded in the electron transport chain, powering the active transport of protons (H^+) from the mitochondrial matrix into the narrow intermembrane space.
3. This leads to a build-up of protons in the intermembrane space. As this space is very narrow and small, the proton concentration here quickly increases, creating a steep concentration gradient across the inner mitochondrial membrane.
4. To move down their concentration gradient, these protons must travel through the specialised protein channel **ATP synthase**. As the protons pass through ATP synthase, they cause the enzyme to spin like a turbine. The kinetic energy of this movement powers the reaction ADP + P_i → ATP, producing 32 or 34 ATP for each original glucose molecule.
5. This process produces large amounts of ATP, but also leads to many free protons and electrons building up in the matrix. Unbound protons and electrons can cause problems for cells in large concentrations – they can damage DNA, interfere with enzyme reactions and create dysfunctional proteins. To prevent this from happening, oxygen acts as the terminal acceptor, binding with these dangerous protons and electrons to form harmless water. Oxygen is therefore required for the electron transport chain to proceed.

ATP synthase an enzyme in the inner mitochondrial membrane that uses the concentration gradient of H^+ to synthesise ATP from ADP and P_i

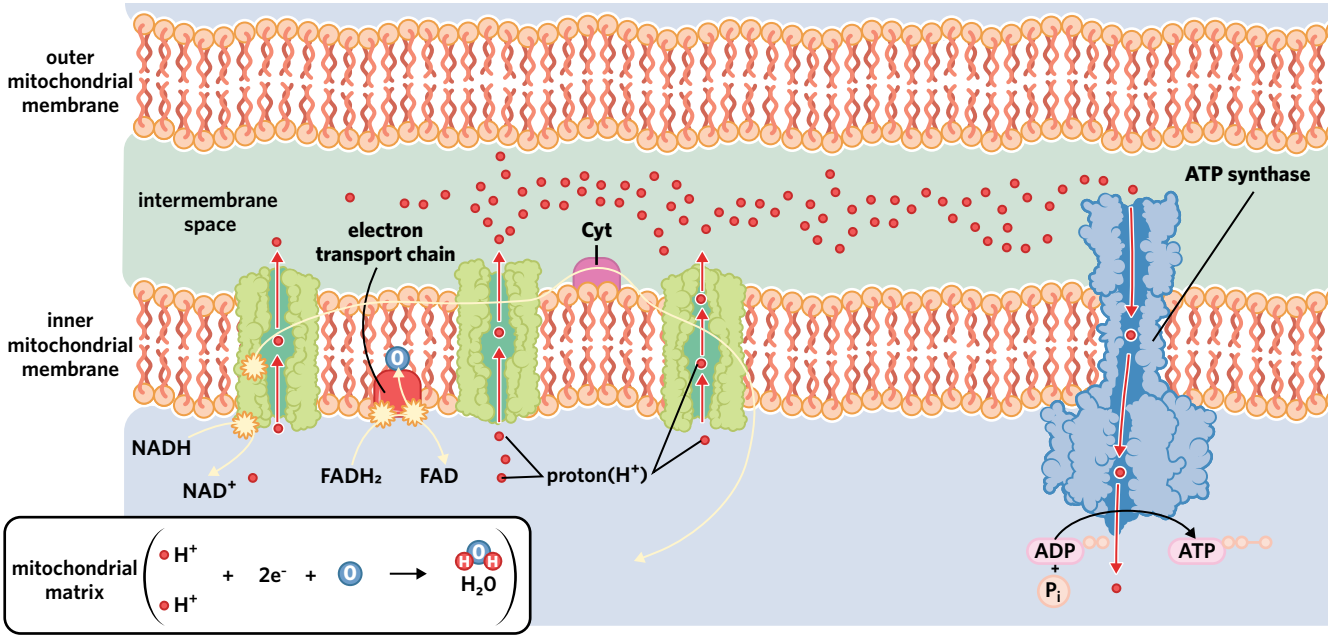


Figure 11 The electron transport chain uses a number of different proteins embedded in the inner mitochondrial membrane to extract the stored energy from NADH and $FADH_2$ and produce 32 or 34 ATP.

Examiners' tip

In exams, it is important to specify that either 32 or 34 ATP is produced at the electron transport chain under ideal conditions, not a range of 32–34. The total number of ATP molecules produced via the electron transport chain depends on how many NADH and $FADH_2$ molecules enter the mitochondrion. However, there are a few different pathways used by the cell to 'shuttle' NADH and $FADH_2$ from the cytosol into the mitochondrial matrix. These mechanisms ultimately result in different overall amounts of ATP production at the electron transport chain, explaining the possibility of 32 or 34 ATP.

Lesson link

The protein complexes embedded in the inner mitochondrial membrane are made up of many different polypeptide chains interacting together. As we discovered in ***lesson 2A***, this means that they have a quaternary structure. In fact, Complex I of the electron transport chain is made up of over 40 different polypeptide chains!

Combined with the 2 ATP produced during glycolysis and the 2 ATP from the Krebs cycle, this means that a total of 36 or 38 ATP can be produced from each original glucose molecule that is committed to aerobic cellular respiration.

Theory in context

WHAT HAPPENS WHEN THE ELECTRON TRANSPORT CHAIN GOES WRONG?

Cyanide is found in different forms in many pesticides, insecticides, tobacco smoke, as well as the smoke often produced during building fires. Cyanide poisoning from exposure to any of these sources inhibits one of the key enzymes in the electron transport chain.

When this enzyme is inhibited, it cannot perform its proper function and the entire electron transport chain is compromised. This means that significantly lower levels of ATP will be produced during aerobic cellular respiration, potentially leading to cell death, and, in extreme cases, organism death.

Enzymes and coenzymes in cellular respiration 3.2.2.3

OVERVIEW

The tightly controlled sequence of biochemical reactions in cellular respiration relies heavily on the use of enzymes and coenzymes to ensure they happen at a fast rate.

THEORY DETAILS

Enzymes, with the help of their coenzyme assistants, **catalyse** the reactions of cellular respiration to allow them to proceed at significantly higher and biologically relevant rates. This means that our cells can break down and extract the energy from glucose fast enough to drive their many diverse, energy-dependent processes.

catalyse to increase the rate of a reaction

Since each enzyme is only capable of catalysing one specific reaction, there is a wide range of enzymes involved in cellular respiration. A few of the key enzymes involved in glycolysis, the Krebs cycle, and the electron transport chain are shown in Figure 12, as well as a brief description of their functions.

Note that these enzymes are under tight regulation to ensure the correct amount of ATP is being produced downstream and that the cell's crucial cellular respiration pathways are operating as efficiently as possible. For example, one key way to regulate enzyme function is via end-product inhibition. In this type of inhibition, the final product in a series of biochemical reactions prevents enzymatic catalysis of an earlier step in the sequence. This means the cell only makes a product when there is a deficit. We will explore more about factors affecting these enzymes, and therefore the rate of cellular respiration, in lesson 6C.

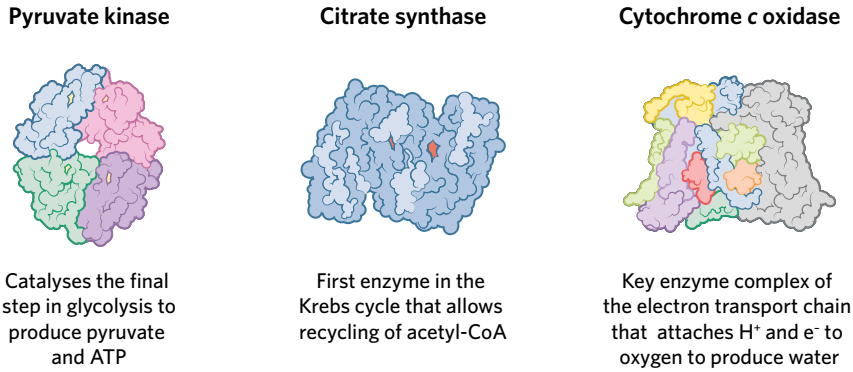


Figure 12 Examples of key enzymes in cellular respiration and their functions

Some cellular respiration enzymes require some extra help to catalyse their reactions. As we learned in lesson 3B, there are a number of different organic, non-protein molecules known as coenzymes that assist enzymes. Three key coenzymes in cellular respiration are ATP, NAD^+, and FAD.

Coenzymes will cycle between unloaded (ADP, NAD^+, FAD, CoA) and loaded (ATP, NADH, $FADH_2$, acetyl-CoA) states as they help catalyse the reactions of cellular respiration (Figure 13). Some enzymatic reactions of cellular respiration will require the unloaded coenzyme to proceed, whereas others may require the loaded coenzyme. In general, coenzymes are unloaded in reactions that need extra energy and become loaded in reactions that produce energy. This ensures that coenzymes can always be efficiently recycled.

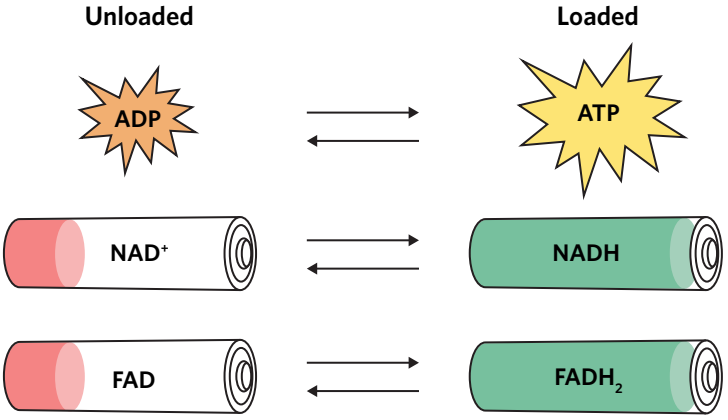


Figure 13 Coenzymes are reduced, reused, and recycled (but not used up) during the reactions of cellular respiration.

Theory summary

Aerobic cellular respiration involves the breakdown of glucose in the presence of oxygen according to the overall equation $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + 36 or 38 ATP. When simplified, we can remember the overall process via the following steps:

1. Glucose is broken down by glycolysis in the cell cytosol to produce two pyruvate, two ATP and two NADH molecules.
2. Pyruvate is first converted to acetyl-CoA in the link reaction before it can enter the Krebs cycle in the mitochondrial matrix and produce four CO_2, two $FADH_2$, six NADH, and two ATP for each original glucose molecule.
3. The high-energy electron and proton carriers NADH and $FADH_2$ are shuttled to the electron transport chain embedded in the inner mitochondrial membrane.

4 The energy from NADH and $FADH_2$ is used to create a proton gradient across the inner mitochondrial membrane, which is harnessed to drive the significant production of ATP by ATP synthase.

5 Any spare electrons and protons at the electron transport chain are 'mopped up' and attached to oxygen to produce water molecules.

Overall, it is important to remember that a range of enzymes and coenzymes are essential to facilitate the reactions of cellular respiration.

Oxygen is a vital component at the end of the electron transport chain, which produces significant amounts of ATP. If a cell can produce energy more efficiently, it can dedicate more energy to powering the complex processes that come with being a multicellular organism.

However, oxygen also has the tendency to oxidise molecules. This involves oxygen "stealing" electrons, which can destabilise molecular structures. The high intracellular oxygen levels that would have resulted from the Great Oxygenation Event could have led to damage and breakdown of important molecules like proteins, especially in anaerobic bacterial cells that lack mitochondria. If enough damage accumulates, these bacteria will die and their species will soon be heading down the express lane to extinction.

Thus, one could say that the entire reason why humans (and billion-year-old early multicellular life-forms) take in oxygen is to protect intracellular molecules by mopping up free protons and electrons at the end of the electron transport chain.

6A QUESTIONS

Theory review questions

Question 1

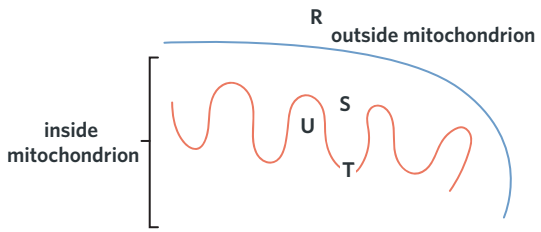
Fill in the blanks in the following sentences.

When _____ is broken down in the presence of _____, carbon dioxide and water are produced. Energy in the form of _____ is also created, which can be used to drive a variety of cellular reactions.

Question 2

Label the parts of mitochondria shown from the list of terms. Terms may be used multiple times or not at all.

- cytosol
- plasma membrane
- mitochondrial matrix
- intermembrane space
- inner mitochondrial membrane
- outer mitochondrial membrane



Adapted from VCAA 2018 Section A Q8

Question 3

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- FAD
- NAD^+
- NADH
- $FADH_2$
- aerobic
- pyruvate
- anaerobic
- acetyl-CoA

During glycolysis, glucose is converted to two _____ molecules via a 10-step reaction sequence. This initial part of the cellular respiration pathway does not require oxygen, and so is described as being an _____ process. As a result of these reactions, two _____ and ATP molecules are also produced.

Question 4

The Krebs cycle produces the high-energy coenzymes, NADH and $FADH_2$, which are shuttled into the mitochondrion for use in

A glycolysis.

B anaerobic fermentation.

C the electron transport chain.

Question 5

Which of the following options correctly lists the inputs of the reactions in aerobic cellular respiration?

	Glycolysis	Krebs cycle	Electron transport chain
A	glucose, ATP + P_i, NAD^+, H^+	acetyl-CoA, NADH, FAD, ADP + P_i	O_2, NAD^+, $FADH_2$, ADP + P_i
B	glucose, ADP + P_i, NAD^+, H^+	acetyl-CoA, CO_2, NAD^+, FAD, ADP + P_i	O_2, NADPH, $FADH_2$, ADP + P_i
C	glucose, ADP + P_i, NAD^+, H^+	acetyl-CoA, NAD^+, FAD, ADP + P_i	O_2, NADH, $FADH_2$, ADP + P_i
D	glucose, ADP + P_i, NAD^+, H^+	acetyl-CoA, NAD^+, FAD, ADP + P_i	O_2, NAD^+, FAD, H^+, ADP + P_i

Question 6

Fill in the blanks in the following sentences.

Each specific reaction within aerobic cellular respiration is catalysed by one particular _____. Some reactions within this pathway also require the presence of a _____, such as NAD^+ or ATP in order to proceed.

Question 7

Match the cellular respiration event to its appropriate location. Terms may be used multiple times or not at all.

Location

- extracellular matrix
- cytosol
- nucleus
- mitochondrial matrix
- cristae
- outer mitochondrial membrane

Cellular respiration event

I _____ glycolysis

II _____ the Krebs cycle

III _____ $FADH_2$ is produced

IV _____ the electron transport chain

V _____ glucose is converted to two pyruvate molecules

VI _____ acetyl-CoA is recycled to create carbon dioxide and other products

Question 8

The majority of ATP produced during aerobic cellular respiration is through the

A conversion of glucose to high-energy pyruvate during glycolysis.

B controlled breakdown of acetyl-CoA within the mitochondrial matrix.

C H^+ gradient across the inner mitochondrial membrane driving the electron transport chain enzyme ATP synthase.

D attachment of free protons and electrons to oxygen to produce water molecules within the electron transport chain.

SAC skills questions

Case study analysis

Use the following information to answer Questions 9–13.

The electron transport chain is made up of a number of different protein complexes that are embedded within the inner mitochondrial membrane. One such complex is cytochrome *c* oxidase, or Complex IV, which is crucial for allowing oxygen to act as the terminal electron and proton acceptor in the electron transport chain to form water.

Complex IV deficiency is an inherited mitochondrial disease that results in a decreased number of functional Complex IV enzymes in the mitochondria of the patient's cells. A reduction in the number of functional Complex IV enzymes disrupts the crucial final step in the electron transport chain.

Mitochondrial diseases generally lead to decreased ATP production. Impeding the electron transport chain also tends to lead to increased amounts of acid (H^+) in the body's cells and their mitochondria, impairing many of their energy-intensive and pH-sensitive life-sustaining processes. If the damage or disease is severe enough, the cells will break down and die.

Mitochondrial diseases are especially damaging to the heart and the brain, which require a large amount of ATP to function properly. Mitochondrial diseases can lead to developmental delays in children, with the most common symptom being extreme fatigue. Over time, if the body does not produce enough energy to maintain its organ systems, mitochondrial diseases can lead to organ failure and, in severe cases, death.

Question 9

Which of the following can be inferred from the information presented?

A If the electron transport chain is disrupted, all cellular respiration pathways will fail.

B Cytochrome *c* oxidase is unique to the electron transport chain in its ability to convert oxygen to water.

Question 10

It is reasonable to conclude that a patient suffering from a Complex IV deficiency will have

A low levels of acid in their bloodstream, known as metabolic alkalosis.

B a high appetite for glucose-rich foods such as honey and dry fruit.

Question 11

When compared to a sample of fluid from the mitochondrial matrix of a well-rested healthy individual, a patient suffering from a mitochondrial disease would be expected to have a

A higher concentration of ATP present in this fluid.

B lower concentration of ATP present in this fluid.

Question 12

Organ failure as a result of severe mitochondrial disease is most likely a result of

A deficits in ATP production compromising cell regulation of nutrient and waste transport.

B excess oxygen intake leading to overactivity of acid-producing metabolic pathways.

Question 13

Using the information presented, which of the following statements is most likely to be true?

A Patients diagnosed with a Complex IV deficiency will have a relative increase in the number of pyruvate kinase enzymes (a key enzyme in glycolytic ATP production) present in their brain cells.

B Patients diagnosed with a Complex IV deficiency will have a complete absence of cytochrome *c* oxidase enzymes, and so will rely entirely on anaerobic fermentation for energy production.

Exam-style questions

Within lesson

Question 14 (1 MARK)

Which of the following gives the inputs and outputs of the Krebs cycle as it would occur in a plant cell?

	Inputs	Outputs
A	acetyl-CoA, ATP, NAD^+, FAD	carbon dioxide, ADP, NADH, $FADH_2$, P_i
B	glucose, ADP, NAD^+, P_i	pyruvate, ATP, NADH
C	oxygen, ADP, $FADH_2$, NADH, P_i	water, ATP, FAD, NAD^+
D	acetyl-CoA, ADP, NAD^+, FAD, P_i	carbon dioxide, ATP, NADH, $FADH_2$

Adapted from VCAA 2018 Section A Q9

Question 15 (1 MARK)

ATP is a coenzyme.

Which one of the following is a correct statement about ATP?

A A large yield of ATP is produced in the electron transport chain.

B There is no ATP produced in the glycolysis stage of aerobic cellular respiration.

C The Krebs cycle produces the greatest yield of ATP in aerobic cellular respiration.

D ATP is responsible for carrying electrons and protons between reactions in cellular respiration.

Question 16 (1 MARK)

An animal cell culture was exposed to radioactively labelled oxygen. The cells were then monitored for three minutes. After this time, the radioactively labelled oxygen atoms would most likely be present in the

A fatty acid tails in the inner membrane of the mitochondria.

B water in the cytosol or mitochondrial matrix.

C mitochondria as part of pyruvate molecules.

D atmosphere as carbon dioxide.

Question 17 (1 MARK)

During cellular respiration, fish use glucose

A in the glycolysis stage.

B to combine with carbon dioxide.

C to build up ATP molecules into ADP molecules.

D as the final acceptor of electrons and hydrogen ions.

Adapted from VCAA 2010 Exam 1 Section A Q18

Question 18 (1 MARK)

Rotenone is a chemical compound that is used as an insecticide and a piscicide (a substance that kills fish). The rotenone molecule disrupts the electron transport chain in animal cells by interfering with one of the essential reactions within the electron transport chain.

After being exposed to rotenone,

A glucose would accumulate in the cytosol.

B ATP would accumulate in the mitochondria.

C NAD^+ would accumulate in the mitochondria.

D NADH would accumulate in the mitochondria.

Adapted from VCAA 2015 Section A Q9

Multiple lessons

Question 19 (1 MARK)

Skeletal muscle cells in the legs contract and relax rapidly during exercise and require a large supply of energy. Which organelle would you expect to see in large numbers in skeletal muscle cells to supply this energy?

A smooth endoplasmic reticulum
B mitochondria
C chloroplasts
D nuclei

Adapted from VCAA 2014 Section B Q3c

Question 20 (1 MARK)

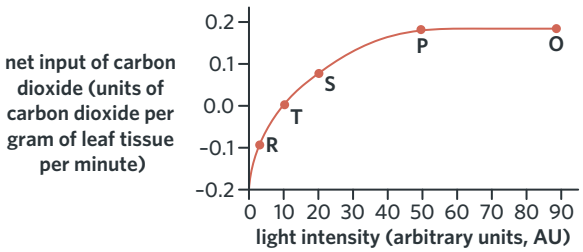
The process that produces the largest number of NADH molecules is

A translation.
B breakdown of glucose during glycolysis.
C the light-dependent reactions of photosynthesis.
D the electron transport chain in cellular respiration.

Adapted from VCAA 2012 Exam 1 Section A Q22

Question 21 (1 MARK)

The graph shows the net input of carbon dioxide in spinach leaves as light intensity is increased. Temperature is kept constant during the experiment.



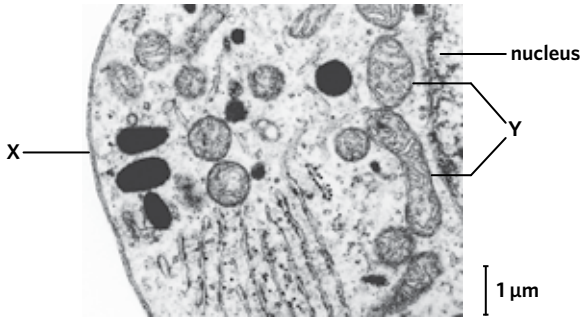
Which one of the following conclusions can be made based on the graph?

A at point O photosynthesis is no longer occurring
B photosynthesis does not occur below a light intensity of 10 AU
C at point S the level of light intensity for photosynthesis is optimal
D at point T the rate of photosynthesis is equal to the rate of cellular respiration

Adapted from VCAA Exam 2017 Section A Q13

Question 22 (5 MARKS)

The electron micrograph shows a portion of a cell.



a Name and describe the role of structure Y. (2 MARKS)

b Structure Y requires a supply of oxygen to undergo a metabolic reaction. Outline the process of how the oxygen molecules would enter the cell with reference to structure X. (2 MARKS)

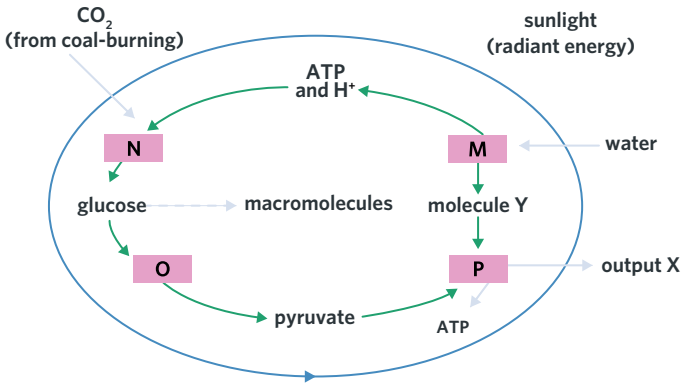
c Researchers have discovered that cardiac muscle cells, found in the heart, contain a larger number of structure Y compared to other cells in the body. Suggest why cardiac muscle cells contain a large number of structure Y. (1 MARK)

Adapted from VCAA 2012 Exam 1 Section B Q1

Question 23 (5 MARKS)

Microalgae such as *Chlorella* can use greater amounts of carbon dioxide than land plants and they do not require prime soil, reliable rainfall, or a particular climate. *Chlorella* can be grown cheaply in existing or engineered ponds which are supplied with carbon dioxide from a coal-burning power station nearby.

The diagram represents a summary of the processes (labelled M, N, O, P) occurring in a *Chlorella* cell.



a Name output X and molecule Y. (2 MARKS)

b With reference to the diagram, complete the following table. (3 MARKS)

Process	Name of process(es)	Site of process
M	light-dependent reactions	
O		cytosol
P	Krebs cycle and electron transport chain	

Adapted from VCAA 2012 Exam 1 Section B Q8

Question 24 (9 MARKS)

Plants require ATP to complete many biochemical reactions.

a Identify the metabolic process that plants use to produce energy in the form of ATP. (1 MARK)

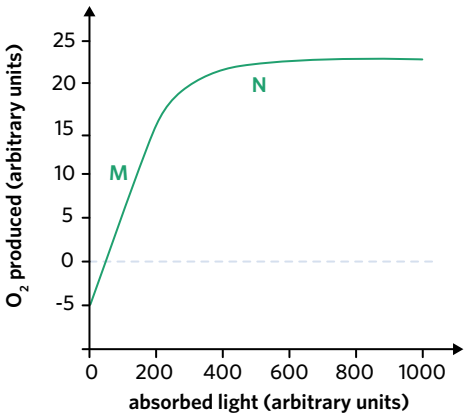
b State the worded equation for the production of energy via aerobic cellular respiration. (1 MARK)

c In the presence of oxygen, the metabolic process to produce energy has three stages.

i Name and state the location for the first stage of this process. (1 MARK)

ii Name and state the location for the stage of this process that requires oxygen as an input. (1 MARK)

d The graph shows the rate of oxygen exchange between a plant and its external environment as light intensity is altered. All other variables are kept constant throughout the experiment.



i With reference to metabolic processes, explain what is occurring when the oxygen produced is zero. (1 MARK)

ii Identify which metabolic pathway is occurring at a faster rate at point M. Justify your response. (2 MARKS)

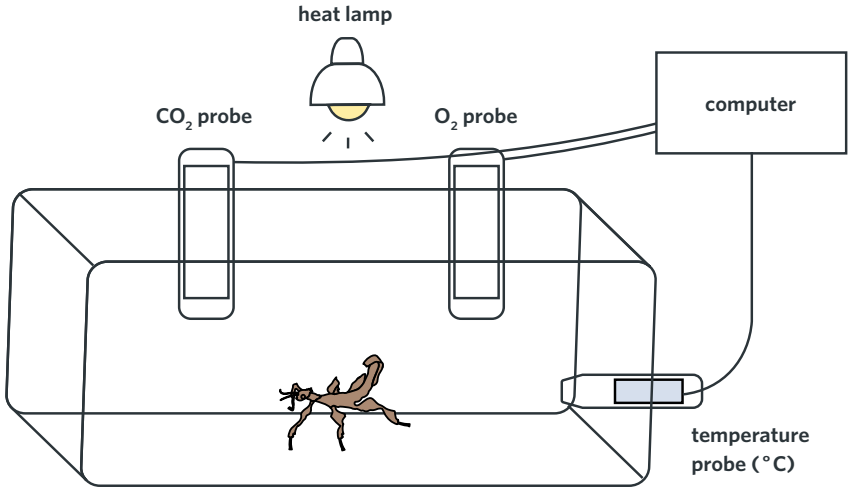
iii At point N, the graph begins to plateau. Explain why this occurs. (1 MARK)

Adapted from VCAA 2011 Exam 1 Section B Q7di

Key science skills and ethical understanding

Question 25 (6 MARKS)

Duncan investigated how changes in environmental temperature affect oxygen and carbon dioxide levels in the air around a spiny stick insect. He used three digital probes linked to a computer, a closed animal chamber, and a heat lamp in the experimental set-up shown.



a Identify the independent and dependent variables in this experiment. (2 MARKS)

b With reference to the inputs and outputs of aerobic cellular respiration, explain why Duncan is measuring the levels of oxygen and carbon dioxide. (3 MARKS)

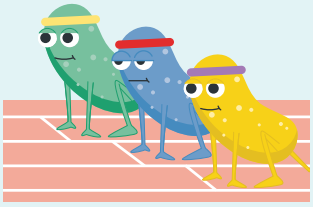
c Duncan tried the experiment again with a different temperature probe. He found that his results were different as the temperature probes produced results that were consistently 2°C higher. Identify what type of error this is. (1 MARK)

Adapted from VCAA 2017 Section B Q11

6B ANAEROBIC FERMENTATION

In the microverse, beyond the reach of our human eyes, eukaryotic yeasts roam the nanoscale earth, searching for nutrients, avoiding predators, and, of course, competing in their biannual 42 µm charity micro-thon. Having trained for days (over half their lifetimes), the yeasts congregate eagerly at the starting line, awaiting the countdown. As the starter's pistol fires, the yeasts pull away at breakneck speed (at least, for non-flagellated unicellular organisms).

But as the race progresses, our microscope picks up something peculiar – the peloton of yeasts begins to veer off course, and the dense mass of cells seems to break down before our very eyes. Not only that, but bubbles of gas start to fizz and envelope the micro-thon! But what, or who, could possibly be behind this disaster?



Lesson 6B

In this lesson you will learn about anaerobic fermentation, a metabolic pathway that occurs in the absence of oxygen. You wil also compare anaerobic fermentation in animals and yeasts.

◀ *Prerequisite knowledge* — *Future applications* ▶

Lesson 6A

The key input for anaerobic fermentation is pyruvate, which is a product of the glycolysis pathway in cellular respiration.

Lesson 6D

The products of anaerobic fermentation in yeasts can be harnessed and used as a biofuel.

Study design dot point

- the location, inputs, and the difference in outputs of anaerobic fermentation in animals and yeasts

Key knowledge units

Overview of anaerobic fermentation	3.2.8.1
Anaerobic fermentation in animals and yeast	3.2.8.2

Overview of anaerobic fermentation 3.2.8.1

OVERVIEW

Anaerobic fermentation involves the breakdown of glucose and ATP production via glycolysis in the absence of oxygen. It allows for the replenishment of NAD^+ for continued use in glycolysis.

THEORY DETAILS

In anaerobic conditions, which describes a lack of oxygen in the environment, cells that require a lot of energy are in trouble. Remember from lesson 6A that the electron transport chain, which produces the bulk of a cell's ATP via aerobic cellular respiration, requires oxygen to accept and 'mop up' free protons and electrons. Without the electron transport chain, the loaded coenzymes, such as NADH and $FADH_2$, cannot drop off their electrons and be converted back to unloaded NAD^+ and FAD, which are required as inputs for the Krebs cycle (Figure 1).

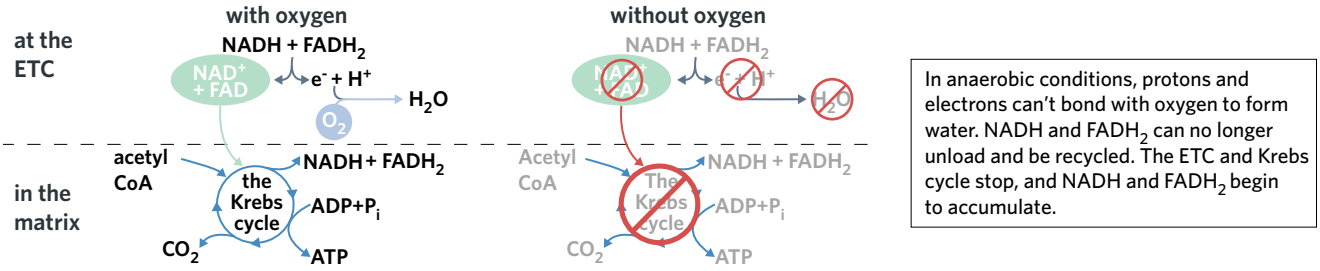


Figure 1 Anaerobic conditions inhibit the electron transport chain (ETC) and the Krebs cycle.

As a result, the oxygen-dependent pathways of the Krebs cycle and the electron transport chain are disrupted under anaerobic conditions, and this key source of ATP is lost. So how does a cell continue to produce ATP and recycle NADH when oxygen is nowhere to be found?

The answer lies in the **anaerobic fermentation** pathway, which consists of glycolysis to generate ATP, followed by one or two extra reactions 'tacked on' to the end to recycle NADH and create a steady supply of NAD^+. Recall that glycolysis involves the breakdown of glucose into two pyruvate molecules. In doing so, it converts two NAD^+ molecules to NADH and produces two molecules of ATP in the absence of oxygen.

anaerobic fermentation a metabolic pathway that occurs in the absence of oxygen. Involves glycolysis, followed by further reactions that convert pyruvate into lactic acid in animals, or ethanol and CO_2 in yeasts

yeast unicellular eukaryotic organisms from the kingdom Fungi

While glycolysis does not generate as much ATP as aerobic cellular respiration, some cells can still live off the energy from this anaerobic pathway. However, for glycolysis to continue indefinitely and keep producing energy, the cell must still find a way to convert NADH back to NAD^+, which is an input of glycolysis, without the use of oxygen. This is where the extra reactions of anaerobic fermentation come in. Animals and **yeast** have developed two different mechanisms to cycle NADH and regenerate NAD^+. These involve the fermentation of pyruvate – the end product of glycolysis – which we will explore in the following section of this lesson (Figure 2).

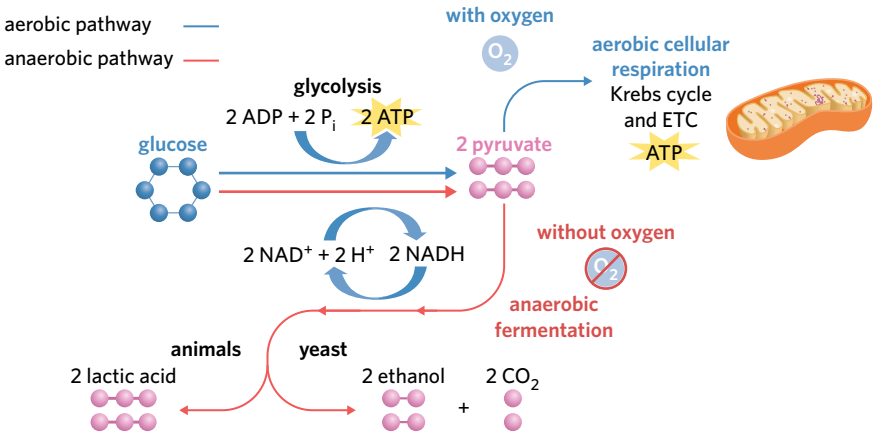


Figure 2 Anaerobic fermentation occurs in the absence of oxygen, regenerating NAD^+ for glycolysis and resulting in different products for animals and yeasts.

Examiners' tip

Justifying why aerobic cellular respiration cannot progress in anaerobic conditions, as well as reasoning why anaerobic pathways of metabolism require stages after glycolysis, helps you to better understand the process of cellular respiration. However, it is unlikely that the VCAA will expect you to discuss these processes to this depth.

Anaerobic fermentation in animals and yeast 3.2.8.2

OVERVIEW

Anaerobic fermentation involves the conversion of pyruvate to lactic acid in animals, and to ethanol and carbon dioxide in yeast. In doing so, both pathways regenerate NAD^+ for anaerobic glycolysis.

THEORY DETAILS

The first stage of anaerobic fermentation is the breakdown of glucose via glycolysis, and is common to both animals and yeasts. The NAD^+ regeneration stage that follows occurs in the cytosol of the cell for both animals and yeasts, just like glycolysis. This allows efficient recycling of NADH and NAD^+ as the coenzymes can freely diffuse in the cytosol without needing to be transported across a phospholipid membrane. The continued production of ATP during glycolysis can therefore be harnessed to keep powering the vital processes of the cell under anaerobic conditions.

Anaerobic fermentation in animals

When oxygen availability is insufficient, such as when working at high intensities, animals undertake **lactic acid fermentation** after glycolysis. This process breaks down pyruvate into **lactic acid** and cycles NADH back to NAD^+ for reuse in glycolysis (Figure 3).

Lactic acid cannot accumulate indefinitely, as it lowers the pH of our cells and blood, and can be toxic in high amounts. To deal with this, once oxygen is present again, lactic acid is metabolised back into pyruvate and used for aerobic cellular respiration.

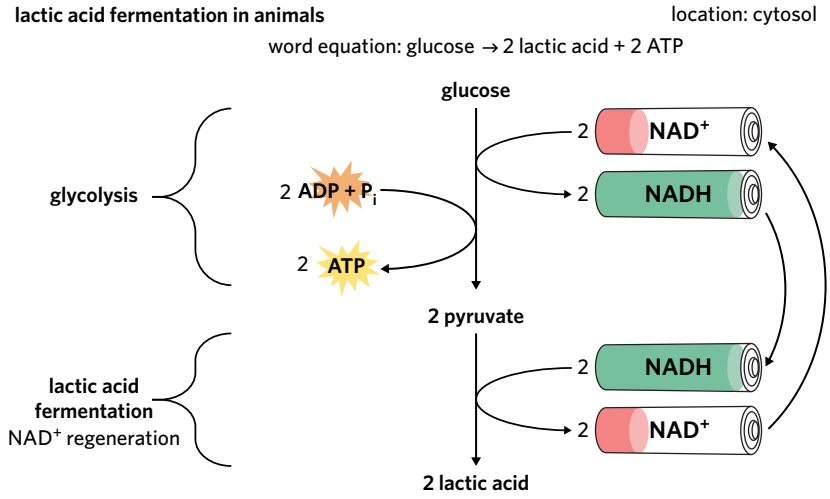


Figure 3 Anaerobic fermentation in animals

lactic acid fermentation the process of anaerobic fermentation in animals, where pyruvate produced via glycolysis is converted to lactic acid

lactic acid a 3-carbon molecule that is the product of anaerobic fermentation in animals. Also known as **lactate**

Lesson link

As you learned in ***lesson 3B***, enzymes require a narrow pH range to function properly and avoid becoming denatured. The lactic acid produced from anaerobic fermentation in animals can therefore lead to a low pH that decreases the activity of enzymes in the bloodstream or around nearby cells.

Theory in context

LACTIC ACID IN MUSCLE STRESS

You'll notice that your cells have been undergoing anaerobic metabolic pathways when you have that heavy feeling in your muscles after intense bursts of physical activity – this is an indication of the presence of lactic acid. Sprinters, for example, need to produce large amounts of ATP in a short span of time. Their muscle cells rapidly use up all the oxygen that is supplied to them. Once there is no oxygen left, the muscle cells must rely on anaerobic fermentation to produce the ATP needed for powerful movements, generating lactic acid as a by-product.

Anaerobic fermentation in yeasts

In yeasts, the anaerobic fermentation pathway also involves glycolysis, but pyruvate is instead converted to **ethanol** and carbon dioxide. As shown in Figure 4, this consists of a two-step process known as **ethanol fermentation**. Once again, these final steps allow the cycling of NADH back to NAD^+ for continued use in glycolysis.

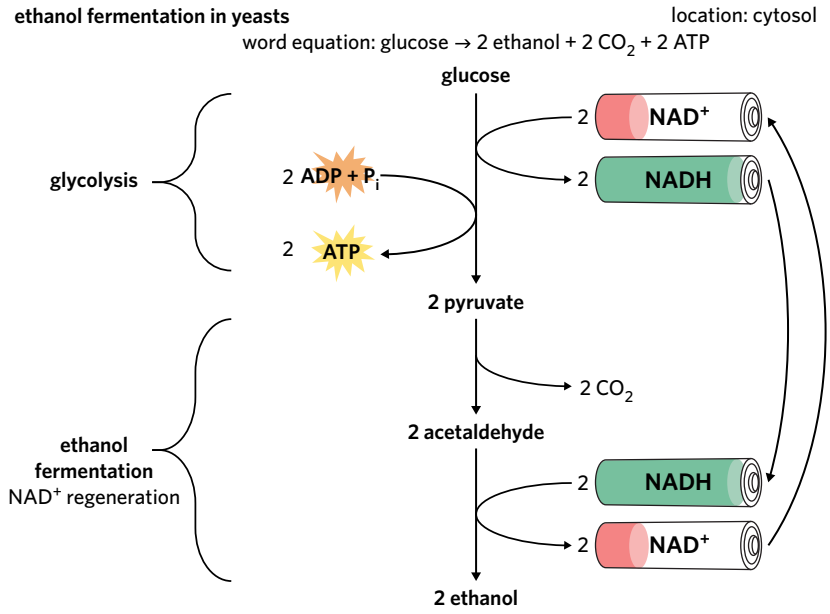


Figure 4 Anaerobic fermentation in yeasts

ethanol a 2-carbon alcohol molecule that is produced along with carbon dioxide during anaerobic fermentation in yeast, bacteria, and plants

ethanol fermentation the process of anaerobic fermentation in yeasts, where pyruvate produced via glycolysis is converted to ethanol and carbon dioxide. Also known as **alcohol fermentation**

Ethanol fermentation by certain yeast species is responsible for producing the ethanol found in alcoholic drinks such as wine, beer, and whiskey. In bread baking, the carbon dioxide produced by yeasts as they metabolise sugars, such as glucose in the dough, allows the bread to expand and rise, while the ethanol is removed during baking.

Yeasts are unable to metabolise ethanol into any useful products. Instead, ethanol diffuses out of cells, but the ethanol concentration of a yeast culture in a confined environment can eventually accumulate to toxic levels.

Examiners' tip

As humans, we are capable of both aerobic cellular respiration and anaerobic fermentation. But anaerobic fermentation is not limited to just animals and yeasts. Different bacteria have different oxygen requirements, and are therefore capable of varying degrees of aerobic cellular respiration, alongside anaerobic fermentation as a source of ATP. While oxygen is essential for the growth of many bacteria, it can be toxic for many others. We can classify these bacteria as:

- anaerobes, which cannot use oxygen
- obligate anaerobes, which are killed by oxygen
- obligate aerobes, which require oxygen
- facultative anaerobes or facultative aerobes, which can grow without oxygen, but grow better in the presence of oxygen.

This concept was examined in the given exam question, requiring students to analyse a graphical representation of the relationship between bacterial growth and environmental oxygen concentration.

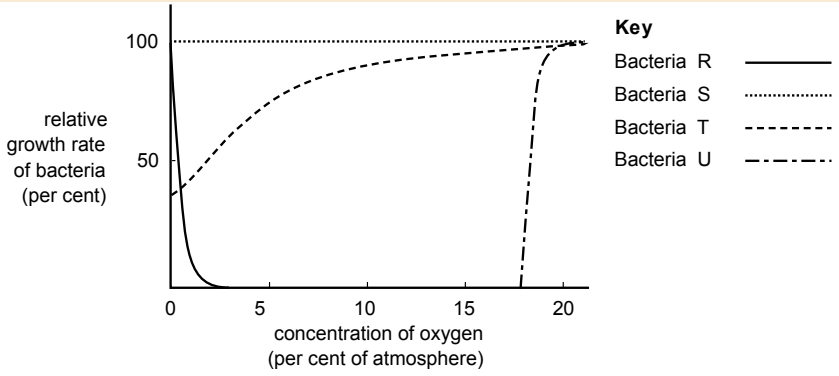


Image: VCAA 2017 Northern Hemisphere Exam Section A Q5

Lesson link

The ethanol produced by certain yeast species during anaerobic fermentation can be harnessed on a mass scale and used as a biofuel. This application of anaerobic fermentation is explored in ***lesson 6D***.

Theory summary

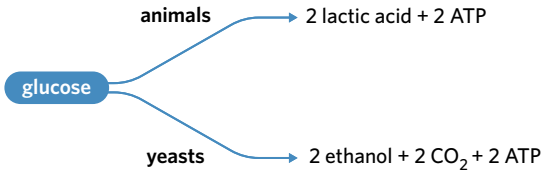


Figure 5 Products of anaerobic fermentation in animals and yeasts

- Anaerobic fermentation takes place in the cell cytosol in the absence of oxygen and involves glycolysis followed by extra steps to regenerate NAD^+ to allow glycolysis to continue producing ATP.
- In animal cells, glucose is converted to lactic acid via lactic acid fermentation.
- In yeasts, glucose is converted to ethanol and carbon dioxide via ethanol fermentation.

Key differences between aerobic cellular respiration and anaerobic fermentation are listed in Table 1, as being able to compare these two processes is commonly assessed in VCE Biology exams.

Table 1 Differences between aerobic cellular respiration and anaerobic fermentation

	Aerobic cellular respiration	Anaerobic fermentation
Stages	glycolysis Krebs cycle electron transport chain	glycolysis lactic acid fermentation (animals) *or* ethanol fermentation (yeasts)
Location	cytosol and mitochondria	cytosol
Inputs	glucose and oxygen	glucose
Outputs	carbon dioxide and water	lactic acid (animals) *or* ethanol and carbon dioxide (yeasts)
ATP Efficiency	36 or 38 ATP per glucose molecule	2 ATP per glucose molecule
Speed	slow	fast
Sustainability	can continue indefinitely	cannot continue indefinitely due to toxic build-up of lactic acid *or* ethanol

How would our micro-thon yeasts produce energy when they need it fast? Anaerobic glycolysis! But remember that ATP, just like coming first in a micro-thon, comes at a price. The carbon dioxide gas yeasts produce via anaerobic glycolysis might lead to just some harmless fizzing, but the ethanol by-product will quickly build up to toxic levels. Soon the yeasts might not just veer off course, but could be looking at a grisly demise – that is, of course, if they don't first win the eternal glory of a podium finish in the yeast-athon!

6B QUESTIONS

Theory review questions

Question 1

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- NAD^+
- NADH
- ethanol
- glucose
- pyruvate
- glycolysis
- lactic acid
- heat and light
- anaerobic fermentation
- aerobic cellular respiration

When oxygen is scarce, animal cells will undergo _____ as their main mechanism of ATP production. This process requires _____ as its primary input, which must first be broken down via _____, followed by an additional reaction that generates _____ as a toxic by-product.

Question 2

Which of the following options correctly identifies the products of anaerobic fermentation in animal and yeast cells?

	Animal cells	Yeast cells
A	lactic acid	ethanol
B	ethanol, carbon dioxide	lactic acid
C	lactic acid	ethanol, carbon dioxide
D	ethanol	lactic acid, carbon dioxide

Question 3

The key role of the final stage(s) of anaerobic fermentation in animal and yeast cells is to

A regenerate ATP from inactive ADP molecules, supplementing ATP production from glycolysis when energy demand is high and oxygen availability is low.

B recycle NADH and generate NAD^+ molecules for continued use in glycolysis when the electron transport chain is inactive.

Question 4

The conversion of pyruvate to ethanol and carbon dioxide during anaerobic fermentation occurs

A in the cytosol in yeast cells.

B in the cytosol in animal cells.

C in the mitochondrial matrix in yeast cells.

D in the mitochondrial matrix in animal cells.

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–9.

Red blood cells (RBCs) are highly specialised cells responsible for the transport of oxygen bound to the protein haemoglobin throughout the body's blood circulatory system. Before maturation, RBCs undergo a series of developmental stages from precursor cells known as haematopoietic stem cells. During development, there is a marked increase in haemoglobin synthesis, and progressive condensation of the nucleus until it is finally ejected from the cell, forming what is known as a reticulocyte. Over the next 2–3 days, reticulocytes lose their remaining organelles, including mitochondria, RNA, and ribosomes to become a mature RBC.

Organelle extrusion maximises the intracellular volume available for haemoglobin and oxygen transport at the cost of more complex metabolic pathways such as aerobic cellular respiration. As a result, the RBC is unique as it is the only cell in the human body that relies exclusively on blood glucose and glycolysis to produce ATP anaerobically. To compensate for this, large numbers of GLUT1 transport proteins are embedded in the RBC membrane that facilitate diffusion of glucose into the cell, after which it can be broken down via anaerobic fermentation. The by-product of this process – lactic acid – is secreted into the bloodstream from where it can modulate blood pH or be recycled.

Question 5

Red blood cells are unique due to their reliance on anaerobic fermentation for ATP production, a process which only occurs

A in the oxygenated haemoglobin-rich cytosol of a mature red blood cell, bounded by a phospholipid bilayer.

B within a specialised cellular compartment formed from the remnants of mitochondrial and nuclear membranes.

Question 6

Which of the following best describes why it might be undesirable for red blood cells to produce ATP via both aerobic and anaerobic metabolic pathways?

A These anaerobic and aerobic pathways are incompatible, as lactic acid production via anaerobic fermentation in red blood cells interferes with the proteins and enzymes necessary for aerobic cellular respiration.

B The primary role of the red blood cell is to optimise transport and delivery of oxygen, a function that would be compromised by its consumption at the electron transport chain embedded in the mitochondrial cristae.

Question 7

Mature red blood cells are only capable of generating ATP via anaerobic fermentation because

A their plasma membranes are impermeable to oxygen, allowing them to maximise oxygen transport, but preventing oxygen from acting as a terminal electron acceptor.

B they do not transcribe the genes and do not contain the organelles that are necessary for aerobic cellular respiration.

Question 8

Consider the following statement.

'Despite having one of the highest rates of utilisation of glucose per kg of tissue (~400% of the body's average), the RBC has one of the lowest overall rates of ATP production per day out of any of the body's cells.'

It would be reasonable to conclude that this is because

A the absence of mitochondria in the RBC prohibits efficient ATP generation via aerobic metabolism, and the remaining biochemical pathway of anaerobic fermentation extracts only a minor proportion of the total energy available from each glucose molecule.

B without ribosomes the RBC is unable to manufacture new glycolytic enzymes, which means that as older enzymes deteriorate, efficient long-term ATP production becomes impossible.

Question 9

Red blood cells also contain enzymes embedded in their cell membranes that use energy from ATP to help maintain a stable pH in their cytosol. If a red blood cell is travelling through a highly acidic artery, which of the following is most likely?

A The red blood cell would increase the transport of glucose across its plasma membrane, promoting glycolytic pathways of ATP production and dedicating this energy to the control of cytosolic pH.

B The red blood cell would upregulate the genes that produce glycolytic enzymes, increasing the level of ATP available for pH regulation via anaerobic fermentation.

Exam-style questions

Within lesson

Question 10 (1 MARK)

Consider the production of ATP molecules in a eukaryotic cell in the absence of oxygen.

The majority of ATP molecules are produced

A during glycolysis.

B in the Krebs cycle.

C at the electron transport chain.

D via the conversion of pyruvate to lactic acid.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q3

Question 11 (1 MARK)

Which of the following is an output of anaerobic fermentation in a yeast cell?

A glucose

B pyruvate

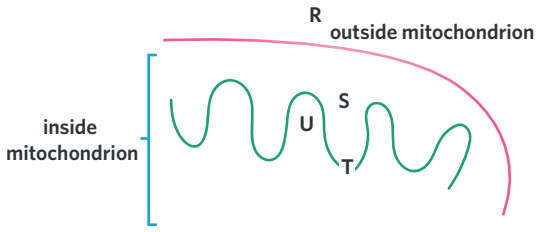
C lactic acid

D carbon dioxide

Adapted from VCAA 2018 Section A Q9

Question 12 (1 MARK)

The diagram shows a section through part of a mitochondrion in a eukaryotic cell.



At which location is the ATP from anaerobic fermentation produced?

A R

B S

C T

D U

Adapted from VCAA 2018 Section A Q8

Question 13 (1 MARK)

During anaerobic fermentation in cells, glucose is broken down into pyruvate. Pyruvate is then converted into other products to regenerate NAD^+ from NADH. Anaerobic fermentation occurs

A on the inner mitochondrial membrane and produces two ATP per glucose molecule.

B on the surface of the outer mitochondrial membrane.

C in the cytosol and cannot be sustained indefinitely.

D in the matrix of mitochondria.

Adapted from VCAA 2014 Section A Q7

Question 14 (1 MARK)

Consider *Saccharomyces cerevisiae*, a yeast species that has been used for centuries in the brewing, baking, and winemaking industries. Which of the following is not true of glycolysis as it would occur in *Saccharomyces cerevisiae*?

A Pyruvate is an input and lactic acid is an output.

B It produces less ATP than the electron transport chain.

C It is a common stage in both aerobic cellular respiration and anaerobic fermentation.

D It produces loaded carriers that can be unloaded in later stages of aerobic cellular respiration.

Adapted from VCAA 2017 Sample Exam Section A Q13

Question 15 (1 MARK)

Which one of the following statements about the process of anaerobic fermentation in yeast cells is incorrect?

A Anaerobic fermentation in yeasts produces only ethanol.

B Anaerobic fermentation cannot be sustained indefinitely.

C The cytosol is the site of glycolysis and produces 2 ATP per glucose molecule.

D Anaerobic fermentation is less efficient than aerobic cellular respiration as it produces fewer ATP per glucose molecule.

Multiple lessons

Question 16 (1 MARK)

The reaction ADP + P_i → ATP

A takes place in glycolysis, the Krebs cycle, and the electron transport chain.

B occurs in aerobic cellular respiration and not in photosynthesis.

C does not occur in plants when no oxygen is present.

D is irreversible.

Adapted from VCAA 2011 Exam 1 Section A Q20

Question 17 (1 MARK)

Aerobic cellular respiration and anaerobic fermentation both involve

A the production of ADP from ATP and P_i.

B the loaded coenzymes NADH and $FADH_2$.

C chemical reactions that occur in the cytosol.

D the use of a proton gradient during the electron transport chain.

Question 18 (1 MARK)

A student was asked to identify differences between the overall processes of aerobic cellular respiration and anaerobic fermentation in eukaryotic cells. The student prepared the given table to outline these differences. Which of the following gives the only incorrect comparison listed by the student?

	Aerobic cellular respiration	Anaerobic fermentation
A	more efficient	less efficient
B	faster	slower
C	involves glycolysis, the Krebs cycle, and the electron transport chain	involves glycolysis
D	can be sustained indefinitely	cannot be sustained indefinitely

Adapted from VCAA 2007 Exam 1 Section A Q7

Question 19 (8 MARKS)

The smoke produced during a house fire is usually more dangerous than the fire itself. Two of the many toxic chemicals in the smoke from a house fire are carbon monoxide and hydrogen cyanide. Carbon monoxide molecules bind to haemoglobin molecules in the blood, which reduces the blood's capacity to transport oxygen. This causes a significant reduction in oxygen supply to the cells of the body. Symptoms of carbon monoxide poisoning include dizziness, drowsiness, and nausea.

a Name and describe the process by which a human cell produces ATP in the absence of oxygen, with reference to the product(s) of this metabolic pathway. (2 MARKS)

b Carbon monoxide poisoning can be treated by inhaling close to pure oxygen.

i State and explain whether carbon monoxide displays the characteristics of a competitive or non-competitive inhibitor of haemoglobin's function. (3 MARKS)

ii Name and briefly describe the three key stages of aerobic cellular respiration as they would occur when human cells are exposed to normal environmental oxygen levels. (3 MARKS)

Adapted from VCAA 2017 Northern Hemisphere Exam Section B Q4

Key science skills and ethical understanding

Question 20 (9 MARKS)

Kombucha tea has risen in popularity within the last decade, with increased levels of commercial and recreational brewing. The drink is often consumed for its supposed health benefits. These include claims for treating AIDS, ageing, anorexia, cancer, and diabetes. However, there is yet to be concrete scientific evidence to support these claims.

Kombucha is a fermented drink that is made from sweetened tea and a symbiotic culture of bacteria and yeast (SCOBY). Part of the health claims come from probiotic bacteria that can be added to the culture during the brewing process. During the brewing process, the yeasts in the SCOBY are responsible for fermenting the glucose present within the tea.

a Name the product(s) produced by yeast during the anaerobic fermentation process. (1 MARK)

Adapted from VCAA 2016 Section B Q2b

b Explain the importance of anaerobic fermentation in yeast cells. (1 MARK)

Adapted from VCAA 2016 Section B Q2a

c State one similarity and one difference between aerobic cellular respiration and anaerobic fermentation. (2 MARKS)

d The SCOBY can contain several different species of bacteria. The culture almost always includes the particular bacterial strain, *Komagataeibacter xylinus*. *K. xylinus* can convert the product of yeast fermentation into acetic acid. This gives Kombucha its sour taste. A group of scientists hypothesised that *K. xylinus* is an obligate anaerobe, meaning that it can only live in an environment with little to no oxygen. They performed an experiment to test this hypothesis, which involved the manipulation of the oxygen content surrounding agar plates that each contained colonies of *K. xylinus* bacteria.

i Identify the independent and dependent variables in this experiment. (2 MARKS)

Adapted from VCAA 2018 Section B Q11c

ii Assuming their hypothesis is supported, describe the environment in which this species of bacteria would thrive. (1 MARK)

iii When the experiment was performed, the samples were not labelled. What type of error is this? (1 MARK)

iv Describe the results that would support the scientists' hypothesis. (1 MARK)

Adapted from VCAA 2016 Section B Q2

6C FACTORS AFFECTING THE RATE OF CELLULAR RESPIRATION

If you've ever been to mountains over 4 000 metres in altitude, you'd be familiar with the symptoms of altitude sickness: insomnia, headaches, and fainting. But did you know that some elite athletes pay money to put themselves in this oxygen-deprived environment? They can do this by training at high altitudes or exercising in special hypoxia gyms. Unfortunately, exercising under these conditions can be very tiring and sometimes even dangerous. Why would anyone want to do that to themselves?

This lady is counting the seconds until she can take a breath of her favourite 21% oxygen air again.

Image: wavebreakmedia/Shutterstock.com

Lesson 6C

In this lesson you will learn about the factors that influence the rate of cellular respiration, including temperature, pH, glucose availability, oxygen concentration, and enzyme inhibitors.

◀ *Prerequisite knowledge* *Future applications* ▶

Lesson 3B
Factors that affect the activities of enzymes, including temperature, pH, and enzyme inhibitors, also influence the rate of cellular respiration.

Lesson 6A & 6B
Glucose and oxygen are the inputs of aerobic respiration, and glucose is the input of anaerobic respiration. As a result, their concentrations can impact the rate of cellular respiration.

Lesson 9B
A factor affecting cellular respiration could act as a selection pressure on a population, leading to natural selection.

Study design dot points

- the factors that affect the rate of cellular respiration: temperature, glucose availability, and oxygen concentration
- the general factors that impact on enzyme function in relation to photosynthesis and cellular respiration: changes in temperature, pH, concentration, competitive and non-competitive enzyme inhibitors

Key knowledge units

Temperature and pH	3.2.9.1
Glucose	3.2.9.2
Oxygen	3.2.9.3
Enzyme inhibition	3.2.3.7

Temperature and pH 3.2.9.1

OVERVIEW

Temperature and pH have a large effect on the rate at which cellular respiration occurs due to their effect on enzymes, which are essential in cellular respiration.

optimal the point at which for a given condition (e.g. temperature), the maximum function of an enzyme occurs. Also known as **optimum**

THEORY DETAILS

As you learned in lessons 6A and 6B, enzymes accelerate the speed at which aerobic respiration and anaerobic fermentation occur. You also learned that each enzyme has an **optimal** temperature at which enzyme-catalysed reactions occur at the greatest rate.

Therefore, cellular respiration rate and ATP production are highest when the temperature aligns with the enzyme's optimal temperature (Figure 1).

Below the optimal temperature, enzymes and substrates have less kinetic energy so there are fewer reaction-inducing collisions. This results in a lower rate of cellular respiration. On the other hand, above the optimal temperature enzymes begin to **denature** and respiration rate drops rapidly due to the loss of enzyme function.

Different enzymes function optimally at different **pH**s. For example, the cytoplasm typically has a pH of around 7.2, so the enzymes involved in glycolysis (which occurs in the cytoplasm) function optimally under this condition. The intermembrane space of the mitochondria usually has a pH of around 7.0-7.4, while the matrix has a pH of 7.8. For this reason, the enzymes that support reactions at these locations may have slightly different optimal pH levels. Above or below the optimal pH, enzymes begin to denature and the rate of respiration slows (Figure 2).

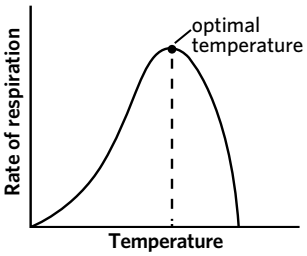


Figure 1 The effect of temperature on the rate of cellular respiration

denature the disruption of a molecule's structure by an external factor such as heat

pH a scale used to measure the acidity or basicity of an aqueous solution

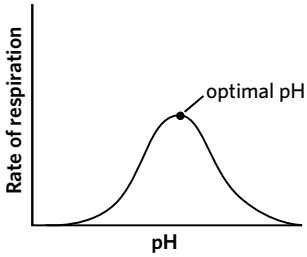


Figure 2 The effect of pH on cellular respiration rate

saturation point the point at which a substance (e.g. an enzyme) cannot receive more of another substance (e.g. a substrate)

Glucose 3.2.9.2

OVERVIEW

Increasing glucose availability increases the rate of cellular respiration until the enzymes reach the saturation point.

THEORY DETAILS

Glucose is the input for glycolysis, the first stage of both aerobic respiration and anaerobic fermentation (Figure 3). As a result, an increase in glucose availability increases the rate of cellular respiration, thereby increasing the rate of ATP production. Conversely, a decrease in glucose availability reduces the rate of cellular respiration, thereby reducing the rate of ATP production.

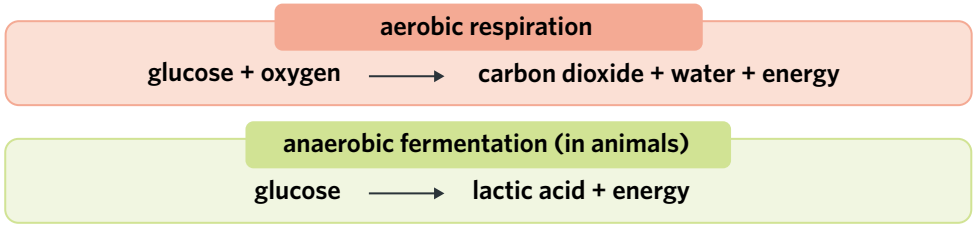


Figure 3 The simplified cellular respiration equations in animals

However, the rate of cellular respiration does not keep increasing indefinitely as the concentration of glucose increases. Increasing glucose concentration will increase respiration rate until a maximum level is reached. At this maximum level, the respiration rate plateaus (Figure 4). This is because the enzymes involved in respiration have reached their **saturation point** and are operating at their maximum capacity.

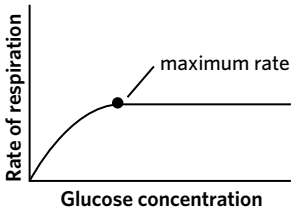


Figure 4 Respiration rate increases with glucose to a point.

Oxygen 3.2.9.3

OVERVIEW

Increasing the concentration of oxygen will increase the rate of aerobic respiration.

THEORY DETAILS

Aerobic respiration requires oxygen for the electron transport chain to function. However, oxygen is not an input of anaerobic fermentation. In animals, low oxygen will induce cells to switch to anaerobic fermentation, while the presence of oxygen will encourage cells to respire aerobically. As oxygen levels rise, the rate of aerobic respiration increases. Therefore, more oxygen results in faster ATP production. At a certain point, assuming unlimited glucose, adding more oxygen does not increase the rate of respiration, as the enzymes involved in the process are saturated and working at their maximum capacity.

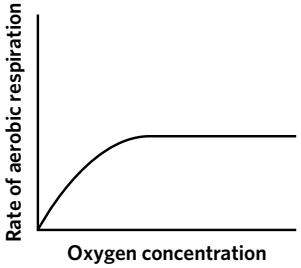


Figure 5 With unlimited glucose, the rate of aerobic respiration increases with oxygen concentration up to a point.

Lesson link

In ***lesson 6A***, you learned that during the electron transport chain, oxygen accepts free protons and electrons that have accumulated from the production of ATP to form water molecules.

Enzyme inhibition 3.2.3.7

OVERVIEW

Enzyme inhibitors decrease the rate of cellular respiration by reducing the activity of enzymes involved in the process.

THEORY DETAILS

Enzyme inhibitors can also influence the function of enzymes and, as a result, the rate of cellular respiration. In lesson 3B, you learned that **competitive inhibitors** bind to the active sites of enzymes to prevent the catalysis of substrates. **Non-competitive inhibitors** bind to the **allosteric sites** of enzymes. This results in a conformational change to the active site so that the substrate can no longer bind. You might have heard of cyanide poisoning before – this is lethal because cyanide allosterically binds to cytochrome c oxidase, an important enzyme in the electron transport chain. Both types of inhibitors slow down the cellular respiration process. This means that as the concentration of enzyme inhibitors increases, the rate of cellular respiration decreases.

enzyme inhibitor a molecule that binds to and prevents an enzyme from functioning

competitive inhibitor a molecule that hinders an enzyme by blocking the active site and preventing the substrate from binding

non-competitive inhibitor a molecule that hinders an enzyme by binding to an allosteric site and changing the shape of the active site to prevent the substrate from binding

allosteric site a region on an enzyme that is not the active site

The effect of competitive inhibitors can be overcome if the substrate concentration is increased (Figure 6). On the other hand, increasing substrate concentration does not reduce the effect of non-competitive inhibitors. This means that the maximum possible rate of reaction (Vmax) is reduced in the presence of non-competitive inhibitors.

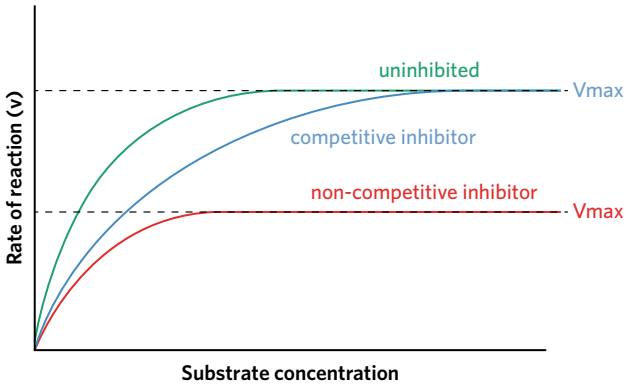


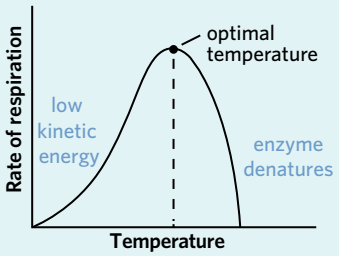
Figure 6 The effect of different inhibitors on the rate of reaction as substrate concentration is increased

Enzyme inhibition of metabolic pathways like aerobic respiration and anaerobic fermentation is not always a bad thing! In fact, allosteric inhibition is very important for regulating respiration. For example, the third step of glycolysis involves an enzyme called phosphofructokinase. It is non-competitively inhibited by ATP. So, when ATP levels are very high – which happens when the cell is producing more ATP than is being used up – glycolysis is paused. By matching their respiration rate to their energy needs through **end-product inhibition** and other feedback loops, cells are more efficient.

end-product inhibition a form of inhibition where the final product in a series of reactions inhibits an enzyme in an earlier reaction in the sequence

Theory summary

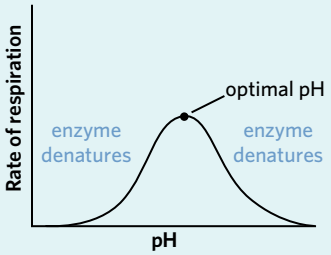
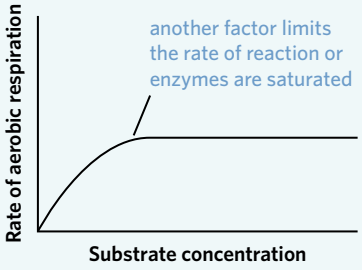
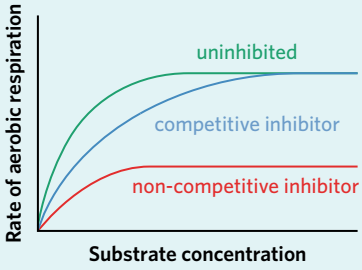
Table 1 Summary of factors impacting the rate of cellular respiration

Factor	How it affects respiration
Temperature	 *cont'd*

Theory in action

Check out scientific investigations 6.1 and 6.2 to put this into action!

Table 1 Continued

Factor	How it affects respiration
pH	
Glucose or oxygen concentration	
Inhibitors	

At high altitude, the air contains a much lower percentage of oxygen than at sea level. This means that there is less oxygen available for aerobic respiration of the cells within the body, causing the rate of aerobic respiration to drop. As a result, the rate of ATP production decreases, making you feel tired more quickly. However, if you train for long enough at high altitude, your kidneys will start to produce a hormone called erythropoietin (EPO). An elevated level of EPO will increase the production of red blood cells (RBC), allowing more oxygen to be carried and transported to cells. When you return to a lower altitude, your RBC count remains higher than usual. This, combined with a higher percentage of oxygen at low altitude, means your muscle cells receive a higher concentration of oxygen than typical for a body at sea level, which increases the rate of aerobic respiration and ultimately increases the rate of ATP production. Therefore, high altitude training is an excellent method utilised by athletes to boost their performance.

Image: Maridav/Shutterstock.com

6C QUESTIONS

Theory review questions

Question 1

Fill in the blanks in the following sentences.

The rate of aerobic respiration is dependent on _____, pH, _____, glucose concentration, and oxygen concentration. _____ does not affect the rate of anaerobic respiration. At the _____ temperature of the enzymes, the rate of cellular respiration is greatest. _____ is the key input in both respiration types, and therefore an increase in its availability will _____ the rate of respiration.

Question 2

Which of the following are all true regarding the rate of cellular respiration?

A	Typically increases as glucose increases.	Aerobic respiration rate is dependent on oxygen concentration.	Anaerobic fermentation rate generally decreases as oxygen concentration increases.	Eventually reaches a maximum rate with increasing amounts of glucose and unlimited oxygen
B	Typically increases as glucose increases.	Aerobic respiration rate is dependent on carbon dioxide concentration.	Anaerobic fermentation rate generally decreases as oxygen concentration increases.	Eventually reaches a maximum rate with increasing amounts of glucose and unlimited oxygen
C	Typically decreases as glucose increases.	Aerobic respiration rate is dependent on carbon dioxide concentration.	Anaerobic fermentation rate generally increases as oxygen concentration increases.	A maximum rate can never be reached.
D	Typically increases as glucose increases.	Aerobic respirationrate is dependent on oxygen concentration.	Anaerobic fermentation rate generally increases as oxygen concentration increases.	Eventually reaches a maximum rate with increasing amounts of glucose and unlimited oxygen

Question 3

Which of the following are all true regarding temperature and cellular respiration rate?

A	Only affects aerobic respiration rate.	Below the optimal temperature, the rate increases as the temperature increases.	Above the optimal temperature, the rate decreases as the temperature increases.	The optimal temperature for the respiration rate is twice the enzyme's optimal temperature.
B	Affects both aerobic respiration and anaerobic fermentation rate.	Below the optimal temperature, the rate increases as the temperature increases.	Above the optimal temperature, the rate increases as the temperature increases.	The optimal temperature for the respiration rate aligns with the enzyme's optimal temperature.
C	Only affects aerobic respiration rate.	Below the optimal temperature, the rate decreases as the temperature increases.	Above the optimal temperature, the rate increases as the temperature increases.	The optimal temperature for the respiration rate is half the enzyme's optimal temperature.
D	Affects both aerobic respiration and anaerobic fermentation rate.	Below the optimal temperature, the rate increases as the temperature increases.	Above the optimal temperature, the rate decreases as the temperature increases.	The optimal temperature for the respiration rate aligns with the enzyme's optimal temperature.

Question 4

Categorise the following statements about respiration rate as either relating to **temperature**, **glucose concentration**, or **oxygen concentration**. Statements may be used in multiple categories or none at all.

I influences aerobic respiration rate _____

II can be seen in both anaerobic fermentation and aerobic respiration equations _____

III an increase can denature enzymes _____

IV is an input in anaerobic respiration _____

V a continued increase can result in a maximum respiration rate being reached _____

VI is key to the ETC functioning in respiration _____

VII directly influences anaerobic fermentation rate _____

VIII a decrease in this factor typically decreases aerobic respiration rate _____

IX is an input in aerobic respiration _____

Question 5

Which of the following is correct regarding the rate of cellular respiration?

A As the amount of non-competitive inhibitors increases, the rate of aerobic respiration does not change because the inhibitors do not block the active site of enzymes.

B Cellular respiration cannot occur at all unless the pH is absolutely perfect for the enzymes involved.

C As the oxygen concentration decreases, the rate of anaerobic fermentation also decreases.

D Competitive inhibition can be overcome by increasing the substrate concentration.

SAC skills questions

Case study analysis

Use the following information to answer Questions 6–11.

Cyanide compounds include sodium cyanide, potassium cyanide, calcium cyanide, and hydrogen cyanide. They are highly toxic and are some of the fastest acting poisons. They are mainly used in gold and silver extraction, metal cleaning, and electroplating.

The electron transport chain, where electrons are transferred from NADH molecules produced during the Krebs cycle, includes the enzyme cytochrome c oxidase. Cyanide hinders the electron transport chain by binding to the ferric ion found in the oxidised form of cytochrome c oxidase's a_3 subunit, changing the structure of the enzyme and therefore reducing its ability to catalyse. When functioning, cytochrome c oxidase transfers electrons to oxygen to help form water, and pumps four protons into the intermembrane space. Without this step, the rate of ATP production drops significantly. While the electron transport chain is disrupted, the glycolysis rate increases markedly. Despite this, pyruvate cannot be used in the impaired Krebs cycle, but is instead turned into lactate, which causes the lactate levels in the body to rise.

Patients with mild cyanide poisoning experience headaches, nausea, vomiting, difficulty breathing, as well as a feeling of general weakness in both the arms and legs. When cyanide poisoning is severe, symptoms include gasping for breath and loss of consciousness. After that, cardiac arrest and ultimately death may result.

Question 6

Why are cyanide compounds considered to be some of the fastest acting poisons?

A because they are highly toxic

B because it can cause severe symptoms

C because it can lead to cardiac arrest and death

D because symptoms appear quickly after exposure

Question 7

Which of the following is true regarding the effect of cyanide on cellular respiration?

A It disrupts the body's ability to inhale oxygen.

B It causes conformational changes to an enzyme in the electron transport chain.

C It disrupts the Krebs cycle by binding to the enzyme subunit cytochrome oxidase a_3.

D It binds to NADH molecules and prevents them from transferring electrons to oxygen.

Question 8

Cyanide is

A an electron carrier.

B a competitive enzyme inhibitor.

C a non-competitive enzyme inhibitor.

D an oxidised form of cytochrome oxidase.

Question 9

It is possible to conclude that

A an increase in cyanide concentration will not change the rate of aerobic respiration.

B an increase in cyanide concentration will increase the rate of aerobic respiration.

C an increase in cyanide concentration will decrease the rate of water production.

D an increase in cyanide concentration will decrease the lactate levels.

Question 10

What is a possible explanation for difficulty breathing and weakness in the arms and legs during mild cyanide poisoning?

A the rate of ATP production decreases

B the rate of oxygen inhalation decreases

C the rate of oxygen production decreases

D the rate of water consumption decreases

Question 11

Why does the rate of ATP production drop significantly with the presence of cyanide?

A Oxygen receives too many electrons, preventing water production, which is required in the ATP production.

B Pyruvate that cannot be utilised in the impaired Krebs cycle deactivates enzymes in the electron transport chain.

C Electrons that cannot be transferred from NADH molecules accumulate in the matrix, which blocks the ATP synthase.

D When the electrons are not transferred effectively along the electron transport chain it is more difficult for water to form, which leads to a less steep proton gradient and ineffective ATP synthase.

Exam-style questions

Within lesson

Question 12 (1 MARK)

Muscle cells in the heart contract and relax more rapidly during exercise and require a constant supply of energy. Heart muscle cells can increase their rate of cellular respiration by

A increasing carbon dioxide supply.

B increasing oxygen concentration.

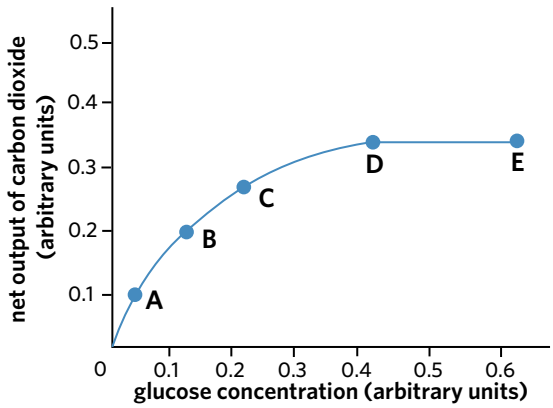
C increasing water concentration.

D increasing pH above 7.2.

Adapted from VCAA 2014 Section B Q3c

Use the following information to answer Questions 13 and 14.

The graph shows the net output of carbon dioxide from yeast as glucose concentration is increased. Temperature is kept constant during the experiment.



Question 13 (1 MARK)

Which one of the following conclusions can be made based on the graph?

A At point E, cellular respiration is no longer occurring.

B The optimal glucose concentration is 0.3 arbitrary units.

C At point A, the amount of carbon dioxide output is half of maximum.

D Below 0.2 arbitrary units of glucose concentration, enzymes involved in cellular respiration are not saturated.

Adapted from VCAA 2017 Section A Q13

Question 14 (1 MARK)

The rate of carbon dioxide output remains constant between points D and E.

This may be because

A there is another metabolic reaction that uses up the carbon dioxide produced.

B the concentration of carbon dioxide increases the rate of cellular respiration.

C glucose competitively inhibits enzymes involved in the Krebs cycle.

D the enzymes involved in cellular respiration are saturated.

Adapted from VCAA 2017 Section A Q14

Multiple lessons

Question 15 (1 MARK)

Glucokinase is an essential enzyme that catalyses the formation of glucose-6-phosphate from glucose during the glycolysis stage of cellular respiration. Human glucokinase's optimal pH range is 8.5–8.7. It is not correct to conclude that

A the production of glucose-6-phosphate can be reduced when the pH is out of the tolerance range of glucokinase.

B the rate of glucose-6-phosphate production is greatest when the pH is between 8.5 and 8.7.

C a change in pH affects the rate of glucose-6-phosphate production.

D when the pH is 1, glucose is produced at a low rate.

Question 16 (1 MARK)

Which of the following is true regarding the impacts of enzyme inhibitors on the rate of cellular respiration?

A Competitive inhibitors decrease the rate of aerobic respiration but do not affect the rate of anaerobic respiration.

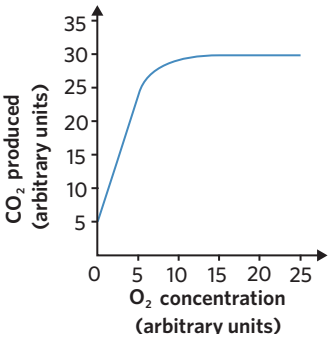
B Non-competitive inhibitors do not affect the rate of cellular respiration because they do not bind to the active sites of enzymes.

C Non-competitive inhibitors decrease the rate of cellular respiration by binding to the active sites of enzymes, which changes their structure.

D Competitive inhibitors decrease the rate of cellular respiration by binding to the active sites of enzymes, preventing them from binding to substrates.

Question 17 (7 MARKS)

The graph shows the rate of carbon dioxide exchange between a plant root system and its external environment as the oxygen concentration is altered. All other variables are kept constant throughout the experiment.



a Danny suggested that aerobic respiration is still occurring when there is no environmental O_2 because the CO_2 produced is greater than zero.

Evaluate Danny's statement and justify your response. (2 MARKS)

b Describe the consequence for plant root cells if they are deprived of O_2 for an extended period of time. (2 MARKS)

c A plateau is seen on the graph.

i Explain why the graph line becomes nearly horizontal from about 10 arbitrary units of oxygen concentration. (1 MARK)

ii Describe the variable(s) that, when altered, could raise the value of the plateau on the graph. (2 MARKS)

Adapted from VCAA 2011 Exam Section B Q7d

Key science skills and ethical understanding

Question 18 (13 MARKS)

Yeast is a single-celled microscopic fungus that uses sucrose as a food source by breaking it down into glucose. An experiment was carried out by four separate groups of students to investigate the cellular respiration rate of four similar species of yeast. Four groups were set up, each with a different species of yeast cells in a container, and a 0.1 M sucrose solution was added to each. The containers were sealed in such a way as to prevent air from entering. The percentages of oxygen and ethanol in the containers were recorded over a one-hour period. The experiment was carried out at a room temperature of 26 °C, which is close to yeast's optimal temperature (30 °C). The results for each group and the mean are shown in the following table.

Group	Percentage of oxygen		Percentage of ethanol	
	At the start of the experiment	At the end of the experiment	At the start of the experiment	At the end of the experiment
1	21	18	0	4
2	8	17	0	5
3	22	19	0	3
4	21	18	0	4
Mean for all groups	18	18	0	4

a State the dependent and independent variables. (2 MARKS)

b Consider the data provided.

i Which type of cellular respiration is being measured by the change in the percentage of oxygen in the container?. (1 MARK)

ii Name and describe the process occurring in the yeast that produces ethanol. (2 MARKS)

c Another student carried out the same experiment at a temperature of 60 °C instead.

i Describe and explain the expected change in the percentage of ethanol. (2 MARKS)

ii To match the other students' results, this student made some adjustments in their recorded values of oxygen and ethanol at the end of the experiment.

Identify the bioethical concept that was not observed by this student. Explain your answer. (2 MARKS)

d Consider the design of the experiment conducted to investigate cellular respiration.

i A student misread the first value in Group 2.

Name the type of error that has occurred in Group 2. (1 MARK)

ii Outline how this error impacts the class results. (1 MARK)

iii Identify and explain a factor other than sucrose availability that could be changed in the original experiment to decrease the rate of both aerobic and anaerobic cellular respiration. (2 MARKS)

Adapted from VCAA 2017 Sample Exam Section B Q1

6D BIOFUEL FROM FERMENTATION

Think of corn on the cob. Now think of a spacecraft to Mars. Now back to the cob.

What do these two things have in common? Nothing? Does Elon eat corn?

Let me ask you this: could corn ever power a spacecraft to Mars? Let's find out!

Image: Alones/Shutterstock.com

Lesson 6D

In this lesson you will learn how the process of anaerobic fermentation can be used to produce biofuel, which is considered a renewable energy alternative to traditional fossil fuels such as petrol and coal.

◀ *Prerequisite knowledge* *Future applications* ▶

Chapter 3
Enzymes are important in facilitating the breakdown of biomass during anaerobic fermentation.

Lesson 6B
Anaerobic fermentation produces ethanol and CO_2 in yeasts, the former of which can be used for biofuels.

Lesson 10A
Fossils are the preserved body, impressions, or traces of a dead organism. Fossil fuels are formed via the fossilisation process of plant matter, which takes millions of years.

Study design dot point

- uses and applications of anaerobic fermentation of biomass for biofuel production

Key knowledge units

What is biofuel?	3.2.11.1
How are biofuels made?	3.2.11.2
The uses and applications of biofuels	3.2.11.3

What is biofuel? 3.2.11.1

OVERVIEW

Biofuels are made from organic material known as biomass, which is plant and animal material that can be sourced from many of our existing industries (such as agriculture and forestry). They offer an alternative to traditional fossil fuels like coal and gas, which are non-renewable.

THEORY DETAILS

We humans require a lot of energy to live comfortably. We like to keep our homes warm, use electricity, and drive cars. All of these things require a huge amount of energy, which we typically get from **fossil fuels** like coal and oil. However, due to the fact that fossil fuels are made from the fossilisation of plants and animals, a process that takes tens of millions of years to complete, the rate of production of fossils fuels is much lower than the rate of their consumption. Therefore, these energy sources are considered **non-renewable**.

fossil fuel fuel that formed over tens of millions of years from the remains of dead organic material. Fossil fuels are considered non-renewable

non-renewable refers to a resource that is replenished at a slower rate than it is being used, meaning that it will eventually run out

Theory in context

HOW FOSSIL FUELS ARE MADE AND WHY THEY ARE NON-RENEWABLE

Fossil fuels are formed from decomposing organic material (such as plant matter) that has fossilised over millions of years. This is where the name 'fossil' fuels comes from. When plants die, they will often retain a lot of carbon, especially if they die in low-oxygenated areas such as lakes and oceans where they do not break down so easily. Over time (tens of millions of years), dead plants will be buried deeper and deeper into the earth, where the pressure and heat turn the high-carbon plant matter into different fossil fuels (Figure 1).

Not only does this take hundreds of millions of years, but when we burn fossil fuels, we combine their carbon with oxygen and release CO_2 into the atmosphere. Burning fossil fuels at such a high rate has led to a wide range of negative impacts on the environment due in part to the high amount of CO_2 that is released. These impacts include things like biodiversity loss, alterations to ecosystems, and climate change.

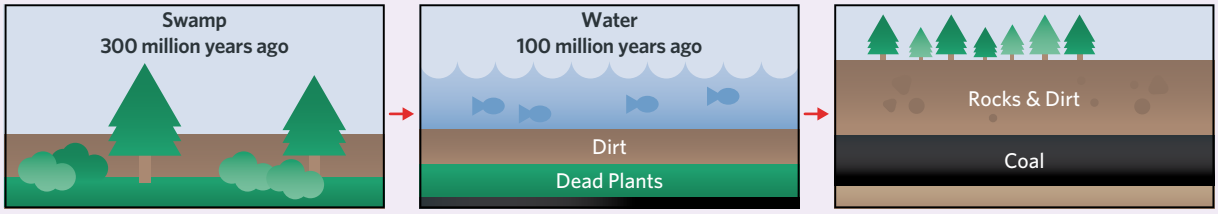


Figure 1 How coal is formed via the process of fossilised organic material

Because fossil fuels are non-renewable, scientists must search for alternative sources of energy that are more sustainable in the long term. One potential source of this alternative energy is **biofuel**, which is fuel that can be made from organic material such as plant matter and animal waste called **biomass**. There are a number of different types of biofuels, including bioethanol, biogas, and biodiesel. We will revisit some different types later in this lesson. For now, it is important to be aware that the biomass which is used to produce the biofuel can be sourced in large amounts from industries such as agriculture, food manufacturing, and forestry, making it readily available and easily replenished. In other words, rather than waiting for the organic material to fossilise, biomass can be harvested today and used in biofuel production tomorrow! Some examples of biomass include edible grains such as corn and sugarcane, and non-edible crops such as waste wood and waste paper.

Images (left to right): gan chaonan,nostal6ie/Shutterstock.com

Figure 2 We create biofuel using biomass, which can be **(a)** edible food crops such as sugarcane, or **(b)** non-edible crops such as waste wood from industry.

So how does this make biofuels **renewable**? The biomass that is used is renewable, given that we can continue sourcing plant material and animal by-products indefinitely, either by growing it ourselves or sourcing it from the action of other industries, such as leftovers from corn harvests, municipal waste, and straw from farms. Biofuels are also believed to have the potential to be better for the environment, given that they are typically **carbon neutral**. This is because the carbon dioxide that is released during combustion was originally captured by the plant during photosynthesis. The carbon, therefore, is cycled back into the atmosphere and can be used again as an input for other photosynthesising plants in the future. This means there is no net increase in the amount of CO_2 released into the atmosphere (Figure 3).

Lesson link

The VCAA includes a study design dot point on biofuels as a way to extend your knowledge of the cycles of photosynthesis and respiration to a real-world example, where fossil fuels are formed from photosynthesising plants, and this stored carbon is released back into the atmosphere when burned. It may be helpful to familiarise yourself with the inputs and outputs of both processes, and to reacquaint yourself with the carbon-cycle across ***chapter 5*** and ***chapter 6***.

biofuel fuel created from organic material known as biomass

biomass organic material, including plants, animal by-products, and biological waste material. Biomass can be sourced from many industries, including farming, forestry, and food manufacturing

renewable refers to a resource that can typically be replenished at the same (or faster) rate than it is being used, meaning it is unlikely to run out

carbon neutral a state in which there is no net release of carbon dioxide into the atmosphere, meaning that there is a balance between the amount of CO_2 that is emitted during combustion of a fuel and how much was originally absorbed during the formation process of that fuel

Table 1 A summary of the differences between fossil fuels and biofuels

	Fossil fuels	Biofuels
Sustainability	Non-renewable	Renewable
Source	Fossilised organic matter that has formed over millions of years	Modern crops, plant residue, organic waste, and animal by-products
Environmental impact	High carbon emissions	Largely carbon neutral

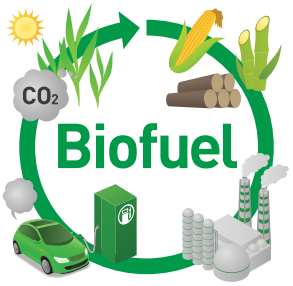


Image: metamorworks/Shutterstock.com

Figure 3 A simplified depiction of the carbon-cycle of biofuels. We can see that biomass is converted to biofuel (a process we will examine later in the lesson) before being used as fuel to power a car (combustion). This releases CO_2 back into the atmosphere which is then absorbed again by photosynthesising plants – and the process continues.

fermentation the anaerobic chemical breakdown of high-energy organic molecules, typically via the action of enzymes. For many plants, fermentation involves the conversion of glucose to ethanol and carbon dioxide

bioethanol a type of biofuel that is produced via the anaerobic fermentation of plants such as sugarcane or corn

How are biofuels made? 3.2.11.2

OVERVIEW

Biofuels are made from biomass via the process of fermentation. The typical steps for the production of bioethanol, a specific type of biofuel, involves the deconstruction of the original biomass, enzyme-catalysed hydrolysis of complex sugars, anaerobic fermentation to produce ethanol, and final distillation and purification of the ethanol for use as fuel.

THEORY DETAILS

As we now know, the purpose of biofuels is to replace traditional fuels with those made from plant material that is renewable. But how can we use plants to produce fuel that can power our cars and planes? Biofuels are generally made via the process of **fermentation**, which breaks down the starches and sugars in plants and converts that glucose into ethanol and carbon dioxide. It is this ethanol, a natural by-product of the fermentation process, which is harnessed and refined to produce much of the biofuel we need. While there are other ways of producing different types of biofuels, for the purposes of VCE Biology, we will be focusing on biofuel produced from ethanol fermentation (known as bioethanol), as this relates to what we learned regarding anaerobic fermentation in lesson 6B.

The process of creating bioethanol

The general process for turning biomass into **bioethanol** is as follows:

Deconstruction

The biomass is treated to help break it down to a point that increases its surface area enough to make the fermentation process more efficient. This is done by breaking down the cell wall and cellulose, and can occur via a range of different methods, including biological approaches such as enzyme breakdown, chemical approaches such as exposure to acids, physical approaches such as mashing and grinding, and/or physiochemical approaches such as heating.

Lesson link

In ***lesson 3A***, you learned about enzymes and the role they play in catalysing reactions. We know that enzymes lower the activation energy of different chemical reactions, and can break large molecules down via catabolic reactions.

Digestion by enzymes

The broken down biomass is then exposed to enzymes (such as amylase) which break down the starch and cellulose and convert them into glucose and other sugars. This breaking down of polysaccharides is aided by the presence of water in a process known as **hydrolysis**.

hydrolysis a chemical reaction in which water is used to break down the chemical bonds of a substance

Lesson link

In ***lesson 2A***, you learned about the structures and function of proteins, including enzymes and the building blocks of amino acids. You learned that monomers are smaller molecules that come together to form polymers. We also discussed what is known as a condensation reaction, where two monomers join to form a larger molecule and produce water as a by-product. Hydrolysis is essentially the opposite of this – water is actually used to help the enzymes break down the polymers into their monomers.

Ethanol fermentation

Yeast is used to facilitate the anaerobic fermentation of the sugars produced in step 2. Here, a large amount of ethanol is produced as a product of this fermentation. The ethanol diffuses out of the yeast cells and is harnessed for biofuel.

> ***Lesson link***
>
> In ***lesson 6B***, we covered anaerobic fermentation, which is a metabolic pathway that occurs in the absence of oxygen and converts pyruvate into either lactic acid in animals, or ethanol and CO_2 in yeasts. The latter pathway is being leveraged in this lesson, as ethanol is used as a biofuel, while the CO_2 is released back into the atmosphere.

Purification and dehydration

The ethanol is distilled via the removal of water, converting it into a usable form called biofuel. The biofuel is then purified and is ready to be used as liquid fuel.

> ***Lesson link***
>
> Over the course of Unit 3, we have considered the different factors that influence biochemical pathways. In ***lesson 3B***, we looked at the factors that influence enzyme activity, and in ***lesson 6C***, we looked at how those factors affect cellular respiration pathways. Given that the production of biofuels is a complex biochemical process involving enzymes and respiration, many of the factors discussed across these lessons directly influence the efficiency of bioethanol production. For example:
>
> - **Temperature**: we know that enzymes have an optimal temperature. In producing biofuels, the enzymes must be kept at the correct temperature to efficiently hydrolyse starches and polysaccharides, and break down glucose.
> - **Substrate**: we know that substrate levels must be high to supply enough reactants for the pathway. In producing biofuels, this means there needs to be high glucose concentration available to the yeast cells.
> - **Oxygen**: we know that fermentation occurs in the absence of oxygen. In producing biofuels, the yeast must be kept free of oxygen to be able to produce ethanol.

The uses and applications of biofuels 3.2.11.3

OVERVIEW

Biofuels can be used as an alternative to current fuels in many ways, including transport, heating, energy, and cleaning. While researchers continue to search for ways to make biofuels more efficient, there are a range of potential implications surrounding their use.

THEORY DETAILS

Types of biofuels

Now that we know what biofuels are, it is important to consider how they might be used. The first thing to be aware of is that there are different types of biofuels. The two main types of biofuels that are currently being produced from biomass are bioethanol and biodiesel. The difference between these two is simply the process by which they are made: bioethanol is derived from the fermentation of plant sugars, while biodiesel is produced via the formation of fatty acids from natural oils like animal fats and vegetable oil. In other words, they differ in the type of macromolecule used – where bioethanol is made from the fermentation of carbohydrates, biodiesel is made via the breakdown of lipids and fats.

Application of biofuels

Biofuels help us meet our transportation needs as they can be used as an alternative to traditional fuels like petrol and diesel, and can theoretically be burned in most combustion engines, including cars, trucks, and even planes. Bioethanol, for example, is blended with gasoline in order to cut down on carbon monoxide and other smog-causing emissions. One of the most common ethanol blends is the fuel E10, which contains 10% ethanol and 90% gasoline.

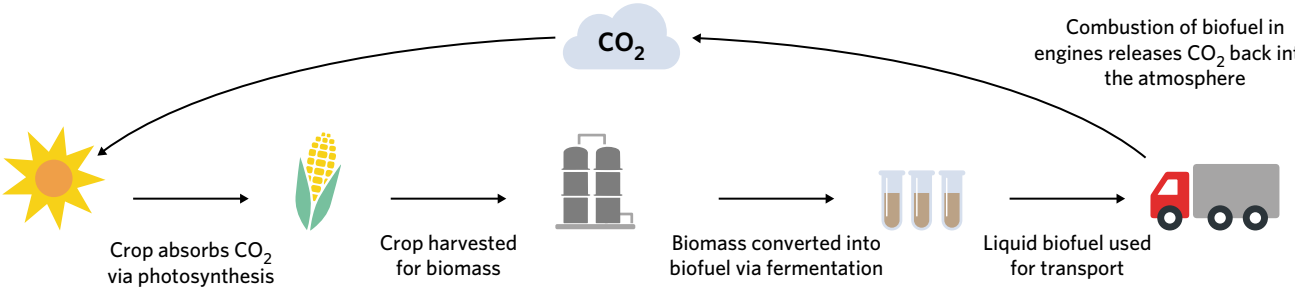


Figure 4 The conversion of biomass into biofuels for use in transport. This demonstrates the carbon neutral cycle of biofuels: during their combustion, the CO_2 produced is released back into the atmosphere for reabsorption by plants during photosynthesis.

Biofuels can also be stored and used for energy generation. In particular, biofuels are often used in backup power systems and generators, where emissions must be kept low while running. Such systems could help power schools, hospitals, and any number of community and residential facilities. As researchers develop more cost-effective and efficient ways to generate biofuels, their applications extend into areas such as cleaning and heating homes as well.

The implications of biofuels

The potential benefits of biofuels can be boiled down to two simple characteristics: the renewability of biomass as a fuel source, and the carbon neutrality of its combustion. Despite this, there are still many concerns that limit the uptake of biofuels and their penetration in the market. Table 2 considers some of the positive and negative implications of the biofuel industry as it stands today.

Table 2 Some of the strengths and weaknesses associated with the biofuel industry

Strengths	Weaknesses
Climate impact: substituting fossil fuels with biofuels may help to reduce carbon emissions and combat climate change, given that biofuels are carbon neutral.	**Food vs fuel:** on a large scale, using viable cropland for harvesting biomass may decrease necessary agricultural output and conflict with growing food demands.
Energy security: as our energy demands continue to increase, we need to consider alternatives to fossil fuels, which are non-renewable. Biofuels reduce our reliance on fossil fuels and could help provide ongoing energy given the relative ease of securing biomass in the long term.	**Cost and difficulty of uptake:** biofuels are typically more costly to produce than traditional fuel, and may not be compatible with all of our current vehicles and energy systems. What's more, the scale of the biofuel industry is small in relation to oil products, and the comparably lower price of oil makes it very difficult for biofuels to penetrate the market.
Localised energy: given that biomass can be sourced and farmed around the globe, biofuels reduce international reliance on the imports and exports of fossil fuels. This has the potential to decentralise control over fuel supplies, allow for community-based control over energy production, increase job opportunities, and reduce the risks associated with fossil fuels transport (such as oil spills).	**Second order environmental impacts**: while biofuels produce lower carbon emissions than traditional fossil fuels, they have been found to produce second order impacts on the environment, such as increased nitrous oxide emissions, deforestation, and a reduction of the genetic diversity of some crop species.

food vs fuel debate a central concern of large-scale biofuel manufacturing that questions the validity of using arable farmland to produce fuel, rather than food

first-generation biofuels biofuels produced from edible food crops such as corn or sugarcane. These compete directly with agricultural land

second-generation biofuels biofuels produced from non-edible crops such as agricultural and forestry residues and municipal waste. These typically compete less with agricultural land

Theory in context

FIRST-GENERATION VS SECOND-GENERATION BIOFUELS

In Table 2, we discuss the food vs fuel debate. In simple terms, using edible food crops such as corn or wheat to create biofuels is in direct conflict with the world's growing food demands. We call this type of biomass **first-generation biofuels**, as it is made from edible food crops which compete directly with agricultural lands and can lead to habitat loss for native species. To circumvent this, researchers have focused their energies on optimising the conversion of non-food crops such as wood waste and other by-products (which we call **second-generation biofuels**) from other industries (Figure 5). However, these crops are often harder to break down due to the presence of high amounts of cellulose and lignin, which often makes the production of biofuels more energy-intensive, requiring more downstream processing technologies, making the process less environmentally sustainable.

Table 3 A comparison of first-generation biofuels with second-generation biofuels

	First-generation biofuel	Second-generation biofuel
Competition with food crops	Yes – made from edible food crops	No – made from non-edible crop waste
Conversion to biofuels	Easy conversion	Difficult – harder to break down

Source: adapted from Abdullah et al. (2019).

Figure 5 A proportional breakdown of the commercial production of biofuel based on generation. One reason why such a high proportion of biofuel comes from agricultural wastes is due to concerns about competition between first-generation biofuels and arable lands.

Theory summary

Biofuel is produced from organic material known as biomass, which is relatively easy to source and can include edible crops like corn and sugarcane, as well as waste material such as wood. Biofuels offer a potential alternative to traditional fossil fuels, given that they are considered renewable and are more readily sourced.

One example of a type of biofuel is bioethanol, which is widely used in a blend with petrol. The process of creating bioethanol can be summarised as follows:

1 sourcing and deconstruction of the biomass
2 breaking down starch and cellulose into glucose molecules via enzymatic hydrolysis
3 ethanol production via anaerobic fermentation
4 dehydration and purification of bioethanol.

Turns out... maybe! Corn is an example of a first-generation biofuel currently being used to help power our transport sector and provide a more renewable and long-term alternative to traditional fossil fuels such as oil and petrol. While much more research is needed to help biofuels be more efficient and cost-effective, the space tourism industry is already looking to biofuel as a potential source of energy to power their rockets! As well as the larger bodies like NASA and SpaceX, many other commercial aerospace companies are already powering some of their rockets with bio-derived fuels (though these rockets are still small and infrequent).

6D QUESTIONS

Theory review questions

Question 1

Which of the following best summarises the relationship between anaerobic fermentation and bioethanol production?

A The anaerobic fermentation of glucose using a microorganism such as yeast produces ethanol which can be harnessed to produce bioethanol.

B The anaerobic fermentation of ethanol involves water hydrolysing carbon dioxide and creating glucose which can be harnessed to produce bioethanol.

Question 2

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- finite
- lower
- higher
- infinite
- ethanol
- biomass
- renewable
- fermentation
- non-renewable
- photosynthesis
- enzymatic hydrolysis

Assuming there is a constant supply of reactants, biofuels are considered _____. In contrast, fossil fuels are considered _____, given that they are used at a much _____ rate than nature can produce them. The _____ that is used to produce biofuels can be sourced from a variety of industries, such as agriculture and food manufacturing, and is typically broken down to produce _____ via the process of _____.

Question 3

Biofuels are often described as being carbon neutral. This is because

A the original biomass used to produce biofuels is inorganic and lacks carbon.

B the CO_2 that is released during the combustion of biofuels is absorbed during photosynthesis.

C the amount of CO_2 that is used as an input for fermentation is equal to the amount of output of photosynthesis.

Question 4

Which of the following provides an accurate representation of two implications of widespread biofuel adoption?

	Potential benefit	Potential challenge
A	Using farmland to produce biomass offsets the need to grow edible crops.	Biomass can be produced locally, leading to fewer international checks and balances.
B	Compared with fossil fuels, biomass represents a more secure energy reserve in the long term.	The production of biofuel comes with a range of potentially detrimental environmental impacts, such as land clearing.

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 5–8.

Indonesia's biofuel transition trajectory

Indonesia has one of the most ambitious fossil fuel-to-biofuel transition plans in the world. The country, which produces its biofuel from locally sourced palm oil, aims to increase its future production of biofuel exponentially and believes, should current projections remain the same, that demand for biofuel across the country could grow to as high as 190 million metric tons by the year 2050.

The transition program calls for the blending of palm oil-based biofuels with diesel oil in larger proportions, and aims to reach a point where all diesel sold within Indonesia is biodiesel. Currently, the diesel sold at pumps contains a 30% blend of palm oil-based biodiesel, and is expected to reach a 50:50 blend by 2025. Increasing production to a larger scale also reduces the cost to be closer in line with fossil fuels, given that the small size of the current biofuel industry makes the cost of palm-based fuel alternatives prohibitively expensive.

However, at the same time as this transition program, there is a real possibility that electric vehicles (EVs) could overtake the biofuel sector and render the program redundant. Not only is the EV market expanding rapidly, but this expansion could be especially true in Indonesia, which could become the world's largest producer of EVs due to its abundant reserves of nickel, a key component of lithium batteries that help power them. The Institute for Essential Services Reform (IESR), which is the same body that predicted the biofuel demand of 190 million metric tons by 2050, states that if the EV market share continues to increase at a similar rate, the biofuel demand might be closer to 90 million metric tons by 2050.

Question 5

According to the information in the text, the diesel alternative currently in use in Indonesia is

A a blend containing palm-oil based biofuel.

B an ethanol based biofuel created from the fermentation of fatty acids.

Question 6

What is the relationship between electric vehicles (EVs) and biofuel manufacturing in Indonesia?

A As the EV industry continues to expand in Indonesia, it challenges the relevancy of the country's biofuel expansion plans.

B EVs represent no challenge to the biofuel industry in Indonesia, given that the predicted demand for biofuels remains the same regardless of the growth of EVs.

Question 7

Which of the following options best summarises two corresponding implications for the expansion of the biofuel industry in Indonesia?

	Potential benefit	Potential challenge
A	As biofuel manufacturing increases, the price of palm-based biofuels will decrease and come in line with oils and gasoline produced from fossil fuels. This will make the uptake of biofuels less expensive.	Given that the future demand for palm-based biofuels is uncertain, increasing the biofuel production too aggressively could risk the manufacturing infrastructure becoming stranded assets amidst an industry more devoted to EV production.
B	As domestic biofuel manufacturing increases from locally sourced palm-based biodiesel, Indonesia's dependence on fossil fuel imports will increase. The program also creates jobs for Indonesians, who can grow and source the biomass using traditional fossil fuels to power their farms.	As Indonesia's reliance on fossil fuel imports lessens, the price of importing fossil fuels and other oil based alternatives, such as palm oil from other countries, will increase exponentially. At the same time, more biofuels means higher prices. This potentially prohibits the country from continuing its transition program of producing more fuel.

Question 8

The Indonesian government has identified biofuels as a cleaner alternative to fossil fuels, and acknowledged the necessity to adopt cleaner alternatives immediately.

Using a consequences-based approach to bioethics, how might the government come to a solution regarding the uncertainty of EVs and biofuels moving forward?

A The development of EVs and biofuel cannot co-exist given the prohibitive price of both, and therefore one must be prioritised over the other moving forward. This ensures that the price and output of whichever industry is chosen is as efficient as possible.

B The government could develop a more flexible biofuel transition program that allows for the switching between platforms such as biofuel and electricity production. This ensures that the two industries do not have to compete with each other and the manufacturing sector is as responsive as possible.

Exam-style questions

Within lesson

Question 9 (1 MARK)

Bioethanol is made from

A the conversion of sunlight into glucose.

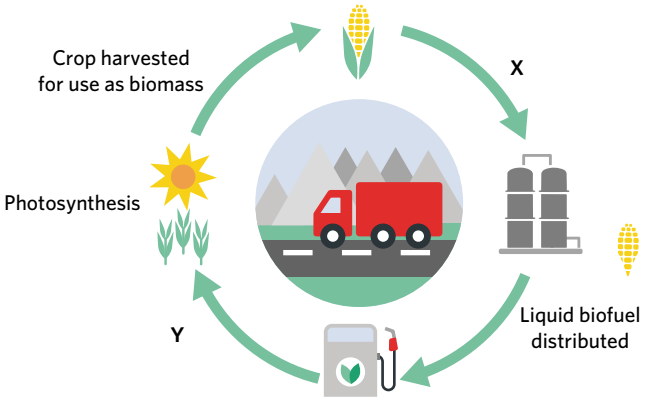
B the anaerobic fermentation of plant sugars.

C the formation of fatty acids from natural oils.

D the combustion of crude oils during transport.

Use the following information to answer Questions 10-12.

The following diagram depicts the carbon neutral cycle of biofuels, from production to use.



Question 10 (1 MARK)

The stage represented by the letter X is

A the harvesting of the crops for biomass.

B the conversion of biomass into biofuel via fermentation.

C the blending of natural oils with fossil fuels to produce liquid biofuel.

D the hydrolysis of biomass to convert carbon dioxide into usable glucose.

Question 11 (1 MARK)

The stage represented by the letter Y results in the

A cellular respiration of yeasts.

B enzymatic hydrolysis of glucose.

C release of CO_2 into the atmosphere.

D absorption of CO_2 through photosynthesis.

Question 12 (1 MARK)

What is the main role of photosynthesis in this pathway?

A Photosynthesis plays no direct role in this pathway.

B Photosynthesis produces the sugar needed for ethanol formation.

C Photosynthesis uses O_2 as an input and transforms H_2O into usable energy for transport.

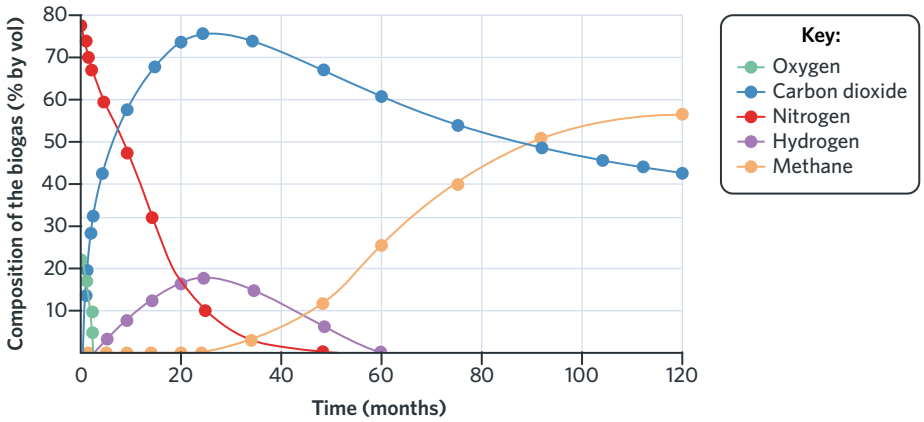
D Photosynthesis releases CO_2 back into the atmosphere following the combustion of biofuels.

Question 13 (5 MARKS)

Biogas is a type of biofuel that is produced via the anaerobic digestion of organic materials such as plant and animal products. This breakdown is facilitated by bacteria, and results in a biogas that is made up primarily (~60–70%) of combustible methane (CH_4) and (~30–40%) non-combustible carbon dioxide (CO_2), with very trace amounts of other gases, such as oxygen, nitrogen and hydrogen (<1%).

CH_4 has a reasonably high calorific value (referring to the amount of energy released when 1 kg of fuel is burnt). On average, the calorific value of biogas is 21.5 MJ/m^3, while that of traditional gas is 35.8 MJ/m^3. Ultimately, the higher the proportion of methane (above a certain point), the less heat is wasted during combustion, meaning the biogas produces energy more efficiently.

Consider the following graph, which depicts the different proportions of biogas components at different periods of time in a digester biogas system.



a State at which point in time the percentage composition of the biogas is highest in CO_2 volume. (1 MARK)

b Describe the role of the bacteria in the production of biogas. (1 MARK)

c With reference to your knowledge of biofuel production, state whether biogas production occurs in the presence of oxygen. (1 MARK)

d With reference to the data in the graph, explain whether the biogas will produce energy more efficiently at 60 months or 120 months. Justify your response. (2 MARKS)

Question 14 (6 MARKS)

Currently, it appears that photosynthetic microalgae represent the best candidate for use in the generation of biofuels long term. For example, some estimates suggest that microalgae could produce biodiesel 200 times more efficiently than traditional biomass crops. Some reasons for this include:

- Agricultural land is not needed for microalgae biomass production.
- Microalgae use light energy to convert CO_2 into organic compounds more efficiently than traditional land plants.
- Microalgae are widespread microorganisms that can be found in freshwater environments around the world.
- Harvesting microalgae is faster than land plants, given that it can be harvested anywhere from ten days to several hours after initial cultivation.
- Microalgae have a high concentration of lipids, which is a source of biodiesel, as well as a high concentration of proteins and carbohydrates, which are sources of bioethanol.

a Based on the information provided, suggest how microalgae could be used to satisfy the food vs fuel debate in biofuel manufacturing. (2 MARKS)

b The production of biofuels from microalgae biomass is complex, and can either involve lipid extraction for biodiesel production, or fermentation distillation for bioethanol production.

Outline the basic difference between the production of biodiesel and bioethanol. (2 MARKS)

c Biofuels from microalgae biomass are faster and easier to produce than traditional land plant biomass. What's more, it requires less high-quality agricultural land.

Assuming biofuel production continues to grow globally, how might the relative ease of microalgae biomass contribute to lowering international reliance on the import and export of fossil fuels? (2 MARKS)

Multiple lessons

Question 15 (4 MARKS)

One reason for the increasing interest of governments and regulatory bodies into the environmental benefits of biofuels has to do with the reputation of biofuels as being carbon neutral.

a State the chemical equation for photosynthesis. (1 MARK)

b State the worded equation for anaerobic fermentation in yeasts. (1 MARK)

c With reference to your understanding of the carbon cycle, explain what is meant by the carbon neutrality of biofuels. (2 MARKS)

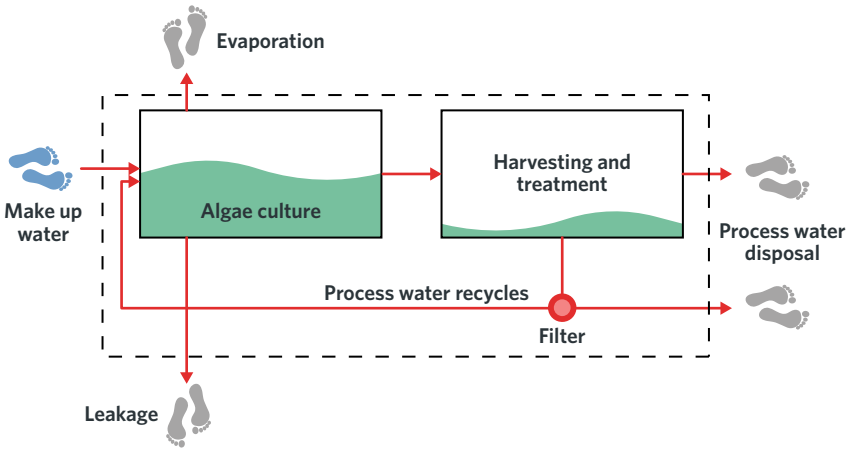
Key science skills and ethical understanding

Question 16 (6 MARKS)

As well as first and second-generation biofuels, researchers are looking into third and fourth-generation biofuels to increase efficiency and lessen the environmental impact of biofuel production. Many of these new methods are not yet commercialised, given that there is insufficient biomass production to make commercialisation feasible.

Fourth-generation biofuels (FGB) use genetically modified (GM) algae. While GM algae biofuel presents a potentially viable alternative to fossil fuels, one particular concern surrounds the water footprint (WF) involved in its production. This includes the level of water consumption involved in the cultivation of the algae, such as consumed rainwater and groundwater, as well as the safe disposal of hazardous water residue arising from the harvesting process, which may contain plasmid or chromosomal DNA that could lead to lateral gene transfer.

In an effort to understand the WF involved in the production of biofuels from GM algae, researchers developed the following model:



Adapted from Abdullah et al. (2019).

a A model is an example of a scientific methodology. Define the purpose of a model. (1 MARK)

b Suggest a potential aim of the researchers in creating this model. (1 MARK)

c Fourth-generation biofuels use genetically modified (GM) algae. Define what is meant by the term 'genetically modified organism'. (1 MARK)

d The researchers found that cultivating the algae in an open-pond resulted in a higher WF than contained systems with zero exposure to the external environment.

With reference to where water is being lost in the model, state one reason why open-pond cultivation might yield a higher WF than closed system cultivation. (1 MARK)

e The researchers found that around 3 700 kg of water was required to produce 1 kg of microalgal biodiesel. This is a ratio of 3 700:1. In contrast, a study in 2009 found that water is consumed at a rate of less than 10 liters for each liter of traditional gasoline produced (Wu et al., 2009).

Consider a hypothetical country facing water restrictions and drought. With reference to these findings, identify a potential bioethical issue for this country in regards to their domestic fuel production. In your answer, use a consequences-based approach to bioethics in order to justify the discontinuing of microalgal biodiesel production in this hypothetical country. (2 MARKS)

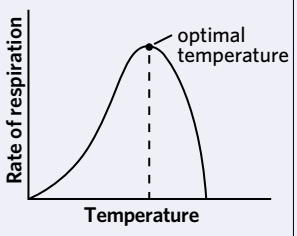
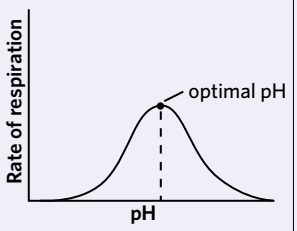
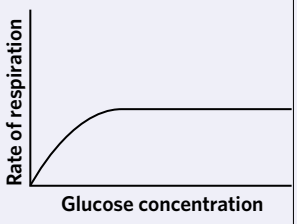
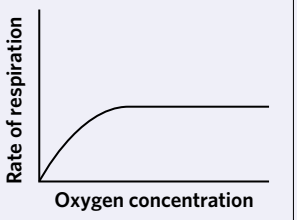
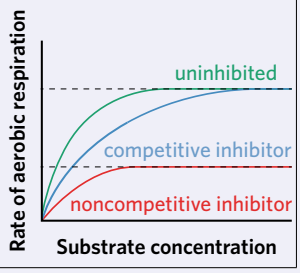
CHAPTER 6 SUMMARY

Comparing aerobic and anaerobic respiration

	Aerobic Respiration
Equations	**Worded equation:** Glucose + Oxygen → Carbon Dioxide + Water + Energy **Chemical equation:** $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + 36 or 38 ATP
Efficiency	36 or 38 ATP per glucose molecule
Locations	Mitochondria and cytosol
Stages	**1 Glycolysis – cytosol** **Inputs:** • 1 glucose ($C_6H_{12}O_6$) • 2 ADP + 2 P_i • 2 NAD^+ + 2 H^+ **Outputs:** • 2 pyruvate • 2 ATP • 2 NADH **2 Krebs cycle – mitochondrial matrix** **Inputs:** • 2 acetyl-CoA (from 2 pyruvate) • 2 ADP + 2 P_i • 6 NAD^+ + 6 H^+ • 2 FAD + 4 H^+ **Outputs:** • 4 CO_2 • 2 ATP • 6 NADH • 2 $FADH_2$ **3 Electron transport chain – mitochondrial cristae** **Inputs:** • 6 O_2 • 32 or 34 ADP + P_i • 10 NADH • 2 $FADH_2$ **Outputs:** • 6 H_2O • 32 or 34 ATP • 10 NAD^+ + 10 H^+ • 2 FAD + 4 H^+

	Anaerobic respiration
Equations	***In animals:*** **Worded equation:** Glucose → Lactic acid + Energy **Chemical equation:** $C_6H_{12}O_6 \rightarrow 2\ C_3H_6O_3$ + 2 ATP ***In plants and yeast:*** **Worded equation:** Glucose → Ethanol + Carbon dioxide + Energy **Chemical equation:** $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$ + 2 ATP
Efficiency	2 ATP per glucose molecule
Locations	Cytosol
Stages	**1 Glycolysis – cytosol** **Inputs:** • 1 glucose ($C_6H_{12}O_6$) • 2 ADP + 2 P_i • 2 NAD^+ + 2 H^+ **Outputs:** • 2 pyruvate • 2 ATP • 2 NADH **2 Further reactions (NADH recycling) – cytosol** **Inputs:** • pyruvate **Output:** • lactic acid (animals) OR • ethanol and CO_2 (plants and yeast)

Factors that affect the rate of respiration

Factor	Type of respiration affected	Graph of factor against reaction rate
Temperature	Both aerobic and anaerobic respiration	
pH	Both aerobic and anaerobic respiration	
Glucose availability	Both aerobic and anaerobic respiration	
Oxygen concentration	Aerobic respiration	
Enzyme inhibition	Both aerobic and anaerobic respiration	

Biofuels

Biofuels are made from biomass, which is plant and animal material that can be sourced from many of our existing industries.

Differences between fossil fuels and biofuels

	Fossil fuels	Biofuels
Sustainability	Non-renewable	Renewable
Source	Fossilised organic matter that has formed over millions of years	Modern crops, plant residue, organic waste, and animal by-products
Environmental impact	High carbon emissions	Largely carbon neutral

Bioethanol production process

1. sourcing and deconstruction of the biomass
2. breaking down starch and cellulose into glucose molecules via enzymatic hydrolysis
3. ethanol production via anaerobic fermentation
4. dehydration and purification of bioethanol

CHAPTER 6 SAC PRACTICE

SAC skills covered in this section:

✓ Case study analysis

DIFFERENT DIETS (21 MARKS)

Ketogenic diet

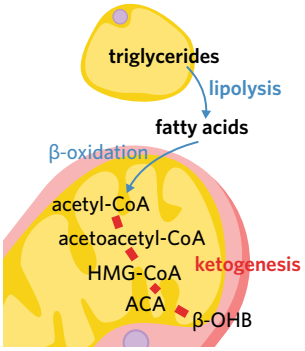
Nowadays, the ketogenic diet is widely accepted as an effective weight-loss method. The diet is high in fat, moderate in protein, and low in carbohydrates. Some studies have indicated that a ketogenic diet is more effective at boosting weight loss than a low-fat diet. It has been found that over a period of 3 months, a ketogenic (low-carb) diet helped participants lose three times as much weight compared to a low-fat diet.

How does a ketogenic diet enhance weight loss? When the glucose levels decrease due to restrictive carbohydrate intake, glucose molecules stored in the liver and muscles are released into the bloodstream to maintain necessary blood glucose levels. When the stored glucose is almost completely used up, the level of blood glucose will drop, subsequently causing insulin levels to decrease and glucagon levels to increase.

This stimulates the release of triglycerides and free fatty acids from stored adipose tissues into the bloodstream. The triglycerides are then broken down into glycerol and fatty acids in the following steps:

- glycerol is converted into one of the intermediate products of glycolysis to produce pyruvic acid which then enters the Krebs cycle.
- fatty acids, together with a large amount of additional fatty acids from the diet, are converted to a large amount of acetyl-CoA.

Normally, acetyl-CoA molecules also enter the Krebs cycle. However, when the level of fatty acids is too high and exceeds the capacity of the Krebs cycle, acetyl-CoA enters a process called ketogenesis, which is summarised in the diagram shown.



ACA, BHB, and acetone produced from ketogenesis will each be taken up by cells in the body and then converted back to acetyl-CoA via ketolysis. These converted acetyl-CoA molecules will be used to produce ATP. In other words, rather than using carbohydrates to produce energy, the body is forced to use its stored fats, meaning that weight will decrease over time.

1 What is a ketogenic diet? (1 MARK)

2 There are three main stages in the process of cellular respiration. Name each of the three stages. (1 MARK)

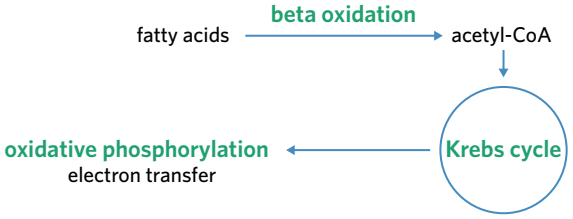
3 Which of the three stages rarely occurs in a ketogenic diet? In your answer, state where in a cell this stage typically occurs. (2 MARKS)

4 List the inputs and outputs of this stage. (2 MARKS)

5 Where does the process of ketogenesis occur? Name the products of this process and describe their role in providing energy to the body. (3 MARKS)

Fat as an energy source

What if you choose a low-carb and low-fat diet? Due to a low level of fatty acids present, acetyl-CoA molecules produced from these fatty acids will enter directly into the Krebs cycle, as opposed to undergoing ketogenesis. The process of cellular respiration where fat is used as an energy source is shown.

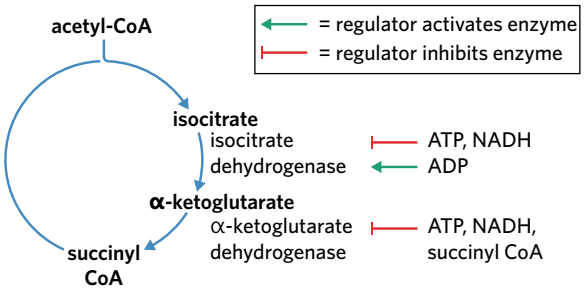


6 What condition will cause acetyl-CoA molecules to enter the Krebs cycle instead of undergoing ketogenesis? (1 MARK)

7 Where does the Krebs cycle take place? List the inputs and outputs of the Krebs cycle. (3 MARKS)

Regulation of the Krebs cycle

The rate of aerobic respiration can be affected by a number of factors, including the presence of enzyme inhibitors. In the Krebs cycle, an enzyme known as isocitrate dehydrogenase catalyses the production of α-ketoglutarate (a ketone) from isocitrate. ATP can inhibit the catalysing function of isocitrate dehydrogenase by binding to the allosteric site of the enzyme. NADH can also act as an enzyme inhibitor by directly displacing NAD^+, which is a substrate of isocitrate dehydrogenase. α-ketoglutarate dehydrogenase, which is the enzyme that catalyses the conversion of α-ketoglutarate to succinyl CoA, is inhibited by ATP, NADH, and succinyl CoA. The regulation of the Krebs cycle is important because if the cycle runs without being carefully controlled, a large amount of energy will be wasted due to the overproduction of ATP. Conversely, if the Krebs cycle runs too slowly, not enough ATP will be produced quickly enough to provide to cells in the body with the energy they need to function.



8 Explain the difference between a competitive and non-competitive enzyme inhibitor. (2 MARKS)

9 Determine whether ATP is a competitive or non-competitive inhibitor of the enzyme isocitrate dehydrogenase. Justify your answer. (2 MARKS)

10 Determine whether NADH is a competitive or non-competitive inhibitor of the enzyme isocitrate dehydrogenase. Justify your answer. (2 MARKS)

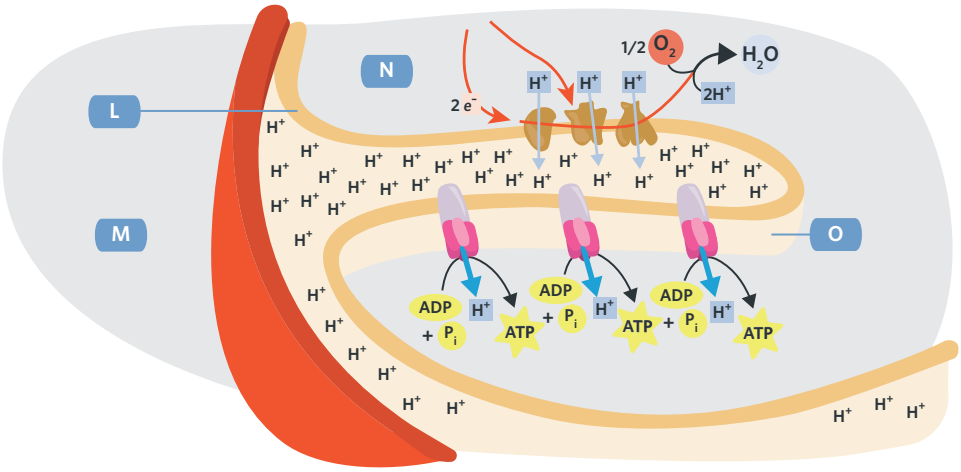
11 Describe the effect of increased levels of ATP, NADH, and succinyl CoA on the rate of the Krebs cycle. Explain your answer. (2 MARKS)

CHAPTER 6 EXAM PRACTICE

Section A (15 MARKS)

Question 1 (1 MARK)

The diagram shows a cross-section of a mitochondrion. The site of each stage of aerobic cellular respiration is labelled (L–O).



The sites of the stages in aerobic cellular respiration are

A L – glycolysis; O – Krebs cycle; N – electron transport chain.

B M – glycolysis; L – Krebs cycle; N – electron transport chain.

C N – glycolysis; M – Krebs cycle; O – electron transport chain.

D M – glycolysis; N – Krebs cycle; O – electron transport chain.

Adapted from VCAA 2018 Section A Q8

Question 2 (1 MARK)

Which of the following correctly lists the inputs and outputs of glycolysis in a plant cell?

	Inputs	Outputs
A	ADP, P_i, NAD^+, glucose	ATP, NADH, pyruvate
B	ADP, P_i, NAD^+, glucose	ATP, NADH, ethanol, carbon dioxide
C	ADP, P_i, NADH, water, glucose	ATP, NAD^+, H^+, oxygen
D	$NADP^+$, H^+, ADP, P_i, glucose	ATP, NADPH, pyruvate

Adapted from VCAA 2018 Section A Q9

Question 3 (1 MARK)

The graph shows the growth rate of four different strains of bacteria when exposed to varying concentrations of atmospheric oxygen.

Based on your knowledge and the information in the graph, which one of the following statements is true?

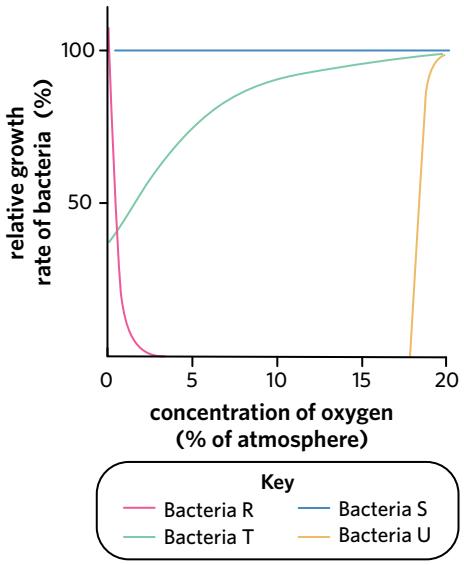
A Bacteria R are able to carry out both aerobic and anaerobic respiration.

B Bacteria T are only able to carry out anaerobic respiration.

C Bacteria U are only able to carry out aerobic respiration.

D Bacteria S can photosynthesise.

Adapted from VCAA 2017 Northern Hemisphere Exam Section A Q5



Use the following information to answer Questions 4 and 5.

The following is a three-dimensional diagram of a mitochondrion found in eukaryotic cells.

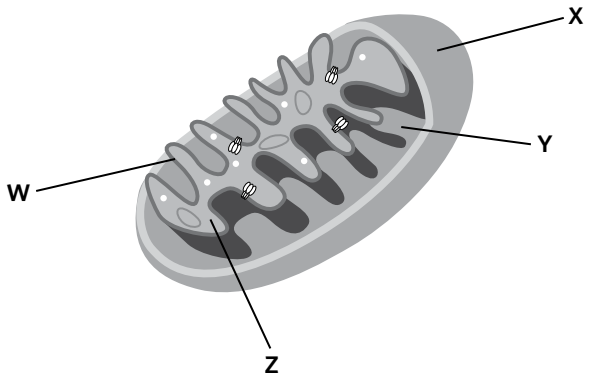


Image: Alila Medical Media/Shutterstock.com

Question 4 (1 MARK)

Which of the following structures represents the cristae?

A W

B X

C Y

D Z

Adapted from VCAA 2017 Section A Q9

Question 5 (1 MARK)

At structure Y

A NAD^+ is converted into NADH.

B there is a high concentration of protons.

C the majority of ATP is produced in the cell.

D pyruvate is broken down, releasing carbon dioxide.

Adapted from VCAA 2017 Section A Q10

Question 6 (1 MARK)

Cellular respiration is influenced by a number of different environmental factors. A factor that would increase the rate of cellular respiration would be

A an increase in water availability.

B an increase in glucose concentration.

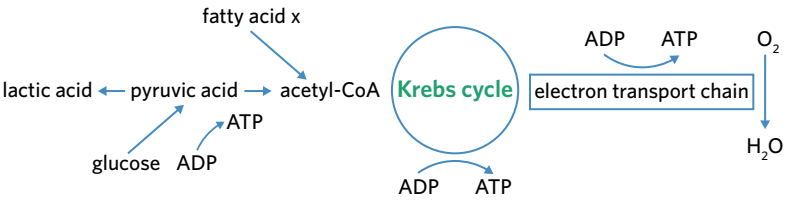
C a reduction in environmental oxygen levels.

D a decrease in the environmental temperature.

Question 7 (1 MARK)

If there is insufficient glucose for cellular respiration, fatty acids can be converted to acetyl-CoA.

The following diagram summarises the pathways for the breakdown of fatty acid X and glucose. Each fatty acid X molecule produces eight molecules of acetyl-CoA. Note that the number of molecules produced in each step is not shown.



Using your knowledge of cellular respiration, which one of the following conclusions can be made?

A Lactic acid is a product of aerobic cellular respiration.

B Fatty acid X is not converted into pyruvate molecules.

C The breakdown of glucose into pyruvate produces one ATP molecule.

D The electron transport chain produces 34 ATP molecules in organisms.

Adapted from VCAA 2014 Section A Q12

Use the following information to answer Questions 8 and 9.

In the laboratory, scientists can isolate animal cells in a test tube. These cells can be burst by submerging them in a hypertonic solution, allowing their cellular organelles to be extracted. In one experiment, mitochondria were collected using this method and were oxygenated. Different substances were added to the suspension and the change in oxygen concentration was recorded.

Question 8 (1 MARK)

Which of the following substances would cause a decrease in oxygen concentration?

A ATP

B glucose

C pyruvate

D NADH

Adapted from VCAA 2016 Section B Q4

Question 9 (1 MARK)

Which suspension would have the greatest increase in lactic acid?

A suspension of cytosol and mitochondria

B suspension of mitochondria

C suspension of cytosol only

D suspension of the nucleus

Adapted from VCAA 2016 Section B Q4

Question 10 (1 MARK)

In a laboratory, mammalian cells were incubated in an anaerobic environment and supplied with glucose-containing radioactive carbon atoms. After four hours, an analysis of the chemicals in and around the mammalian cells was undertaken. Which one of the following molecules would contain the radioactive carbon atoms after four hours?

A water
B ethanol
C lactic acid
D carbon dioxide

Adapted from VCAA 2016 Section A Q10

Question 11 (1 MARK)

Which of the following statements is true?

A An increase in pH will always increase the rate of aerobic respiration.
B Only competitive enzyme inhibitors affect the rate of cellular respiration.
C A decrease in oxygen concentration will decrease the rate of anaerobic respiration.
D The rate of cellular respiration is highest when the temperature is at the optimal temperature of the enzymes involved.

Question 12 (1 MARK)

Which one of the following is true of the electron transport chain?

A It is involved in both aerobic and anaerobic respiration.
B It requires oxygen, NADPH, and $FADH_2$ as inputs.
C It produces more ATP than glycolysis.
D It occurs in the cytosol of the cell.

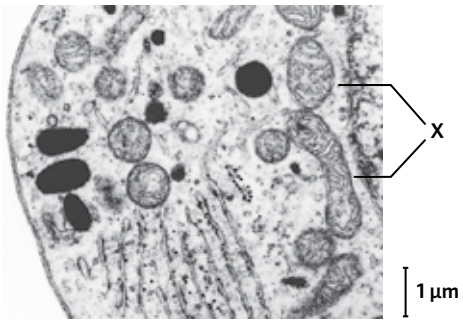
Question 13 (1 MARK)

The electron micrograph provided shows a portion of a cell.

In organelle X

A the cycling of NADPH occurs.
B lactic acid or ethanol is produced.
C the Krebs cycle occurs on the cristae.
D a proton gradient is coupled with ATP production.

Adapted from VCAA 2017 Sample Exam Section A Q11



Question 14 (1 MARK)

Which of the following correctly describes the order of one potential pathway for the production of bioethanol?

The following steps to produce bioethanol are written out of order.

W: Ethanol is produced from fermentation.

X: Ethanol is distilled via the removal of water.

Y: Biomass is broken down into smaller pieces with a higher SA:V ratio.

Z: Small pieces of biomass are broken down further via the presence of enzymes.

A Y, Z, W, X

B W, Z, X, Y

C X, Y, W, Z

D Y, Z, X, W

Question 15 (1 MARK)

Which of the following is false regarding biofuels?

A Biofuel combustion results in no net increase in CO_2.

B Biodiesel is made via the formation of fatty acids from natural oils.

C Biofuel production can occur in many places due to the availability of biomass.

D Bioethanol has more negative impacts on the environment than fossil fuels due to the increased release of greenhouse gases during the combustion process.

Section B (25 MARKS)

Question 16 (6 MARKS)

At high altitudes, the air gets 'thinner'. This means that the concentration of oxygen is reduced and less oxygen is available for the lungs. This causes a significant reduction in oxygen supply to the cells of the body.

a Identify a metabolic process that requires oxygen as an input to produce large amounts of energy. (1 MARK)

b With reference to the lack of oxygen, suggest how the pH of human cells may change at high altitudes. (2 MARKS)

c Ethan moved house and now lives at a higher altitude. In the process of relocating, he dug up one of his saplings and transplanted it into his new garden. Ethan noticed that the plant did not grow as well as it had at lower altitudes, and eventually it began to die. With reference to anaerobic respiration, explain how lower oxygen levels may have contributed to the death of the sapling. (3 MARKS)

Question 17 (6 MARKS)

Plant materials containing starch and other polysaccharides are broken down during digestion in order to produce glucose. Glucose can then be used by mammalian cells during anaerobic respiration.

a Why is anaerobic respiration important? (1 MARK)

Adapted from VCAA 2016 Section B Q2a

b What is/are the product/s of anaerobic respiration in mammalian cells? (1 MARK)

Adapted from VCAA 2016 Section B Q2b

c Temperature is a factor that affects the rate of cellular respiration. Describe and explain the relationship between temperature and the rate of respiration. (2 MARKS)

d State one similarity and one difference between aerobic and anaerobic cellular respiration. (2 MARKS)

Adapted from VCAA 2005 Exam 1 Section B Q3d

Question 18 (7 MARKS)

Oxygen is required for the process of aerobic cellular respiration.

a The three stages of aerobic respiration are listed in the table. Complete the table by naming the missing stage, location, and inputs and outputs of each stage of cellular respiration when normal oxygen levels are available. (3 MARKS)

Stage	Location	Inputs	Outputs
Glycolysis	**M**	ADP + P_i **N** $NAD^+ + H^+$	ATP Pyruvate NADH
L	Matrix	ADP + P_i $NAD^+ + H^+$ Acetyl-CoA FAD + 2 H^+	ATP NADH **P** $FADH_2$
Electron transport chain	Cristae	$FADH_2$ 32 or 34 ADP + P_i NADH **O**	FAD + 2 H^+ 32 or 34 ATP $NAD^+ + H^+$ **Q**

Adapted from VCAA 2017 Northern Hemisphere Exam Section B Q4a

b Cytochrome c oxidase is an essential enzyme in the electron transport chain. It is the last enzyme in the electron transport chain, and catalyses the transfer of electrons to oxygen. Hydrogen cyanide is a non-competitive inhibitor of this enzyme.

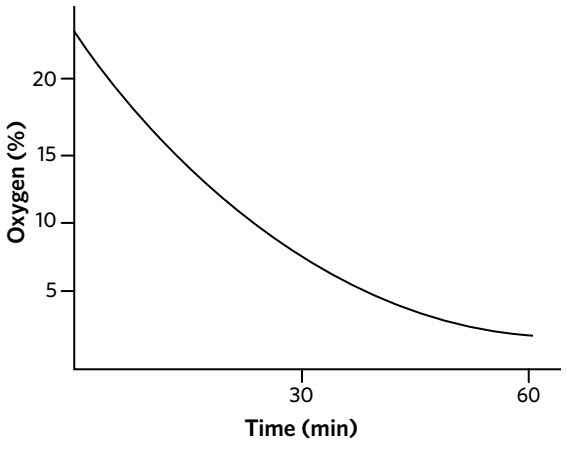
Outline the effect on the structure of the active site when hydrogen cyanide binds to the enzyme. (2 MARKS)

c Justify how this inhibitor could be lethal. (2 MARKS)

Adapted from VCAA 2017 Northern Hemisphere Exam Section B Q4

Question 19 (6 MARKS)

Yeast is a single-celled, microscopic fungus that can use sucrose as a food source. An experiment was carried out to investigate the rate of cellular respiration in a particular species of yeast. Yeast cells were placed in a container, a sucrose solution was added, and an airtight lid was placed on the container. The experiment was carried out at room temperature and the percentage of oxygen and ethanol in the container were recorded over a one-hour period. The change in oxygen percentage within the container is shown in the graph.



a State where ethanol is produced in a yeast cell. (1 MARK)

b Predict whether the ethanol concentration inside the airtight container would change within the time the experiment was carried out. Explain the reasoning behind your prediction. (2 MARKS)

Adapted from VCAA 2013 Exam 1 Section B Q1a

c The percentage of carbon dioxide was also monitored during the experiment. It was noted that there was virtually no carbon dioxide in the container at the start of the experiment. Redraw the curve for oxygen concentration in your own workbook. Then, draw the expected change in carbon dioxide concentration on the same set of axes. (2 MARKS)

Adapted from VCAA 2013 Section B Q1

d Identify the stage of aerobic cellular respiration that carbon dioxide is produced in and identify where in the yeast cell this occurs. (1 MARK)

UNIT 4

How does life change and respond to challenges?

In this unit, students consider the continual change and challenges to which life on Earth has been, and continues to be, subjected to. They study the human immune system and the interactions between its components to provide immunity to a specific pathogen. Students consider how the application of biological knowledge can be used to respond to bioethical issues and challenges related to disease.

Students consider how evolutionary biology is based on the accumulation of evidence over time. They investigate the impact of various change events on a population's gene pool and the biological consequences of changes in allele frequencies. Students examine the evidence for relatedness between species and change in life forms over time using evidence from paleontology, structural morphology, molecular homology, and comparative genomics. Students examine the evidence for structural trends in the human fossil record, recognising that interpretations can be contested, refined, or replaced when challenged by new evidence.

Students demonstrate and apply their knowledge of how life changes and responds to challenges through investigation of a selected case study, data analysis, and/or bioethical issue. Examples of investigation topics include, but are not limited to: deviant cell behaviour and links to disease; autoimmune diseases; allergic reactions; development of immunotherapy strategies; use and application of bacteriophage therapy; prevention and eradication of disease; vaccinations; bioprospecting for new medical treatments; trends, patterns, and evidence for evolutionary relationships; population and species changes over time in non-animal communities such as forests and microbiota; monitoring of gene pools for conservation planning; role of selective breeding programs in conservation of endangered species; or impact of new technologies on the study of evolutionary biology.

UNIT 4

AOS1

How do organisms respond to pathogens?

In this area of study, students focus on the immune response of organisms to specific pathogens. Students examine unique molecules called antigens and how they elicit an immune response, the nature of immunity, and the role of vaccinations in providing immunity. They explain how technological advances assist in managing immune system disorders and how immunotherapies can be applied to the treatment of other diseases. Students consider that in a globally connected world there are biological challenges that can be mediated by identification of pathogens, the prevention of spread and the development of treatments for diseases.

Outcome 1

On completion of this unit, the student should be able to analyse the immune response to specific antigens, compare the different ways that immunity may be acquired, and evaluate challenges and strategies in the treatment of disease.

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CHAPTER

7

Dealing with disease

Key knowledge

- initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens, and allergens
- physical, chemical, and microbiota barriers as preventative mechanisms of pathogenic infection in animals and plants
- the innate immune response including the steps in an inflammatory response and the characteristics and roles of macrophages, neutrophils, dendritic cells, eosinophils, natural killer cells, mast cells, complement proteins, and interferons
- the characteristics and roles of the components of the adaptive immune response against both extracellular and intracellular threats, including the actions of B lymphocytes and their antibodies, helper T, and cytotoxic T cells
- the role of the lymphatic system in the immune response as a transport network and the role of lymph nodes as sites for antigen recognition by T and B lymphocytes

7A DETECTING PATHOGENS

Meet Bruce the bacterium. He's a nasty strain of Staphylococcus aureus*, which commonly inhabits the surface of our skin. While Bruce is relatively harmless outside the body, if he enters our body, the immune system is immediately kicked into overdrive to help find and destroy him to prevent disease from occurring. But how does our immune system recognise Bruce? Is there something special on Bruce that allows our immune system to recognise him?*

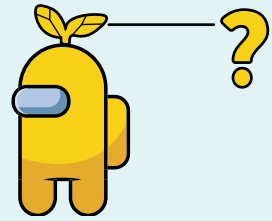


Image: Maybielater/Shutterstock.com

Lesson 7A

In this lesson you will learn how the body distinguishes between self and non-self molecules, as well as the types of pathogens our body fights.

◀ *Prerequisite knowledge*

Year 11
The characteristics of biological cells, including the differences between prokaryotic and eukaryotic cells, helps to identify different types of pathogens.

Chapter 2
Antigens can exist as proteins, which are coded for by DNA.

Future applications ▶

Chapter 7
The ability to differentiate between self and non-self antigens and identifying different types of pathogens is crucial for initiating an effective immune response against invading pathogens.

Lesson 8A
Identifying or isolating specific pathogenic antigens can be useful in the development of vaccines.

Lesson 8D
When the body fails to distinguish between self and non-self antigens, autoimmune diseases can develop.

Study design dot point

- initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens, and allergens

Key knowledge units

Self vs non-self	4.1.3.1
Types of pathogens	4.1.3.2

Self vs non-self 4.1.3.1

OVERVIEW

The immune system uses antigens to recognise if a cell or molecule is self or non-self. If it is identified as non-self, an immune response is initiated.

THEORY DETAILS

The immune system protects our body by scanning for and destroying **pathogens**. This is a classic case of 'easier said than done', as our immune system must recognise a vast variety of different pathogens while ensuring that it doesn't harm any of our own self-cells. When we consider that the average human body contains approximately 37.2 trillion cells, it begs the question – how does it do this? The answer – **antigens**.

pathogen an agent that causes disease

antigen any molecule that may trigger an immune response

Antigens are molecules that interact with the immune system. Depending on their source, antigens can exist as many different types of molecules, including proteins, sugars, and DNA or RNA. Additionally, antigens do not need to be attached to a pathogen or cell, but they can simply be free-floating molecules. Overall, there are two different types of antigens – self-antigens and **non-self antigens**.

non-self antigen a molecule from outside the body that is recognised by the immune system and initiates an immune response. Also known as a **foreign antigen**

Self-antigens

Self-antigens, which are located on the surface of cells, mark the cells of an organism as 'self' so that the immune system doesn't attack them. In humans, the most important self-antigens take the form of **major histocompatibility complex (MHC) proteins**, which can be divided into two different classes:

- MHC I proteins are expressed on all nucleated cells in the body. Therefore, virtually all cells in the human body except for those without a nucleus (e.g. red blood cells) express MHC I proteins.
- MHC II proteins are found on specialised cells of the immune system.

major histocompatibility complex (MHC) proteins a group of proteins present on the surface of all self-cells that enables the immune system to distinguish it from non-self material. Also known as **self-antigens**

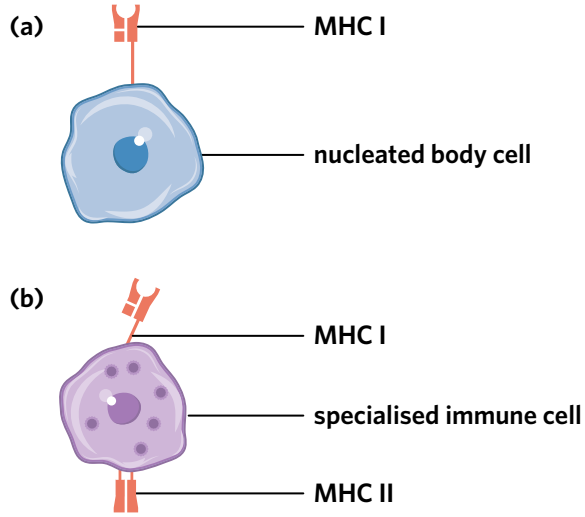


Figure 1 **(a)** A regular nucleated body cell with only MHC I proteins and **(b)** a specialised immune cell with both MHC I and MHC II proteins

> ***Lesson link***
>
> The specialised immune cells that express MHC II are known as antigen-presenting cells. These cells are explored in ***lesson 7C*** and the significance of MHC II in the activation of the adaptive immune system is explored in ***lesson 7D***.

Non-self antigens

Non-self antigens are antigens that the immune system reads as 'foreign' or not belonging to that individual. If a non-self antigen is recognised within the body, the immune system is activated and attempts to eliminate it. For example, if a pathogen such as a **bacterium** enters the body, the immune system will recognise specific bacterial proteins present on its surface as foreign and launch an attack in response. In this case, the bacterial proteins are serving as antigens.

Additionally, the MHC proteins found on our cells differ between individuals. For example, in an organ transplant, the MHC I proteins expressed on the donor organ will be different to the MHC I proteins of the organ receiver, which can stimulate the receiver's immune system to recognise the transplanted organ as non-self and launch an attack. Therefore, organ transplant recipients must routinely take immunosuppressants in order to prevent the immune system from attacking the donated organ.

> ***Lesson link***
>
> Antigens are an important component of triggering the adaptive immune response, interacting with receptors on B and T cells. This process is covered in ***lesson 7D***.

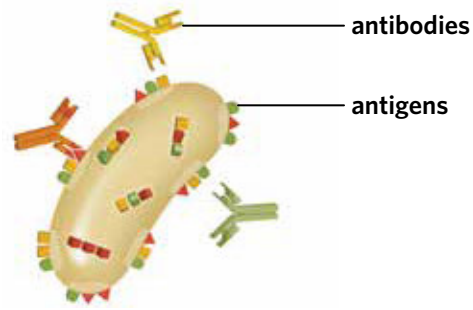


Image: Aldona Griskeviciene/Shutterstock.com

Figure 2 A bacterium with antigens on its surface. The antigens are interacting with antibodies as part of the adaptive immune response. You'll learn more about this in lesson 7D.

Malfunctions involving antigens

One type of malfunction involving antigens can occur when an error in the immune system results in the recognition of self-antigens as non-self. This results in the immune system attacking self-cells and is known as an **autoimmune disease**. Autoimmune diseases include rheumatoid arthritis and lupus, and are especially detrimental to our body due to the destruction of our own cells.

Another type of malfunction is allergies. Allergies involve an overreaction to the presence of an **allergen** – an important subcategory of antigens. Allergens are antigens that the immune system recognises as non-self and initiates a strong immune response towards. In actual fact, allergens aren't pathogenic and can't cause the body any harm. The immune response they generate, therefore, is unwarranted and is what we call an **allergic reaction** (Figure 3). Common types of allergens include pollen, dust, and peanuts. Mild symptoms of allergic reactions can include: an itchy rash, runny nose, sneezing, shortness of breath, and swelling. However, in more severe cases, constriction of airways, increased permeability of blood vessels, difficulty breathing, and decreased blood pressure may also be observed.

autoimmune disease a disease in which an individual's immune system initiates an immune response against their own cells

allergen a non-pathogenic antigen that triggers an allergic reaction

allergic reaction an overreaction of the immune system to a non-pathogenic antigen

Image: Africa Studio/Shutterstock.com

Figure 3 This unfortunate soul is allergic to dogs. Her immune system recognises some aspect of the dog's fur as a foreign antigen. In this instance, however, the fur is serving as an allergen, since it isn't actually harmful to the body.

Theory in context

RED BLOOD CELLS (PART 1)

Because red blood cells (RBCs) don't have MHC proteins on their surface to serve as self-antigens, they instead have different glycoproteins on their surface that label them as 'self'. These glycoproteins are the basis of how we categorise blood types.

- Type A individuals display the A antigen on the surface of their RBCs.
- Type B individuals display the B antigen on the surface of their RBCs.
- Type AB individuals display both the A and B antigens on the surface of their RBCs.
- Type O individuals display neither the A nor B antigens on the surface of their RBCs.

When giving a blood transfusion to a patient, we must be careful to match the blood type of the sample with their own blood type. If they are given blood with different antigens to their own, their immune system will recognise the different RBC antigen(s) as non-self and launch an attack against these cells. This means that someone with AB blood can safely accept all blood types due to the presence of both A and B antigens. Additionally, someone with type O blood can safely give blood to everyone due to the absence of A and B antigens. If the body does initiate an immune response, it can make the patient who received the blood extremely ill, and in some cases, die.

The second part of this theory in context is continued in lesson 7D.

	Type O	Type A	Type B	Type AB
Red blood cell type				
Antigens in red blood cells	None	Antigen A	Antigen B	Antigen A and Antigen B

Image: Designua/Shutterstock.com

Figure 4 Antigens present in each blood type

Lesson link

Autoimmune diseases are explored in more detail in ***lesson 8D***.

Types of pathogens 4.1.3.2

OVERVIEW

There are many different types of pathogens that can infect organisms and make them sick, including bacteria, fungi, worms, protozoa, viruses, and prions.

THEORY DETAILS

Pathogens come in all shapes and sizes – not to mention domains of life! Pathogens can be categorised as:

- **Cellular pathogens** have a cellular structure and are living organisms.
- **Non-cellular pathogens** do not have a cellular structure and are non-living.

cellular pathogen a pathogen that has a cellular structure and exhibits the processes of a living organism. Examples include bacteria, fungi, protozoa, and parasites

non-cellular pathogen a pathogen that neither has a cellular structure nor exhibits the processes of a living organism. Examples include viruses and prions

Whenever pathogens affect the normal functioning of our cells, we say that a disease is occuring. For example, some pathogens may produce toxins which cause the **lysis** of cells, some may inhibit protein or nucleic acid synthesis, and others may affect cellular respiration, preventing cells from producing energy. Table 1 summarises the key pathogens you need to know.

lysis the disintegration or rupturing of a cell

Examiners' tip

The VCAA often assess your knowledge of these pathogens, so it's important to understand what they are and general properties about them. For example, in the 2016 VCAA Exam Section A Q21, you were required to identify the type of pathogen that *Salmonella anatum* was based on information given in the scenario.

Table 1 Summary of key pathogen types

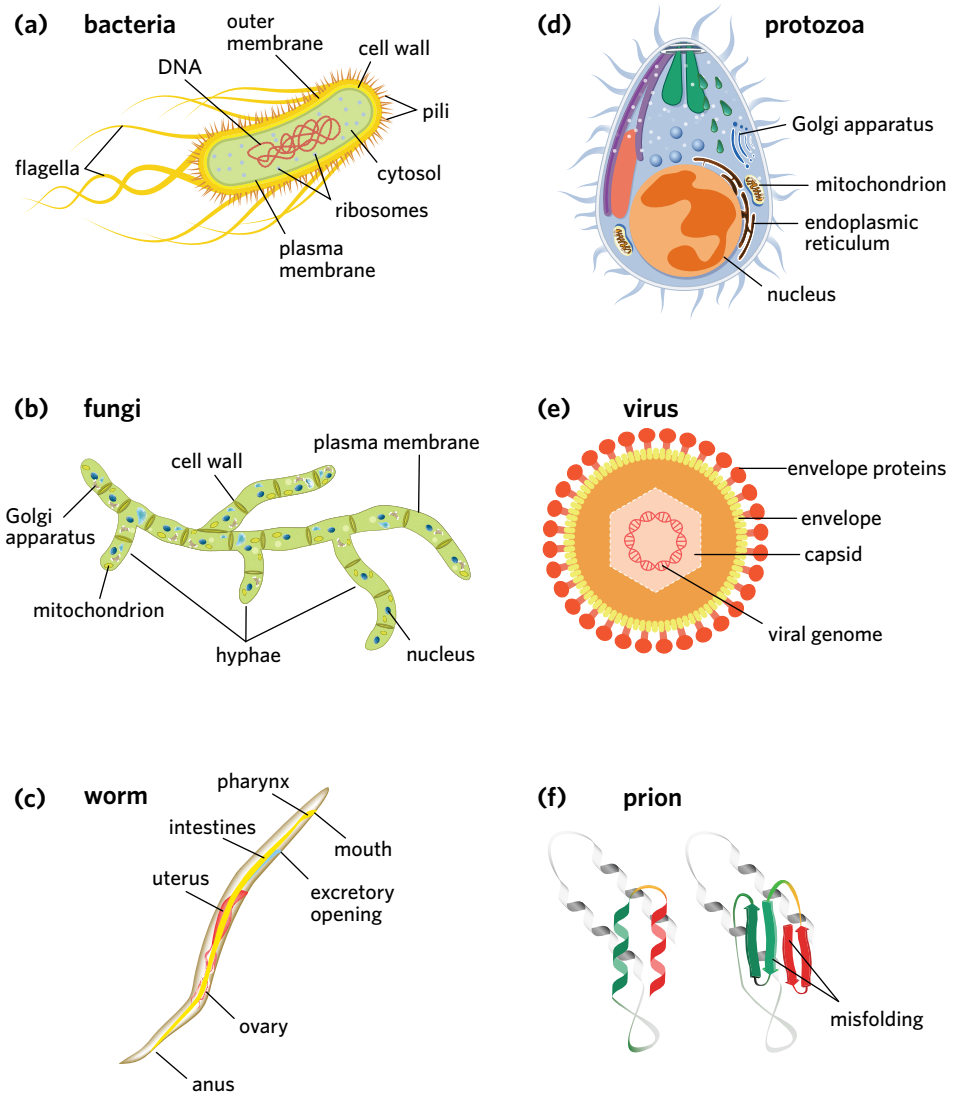
Pathogen	Description	Examples
Cellular pathogens		
Bacteria	Unicellular prokaryotes that can infect almost any part of the body. Bacteria can cause disease through the production of toxins and enzymes which either affect the functioning of cells or cause their death.	• *Neisseria meningitidis* causing meningitis • *Clostridium tetani* causing tetanus
Fungi	Eukaryotic organisms that include yeasts and moulds and contain long, branching filaments called **hyphae**.	• Thrush • *Trichophyton* spp. causing athlete's foot (Tinea pedis) • Ringworm (Tinea)
Worms	Multicellular invertebrate parasites whose development include egg, larval, and adult stages. Can vary in length, with the longest worms being over 55 m in length.	• **Parasite** (e.g. tapeworm) infection leading to malnutrition • Roundworm (Ascaris)
Protozoa	Single-celled eukaryotes that can be free-living or parasitic. Protozoa have many different mechanisms of action – for example, some can inhibit nucleic acid synthesis, protein synthesis, and various stages of cellular respiration.	• *Plasmodium* causing malaria
Non-cellular pathogens		
Viruses	An infectious agent composed of genetic material (DNA or RNA) inside a protein coat (capsid). In some instances the protein coat is surrounded by a lipid envelope. Viruses are not able to independently reproduce, instead they insert their genetic material into a host's cell and use the cell to replicate. Viruses can cause disease through the lysis of cells during viral replication, the formation of cancer by affecting gene expression, and the over-stimulation of the immune system leading to organ damage.	• Rhinovirus causing the common cold • Influenza causing the flu • Ebola virus causing ebola • SARS-CoV-2 causing COVID-19
Prions	Abnormally folded proteins that have the ability to induce normal proteins nearby to become misfolded. They only occur in mammals and affect only the brain and other neural structures. They are currently the only known infectious agents that don't contain nucleic acids.	• Creutzfeldt-Jakob disease • Bovine spongiform encephalopathy (also known as mad cow disease)

hyphae branching filaments of a fungus which help absorb nutrients from the environment

parasite an organism that lives in or on another organism, usually deriving nutrition from the host organism

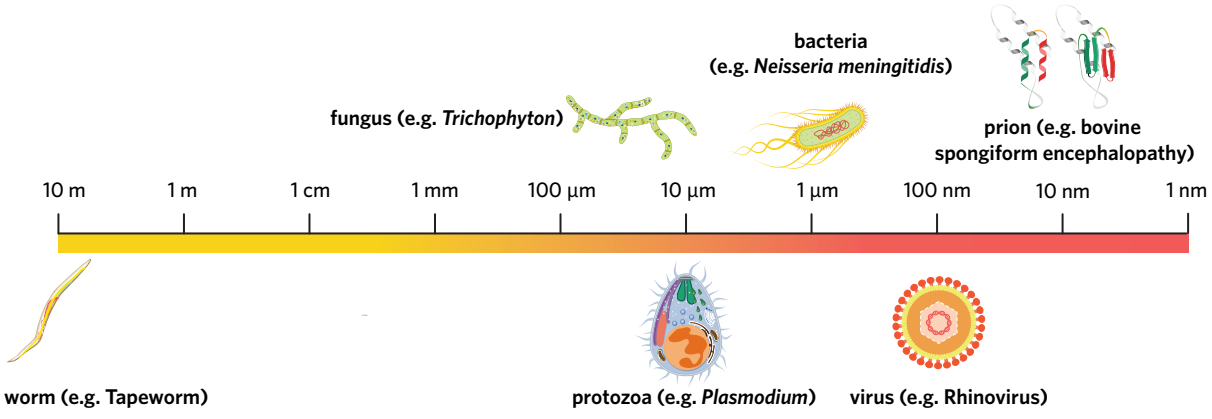
Lesson link

Pathogens can be transmitted through many different ways, such as by ticks and mosquitos. These organisms are called vectors, and will be explored in ***lesson 8C***.



Images: (a) VectorMine/Shutterstock.com (b) Designua/Shutterstock.com (c) Timonina/Shutterstock.com (d) Designua/Shutterstock.com (e) VectorMine/Shutterstock.com (f) Designua/Shutterstock.com

Figure 5 The various types of pathogens: **(a)** bacteria, **(b)** fungi, **(c)** worms *(Ascaris)*, **(d)** protozoa *(Plasmodium)*, **(e)** virus, and **(f)** prion. Note that in (f) the two coloured alpha-helices have become two beta-pleated sheets, a misfolding that can be transmitted to other nearby proteins.



Images: Timonina, Designua, Designua, VectorMine, VectorMine, Designua/Shutterstock.com

Figure 6 The relative sizes of different pathogens

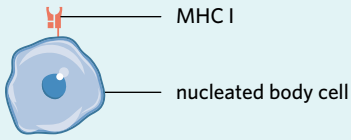
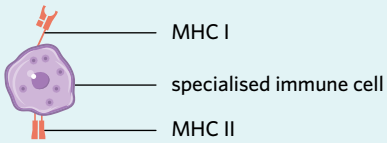
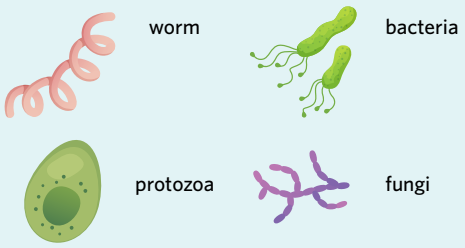
Theory summary

There is a large variety of pathogens that can cause disease and make us sick. Our bodies respond after recognising antigens on a pathogen's surface. Over the next few lessons, you'll be learning in detail about the variety of ways in which the immune system protects against the millions of pathogens that organisms are exposed to each day, and will come to appreciate just how fully sick the immune system is.

Lesson link

Lesson 8C details the ways in which modern medicine combats a number of the pathogens introduced in this lesson.

Table 2 Summary of self and non-self antigens

Identifying self	Identifying non-self
Self-antigens in humans are MHC proteins which mark the body's cells as self. These include: • Nucleated cells  • Specialised immune cells  	Pathogens containing non-self antigens are recognised as foreign by the immune system. These include: • Cellular pathogens  • Non-cellular pathogens  Image: Tartila/Shutterstock.com

Fortunately, the immune system is able to differentiate between self and non-self cells with the use of antigens. Therefore, as soon as the foreign antigens on Bruce's head are detected, the immune system can launch an attack and destroy him. Bruce was the imposter.

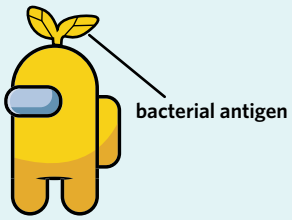


Image: Maybielater/Shutterstock.com

7A QUESTIONS

Theory review questions

Question 1

Pathogens

A are agents that cause disease.

B trigger the immune system via the recognition of self-antigens.

Question 2

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- autoimmune disease
- allergic reaction
- MHC proteins
- antigens
- non-self
- high
- low
- self

_____ are molecules that are recognised by the immune system as either foreign or self. In humans, self-antigens take the form of _____, which show a _____ degree of variability between individuals. If _____ antigens are detected, the immune system is activated and launches an attack in response. If the immune system mistakenly attacks self-cells, this is known as an _____.

Question 3

Categorise the following as **cellular** or **non-cellular**.

I protozoa _____
II bacteria _____
III viruses _____
IV worms _____
V prions _____
VI fungi _____

Question 4

Match the type of pathogen to its description.

Pathogen	Description
• bacterium	**I** _____ composed of genetic material (DNA or RNA) inside a protein coat (capsid)
• protozoa	**II** _____ single-celled eukaryote that can cause disease
• fungus	**III** _____ contain long branching filaments called hyphae
• prion	**IV** _____ a prokaryote which often produces toxins
• virus	**V** _____ abnormally folded proteins

Question 5

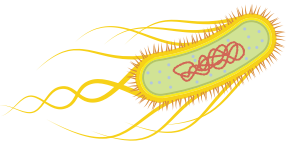
Match the type of pathogen to its corresponding image.

Pathogen

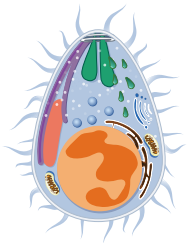
- bacterium
- protozoa
- fungus
- prion
- virus

Image

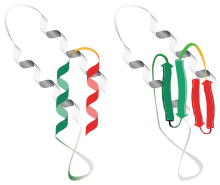
I



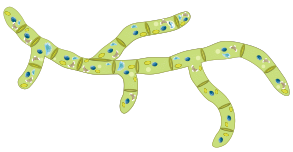
II



III



IV



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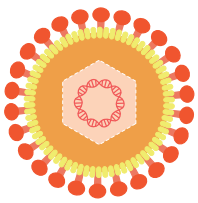


Images (top to bottom): VectorMine, Designua, Designua, Designua, VectorMine/Shutterstock.com

SAC skills questions

Data analysis

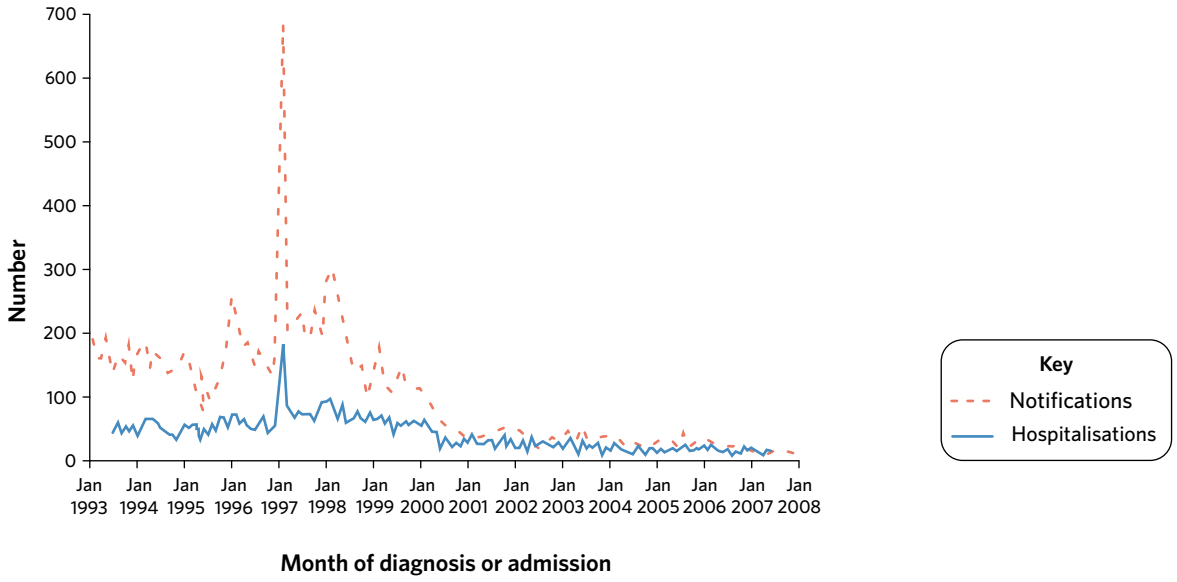
Use the following information to answer Questions 6–11.

Hepatitis A is a viral infection of the liver that causes symptoms such as fever, nausea, diarrhoea, stomach pain, and jaundice, which involves the yellow discolouration of the eyes and skin. In severe cases, acute liver failure can develop and a liver transplantation is required. Transmission of hepatitis A occurs through either bodily fluids or the faecal-oral route, where individuals come into contact with human sewage or excrement particles.

In 1997, a sudden increase in the presence of locally-acquired viral hepatitis A in New South Wales prompted an investigation by the NSW Health Department. When outbreaks occur, investigators begin by defining and identifying cases. Information crucial to locating the source of the infection include where the individual has been and the time when symptoms first presented themselves. In doing so, investigators can calculate the time of first infection by factoring in the incubation period, during which the virus is latent and does not cause any symptoms.

After gathering all the relevant information, investigators identified a pattern in the 467 cases of hepatitis A in 1997 and formed a hypothesis – they had all consumed oysters cultivated in Wallis Lake, NSW. After extensive testing, it was determined that the lake had been contaminated with human faecal pollution, thereby confirming their hypothesis.

The following graph depicts hepatitis A notifications and hospitalisations in Australia from 1993 to 2007.



Source: adapted from the Australian Department of Health 2010

Question 6

Infected individuals who consumed oysters from Wallis Lake all

A require a liver transplantation due to infection with hepatitis A.

B ingested human faecal matter containing hepatitis A.

Question 7

Surface proteins of hepatitis A can be detected by the immune system as

A non-self antigens.

B MHC proteins.

C self-antigens.

D allergens.

Question 8

Hepatitis A is considered to be a

A non-living, non-cellular pathogen.
B living, non-cellular pathogen.
C non-living, cellular pathogen.
D living, cellular pathogen.

Question 9

The structure of hepatitis A is comprised of

A a tough cell wall and a flagella for locomotion.
B genetic material enclosed within a capsid.
C branching filaments called hyphae.
D abnormally folded proteins.

Question 10

Based on the graph, in January of 1996, there were

A 300–400 hospitalisations.
B 100–200 hospitalisations.
C 200–300 notifications.
D 0–100 notifications.

Question 11

Based on the graph, if notifications associated with the Wallis Lake outbreak were excluded, there would be

A 100–200 notifications in 1997.
B 200–300 notifications in 1997.
C 300–400 notifications in 1997.
D 600–700 notifications in 1997.

Exam-style questions

Within lesson

Question 12 (1 MARK)

When making vaccines, scientists search for a molecule that is unique to a pathogen and stimulates the immune system.

Based on this information, which type of molecule could scientists use?

A an antigen
B an allergen
C a self-molecule
D an MHC protein

Adapted from VCAA 2016 Section A Q24

Question 13 (1 MARK)

Which one of the following is considered 'non-self' in humans?

A cells lining the oesophagus
B a red blood cell
C an allergen
D a neuron

Adapted from VCAA 2014 Section A Q14

Question 14 (1 MARK)

Which one of the following is considered 'self' in humans?

A liver from a transplant
B bacterial colonies in the gut
C lung cells obtained in a tissue sample
D viral particles circulating in the bloodstream

Adapted from VCAA 2014 Section A Q14

Question 15 (1 MARK)

In the future, scientists aim to grow full-size kidneys for transplantation use in patients with kidney disease using the patient's own skin cells. This would overcome the problem of rejection of the transplanted kidney by the immune system.

Which of the following is responsible for causing organ rejection?

A pathogens from the donor's kidney
B the MHC proteins on the donor's kidney
C the MHC proteins on the receiver's kidney
D self-antigens from the receiver's body not recognising the donor kidney cells

Adapted from VCAA 2014 Section A Q19

Question 16 (1 MARK)

Which one of the following statements is correct?

A Prions are eukaryotic cells that contain membrane-bound organelles.
B Prions insert their genetic material into a host cell.
C Prions do not contain genetic material.
D Prions are cellular pathogens.

Question 17 (1 MARK)

Which one of the following statements about allergens is correct?

A All antigens are allergens.
B Allergens are antigens that cause an allergic reaction.
C Allergens are pathogenic because they can cause disease.
D Allergens involve an error where the immune system cannot distinguish between self and non-self.

Use the following information to answer Questions 18 and 19.

Cavendish bananas are predominantly grown in Queensland, where the tropical conditions allow for banana trees to flourish. However, because all bananas are genetically identical, pathogens that threaten their survivability are a great concern to farmers. One significant disease is Panama Disease (Tropical Race 4), caused by the fungus *Fusarium oxysporum*, which leads to the wilting and death of banana trees.

Question 18 (1 MARK)

The structure of the *Fusarium oxysporum* pathogen would consist of

A a lipid envelope.
B abnormally folded proteins.
C MHC proteins on its surface.
D cells containing membrane-bound organelles.

Question 19 (1 MARK)

Which one of the following could be an antigen originating from *Fusarium oxysporum*?

A a toxin

B a larva

C a capsid protein

D a bacterial ribosome

Adapted from VCAA 2017 Northern Hemisphere Exam Section A Q16

Question 20 (3 MARKS)

Haemolytic disease of the newborn (HDN) can occur if a Rhesus-negative mother is pregnant with a Rhesus-positive foetus. A person who is Rhesus-positive has certain proteins on the surface of their red blood cells whereas a person who is Rhesus-negative does not have these proteins. During pregnancy and birth some foetal blood cells may enter the mother's bloodstream, causing an immune response in her body.

a Identify and explain the role that Rhesus proteins play in the mother's immune response. (2 MARKS)

b Do foetal red blood cells serve as pathogens in the mother's body? Justify your response. (1 MARK)

Adapted from VCAA 2014 Section B Q5

Multiple lessons

Question 21 (10 MARKS)

In organ transplants, MHC proteins must be matched as closely as possible to increase the chances of a successful transplant. Following a transplant, immunosuppressants are also used to prevent the chances of organ rejection. However, the use of immunosuppressants can leave individuals vulnerable to infection.

a Define the term 'disease'. (1 MARK)

b Major histocompatibility complexes are a type of protein embedded in cell membranes. Explain how a single gene can produce many different proteins. (1 MARK)

c Due to the risk of infection, individuals using immunosuppressants often take antibiotics to help prevent the occurrence of bacterial infections.

i Rifampicin is a type of antibiotic which inhibits bacterial RNA polymerase. Identify and outline the process which rifampicin inhibits. (4 MARKS)

ii Clarithromycin and doxycycline are two types of antibiotics which target bacterial ribosomes. Identify and outline the process inhibited by these antibiotics. (4 MARKS)

Key science skills and ethical understanding

Question 22 (5 MARKS)

Sharon wanted to investigate the effect of increasing the concentration of a medication against the growth of an unknown pathogen. For her experiment, she prepared five different concentrations of the medication (5%, 10%, 15%, 20%, and 25%) which is known to kill pathogens by interfering with the production of their cell wall. Five agar plates containing each concentration of medication and the pathogen sample were set up and left for a week.

From her experiment, Sharon found that the medication had influenced the growth of the pathogen. Plates that had higher concentrations of the medication had lower rates of pathogenic growth.

a Based on the information provided, which two kinds of pathogens could Sharon be using in her experiment? (1 MARK)

b Identify a possible control for Sharon's experiment. (1 MARK)

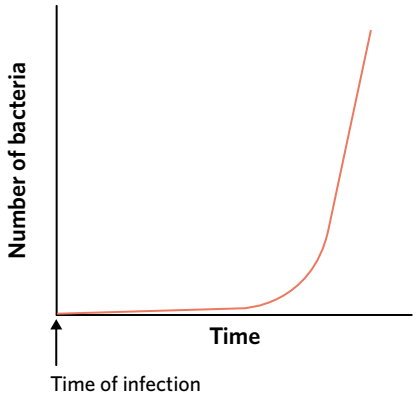
c Explain why having a control is important in this experiment. (2 MARKS)

d While observing her results, Sharon discovered another pathogen growing on the agar. Suggest where this pathogen may have originated from. (1 MARK)

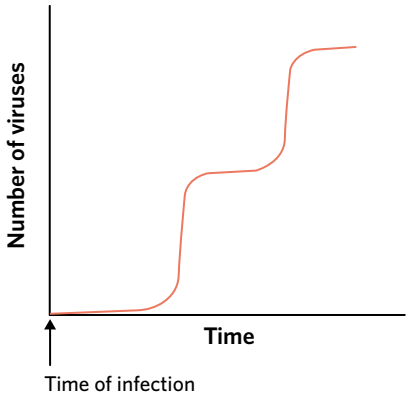
Question 23 (5 MARKS)

There are many differences between the replication methods of bacteria and viruses. Bacteria are considered to be extracellular pathogens, where they are free to replicate outside of cells. Conversely, viruses are intracellular pathogens which must use host cells to replicate. One of the many effects of the accumulation of viruses inside a cell include the weakening of the cell's cytoskeleton, which can lead to the bursting of the cell.

Graph X



Graph Y



a Provided that the virus is composed of RNA, suggest how viral proteins may be produced within the infected cell. (1 MARK)

b Explain the difference between the patterns of growth of bacteria and viruses after infection. (2 MARKS)

c When creating vaccines, deactivated viral particles are often used. However, in the research and development of these vaccines, there are many risks. Describe how the bioethical concept of non-maleficence may influence researchers. (2 MARKS)

Adapted from VCAA 2004 Exam 1 Section B Q8

7B THE FIRST LINE OF DEFENCE

Bruce, our local resident bacteria also known as Staphylococcus aureus*, is an extremely common strain of bacteria that lives all over our skin. Therefore, we must take a series of precautions to ensure that he never enters our body and most certainly does not enter our bloodstream, as if he did he could rapidly travel around our body and cause disease. Do we have any specialised structures which help prevent Bruce from entering? What are some of these precautions our body has?*

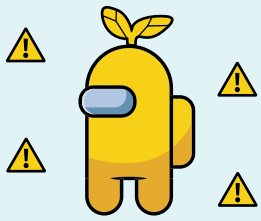


Image: Maybielater/Shutterstock.com

Lesson 7B

In this lesson you will learn about the components of the first line of defence in animals and plants.

◀ *Prerequisite knowledge*

Chapter 3
Many immune defences rely on the presence of enzymes that combat pathogens. The enzymes found within pathogens can also be affected by the internal environment of an organism, reducing that pathogen's ability to cause disease.

Lesson 7A
Pathogens are causative agents of disease and first encounter the immune system at the first line of defence, which provides a non-specific and immediate response.

Future applications ▶

Lesson 7C
The second line of defence is another component of the innate immune system that is activated when pathogens break through the barriers of the first line of defence.

Study design dot point

- physical, chemical, and microbiota barriers as preventative mechanisms of pathogenic infection in animals and plants

Key knowledge units

Introduction to the innate immune system	4.1.1.1
Barriers in plants	4.1.1.2
Barriers in animals	4.1.1.3

Introduction to the innate immune system 4.1.1.1

OVERVIEW

The first line of defence is a component of the innate immune system, providing physical barriers, chemical barriers, and microbiological barriers to prevent pathogenic invasion.

THEORY DETAILS

The **innate immune system** is composed of two different defences known as the **first** and **second lines of defences**. Both of these mechanisms involve a **non-specific** response to foreign antigens, responding the same way regardless of the type of pathogen or antigen present. Additionally, another characteristic of these two mechanisms is that they respond to injury and antigens extremely quickly – within minutes to hours they already begin to limit the spread of infection and stimulate local changes at the site of injury. In this lesson, we will explore the first line of defence in plants and animals, continuing onto the second line of defence in lesson 7C.

innate immune system a component of the immune system that is composed of generalised and non-specific defences and/or responses to pathogens. Also known as the **non-specific immune system**.

first line of defence a component of the innate immune system characterised by the presence of physical, chemical, and microbiological barriers to keep pathogens out of the host organism

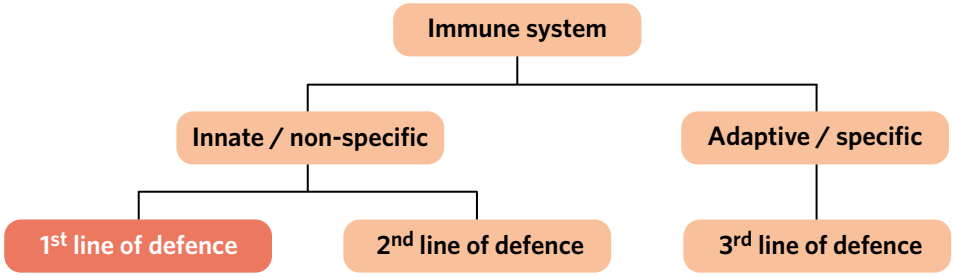


Figure 1 Breakdown of the immune system

second line of defence a component of the innate immune system characterised by the non-specific response to injury and/or pathogens by a variety of cells and molecules

non-specific describes a component of the immune system that responds the same way to all pathogens

Barriers in plants 4.1.1.2

OVERVIEW

Plants have a number of first line defences against pathogens, including physical and chemical barriers.

THEORY DETAILS

Plants need to protect themselves from the types of pathogens identified in lesson 7A and herbivory (being eaten by herbivores), but cannot move or run away like we can. Plants also don't have the more advanced forms of immunity that animals do. For them, the best way to avoid becoming infected is to prevent pathogens from entering in the first place. This is where the first line of defence comes into play!

There are two types of barriers present in the first line of defence of plants – **physical** and **chemical** barriers. Key components of these barriers are summarised in Table 1.

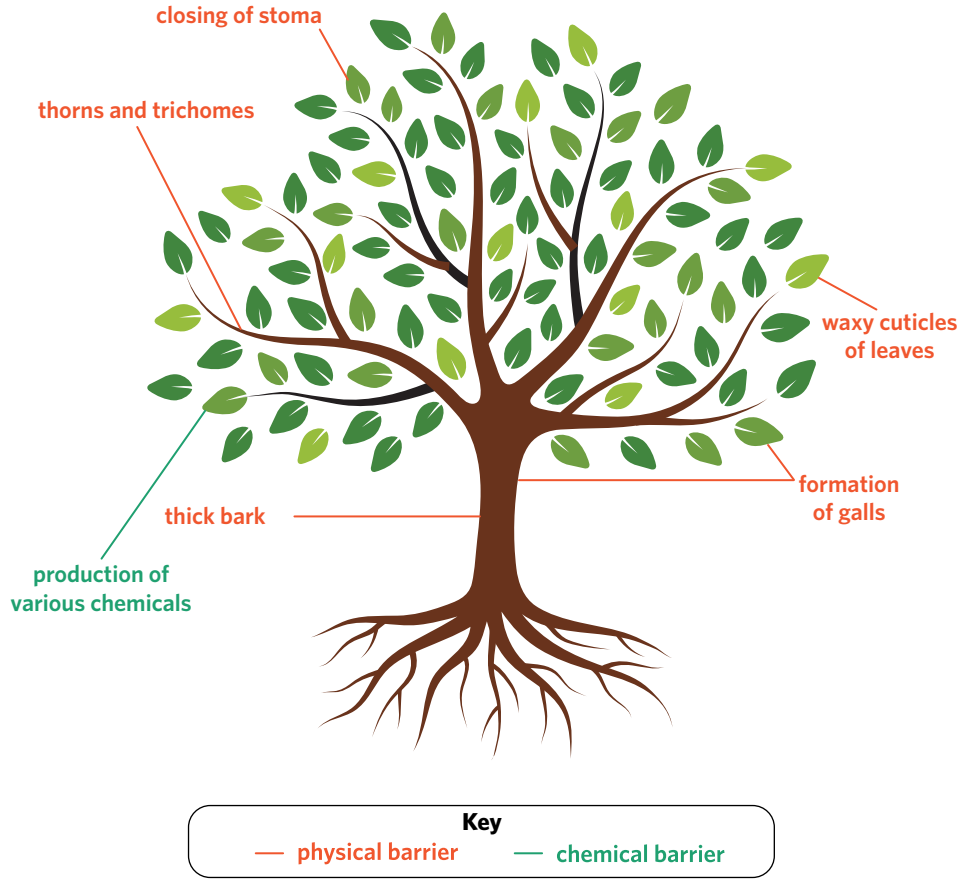


Image: Alazur/Shutterstock.com

Figure 2 Barriers present in plants

physical barrier a component of the first line of defence that features solid or fluid obstacles that block pathogen entry such as skin or mucus

chemical barrier a component of the first line of defence that features the use of enzymes, toxins, and acids to protect against pathogen invasion

cuticle a waxy protective film covering the surface of a plant leaf

gall an abnormal outgrowth of tissue in plants designed to limit the spread of an invading pathogen

trichomes small hairs on the surface of plants used to deter pathogens and/or insects

stoma (pl. stomata) a small pore on the leaf's surface that opens and closes to regulate gas exchange

Table 1 First line of defence in plants

Barrier type	Description	Examples
Physical	Barriers that prevent pathogens from physically entering the organism	• Thick bark • Waxy **cuticles** of leaves • Formation of **galls** to prevent the spread of infection • Presence of thorns and **trichomes** to deter insects and grazers • Closing of **stomata** to prevent pathogen invasion during carbon dioxide uptake
Chemical	Barriers that involve the production of chemicals (e.g. toxins) which are harmful to the pathogen and/or enzymes that affect the functioning or development of the pathogen. Some chemicals can also act to repel insects or animals that may damage the plant	• Chitinases – enzymes that occur in a number of different plants and have antifungal properties • Phenols – secreted by wounded plants, repelling or killing invading microorganisms • Defensins – small peptides that are toxic to microbes and fungi • Saponins – disrupt the cell membranes of various fungi • Oxalic acid – a substance that can be toxic if ingested • Glucanases – defend plants against fungi

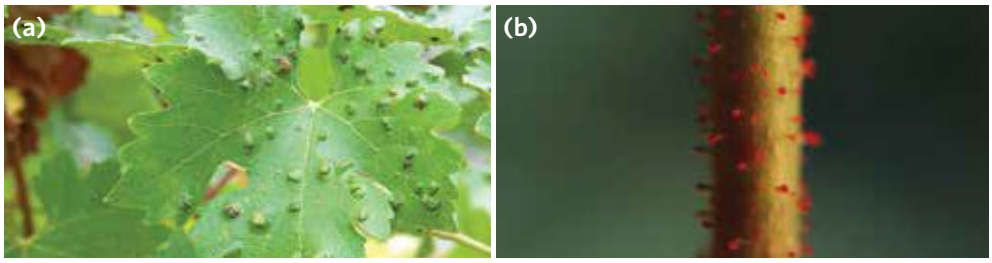


Image: Sheila Fitzgerald/Shutterstock.com

Figure 3 **(a)** Galls on a grape leaf and **(b)** trichomes on the stem of a rose

Barriers in animals 4.1.1.3

OVERVIEW

Animals have a number of first line defences against pathogens, including physical, chemical, and microbiological barriers.

THEORY DETAILS

Just like plants, animals employ a large range of first line defences against pathogens. However, not only do they have physical and chemical barriers, but they also have **microbiological barriers**. Key components of these barriers are summarised in Table 2.

microbiological barrier a component of the first line of defence in which the presence of normal flora limits the growth of pathogenic bacteria

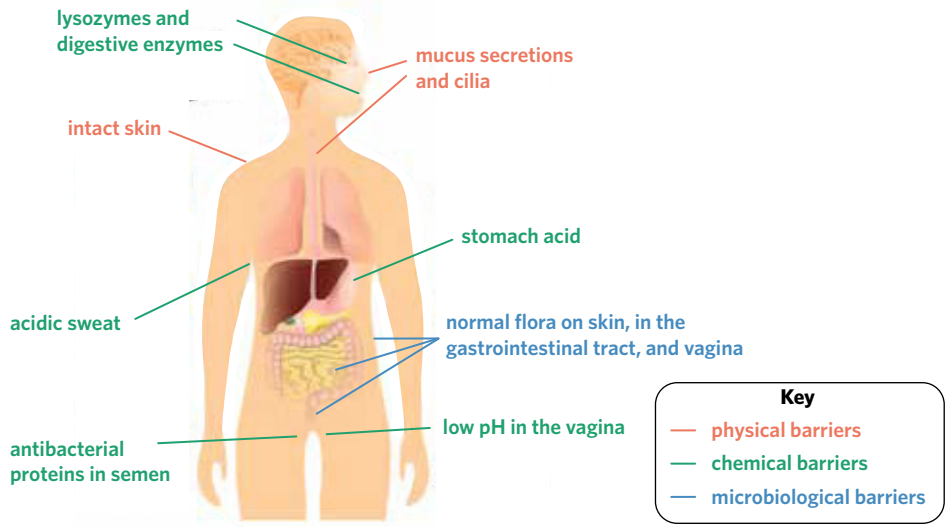


Image: Designua/Shutterstock.com

Figure 4 Barriers present in animals

Table 2 First line of defence in animals

Barrier type	Description	Examples
Physical	Barriers that block or hinder pathogens from entering the organism	• Intact skin and surfaces between external and internal environments (e.g. integumentary, respiratory, gastrointestinal, and genitourinary tracts) • Mucous secretions and/or hairs in the respiratory tract that trap organisms, and **cilia** that sweep them away from the airways and into the throat where they are swallowed and destroyed by the gastrointestinal tract
Chemical	Barriers that work by producing chemical substances that make an environment unlivable for a pathogen	• Presence of lysozyme enzymes in tears and saliva that destroy bacterial cell walls • Acidic sweat that destroys pathogens growing on the surface of the body • Stomach acid that destroys pathogens that have been eaten/swallowed • Antibacterial compounds in earwax • Antibacterial proteins in semen • Low pH in the vagina
Microbiological	The presence of non-pathogenic bacteria (known as normal **flora**) in the body can prevent the growth of pathogenic bacteria as they compete for space and resources	• Presence of bacteria on the skin, in the lower gastrointestinal tract, and the vagina

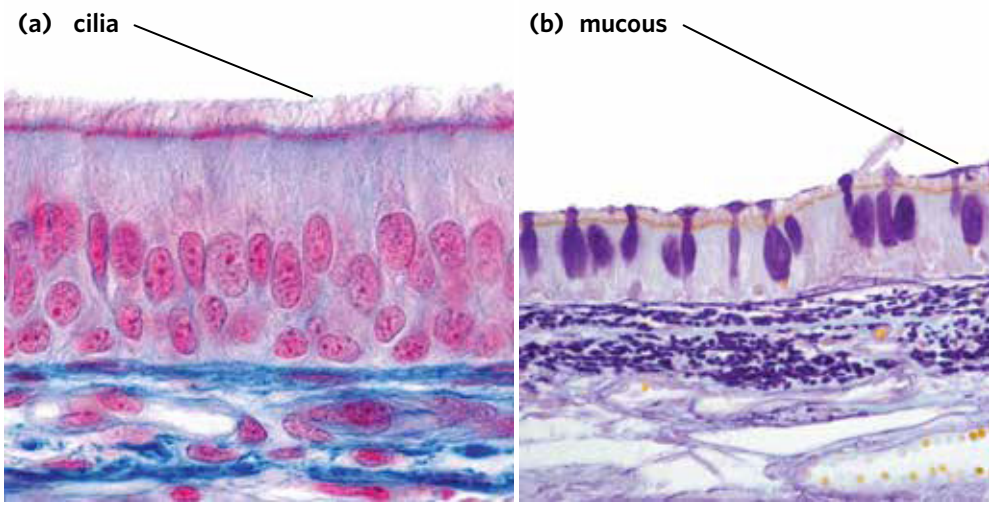


Image: Jose Luis Calvo/Shutterstock.com

Figure 5 **(a)** Cilia and **(b)** mucous secretions within the trachea

cilium (pl. cilia) thin, hair-like projection that protrudes from eukaryotic cells

flora naturally occurring, non-pathogenic bacteria present in an organism

Theory summary

Animals and plants have a variety of first line defences designed to prevent pathogens from infecting them. These defences are composed of various physical, chemical, and microbiological barriers. Fortunately for animals, if these first line defences fail, there's a backup plan – the second line of defence. You'll learn about this in the next lesson.

Table 3 Summary of the first line of defence in plants and animals

Category	Plant	Animal
Physical	• Thick bark • Waxy cuticles • Formation of galls • Presence of thorns and trichomes • Closing of stomata	• Intact skin • Mucous secretions and cilia

cont'd

Table 3 Continued

Category	Plant	Animal
Chemical	• Chitinases • Phenols • Defensins • Saponins • Oxalic acid • Glucanases	• Lysozymes in tears • Acidic sweat • Stomach acid • Earwax • Semen • Low vaginal pH
Microbiological		• Normal flora found on the skin, in the gastrointestinal tract, and in the vagina

Fortunately for us, we have a series of first line defences including physical, chemical, and microbiological barriers which help prevent Bruce from entering our body and causing disease. Likewise, Bruce may even help us prevent skin infections by acting as a microbiological barrier and competing with other potential pathogens. But as with any component of our normal flora, he has the potential to become pathogenic and when this occurs, our body must employ a series of more advanced maneuvers.

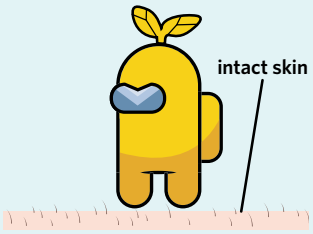


Image: Maybielater/Shutterstock.com

7B QUESTIONS

Theory review questions

Question 1

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- innate immune system
- second line of defence
- first line of defence
- non-specific
- immediate
- delayed
- specific

The _____ is a component of the _____ and provides a/an _____ and _____ form of protection against pathogens. Examples of these defences include physical, chemical, and microbiological barriers.

Question 2

Plants and animals both

A have advanced forms of immunity beyond the first line of defence.

B produce chemicals that can inhibit pathogens.

Question 3

Categorise the following as either a **physical**, **chemical**, or **microbiological** barrier.

I presence of lysozyme in tears _____

II mucous secretions in airways _____

III fine hairs present in the nose _____

IV intact lining of the airway _____

V stomach acid _____

VI normal flora _____

Question 4

Match the type of first line defence to its description.

First line defence	Description	
• waxy cuticle	**I** _____	non-pathogenic bacteria which inhabit the body and compete with potential pathogens
• normal flora	**II** _____	hair-like structures which line the trachea and sweep mucous secretions away
• lysozymes	**III** _____	growths that occur in response to infection to limit the spread of the pathogen
• galls	**IV** _____	a thick layer covering leaves of plants to prevent the entrance of pathogens
• cilia	**V** _____	present in tears and saliva, capable of destroying bacterial cell walls

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–9.

In humans, the large intestine is inhabited by over 100 trillion bacteria from more than 1 000 different species. These bacteria form a crucial component of our microbiota, providing us with many benefits such as protection against pathogenic agents, improved lipid metabolism, and even the production of vitamins. But what happens when our microbiota is disturbed?

Whenever we are diagnosed with a bacterial infection and require antibiotics, not only are the pathogenic bacteria destroyed, but a portion of our healthy gut flora may also be harmed. When this occurs, the careful balance within our microbiota is disturbed, sometimes allowing harmful pathogens to colonise and cause disease. This is known as an opportunistic infection. For example, a bacterium known as *Clostridium difficile* often causes an infection when the gut microbiota is disturbed, leading to symptoms such as diarrhoea, stomach pain, and nausea.

While *C. difficile* can be treated with antibiotics, some people experience what is known as a recurrent *C. difficile* infection where, after being cured of the infection, it quickly returns. One reason it's thought this occurs is because after being destroyed by antibiotics, the normal flora of the gut do not regenerate, allowing *C. difficile* to again colonise the intestines. This leaves doctors with an important question – how do they restore a healthy gut microbiota? Faecal microbiota transplants!

A faecal microbiota transplant involves the transplantation of faeces from one individual to another. Why do doctors do this? Flora are excreted in faeces, and so by taking faeces from an individual with a healthy microbiome and transplanting it to the large intestine of an individual with an unhealthy microbiome, doctors can repopulate their gut with good bacteria. In this way, the normal flora are restored, reinstituting the microbiological barrier and reducing the risk of *C. difficile* returning.

Question 5

Antibiotics

A are prescribed for all types of infections.

B can destroy healthy bacteria in our gastrointestinal tract.

Question 6

The primary aim of faecal microbiota transplants is to

A eliminate existing healthy bacteria with the introduction of new bacteria.

B correct the distribution of bacteria within the gastrointestinal tract.

Question 7

Gut flora serves as

A a microbiological barrier.

B an adaptive barrier.

C a chemical barrier.

D a physical barrier.

Question 8

Gut flora protects against pathogens by

A reducing the availability of nutrients, minerals, and space.
B increasing lipid metabolism.
C producing vitamins.
D forming phenols.

Question 9

The high rate of recurrent infection by *C. difficile* is caused by

A increased numbers of *C. difficile* bacteria in the body.
B a disruption to the normal flora inhabiting the skin.
C an increase in the number of gut flora.
D the continued imbalance of gut flora.

Exam-style questions

Within lesson

Question 10 (1 MARK)

Which one of the following is not a barrier to invading pathogens in humans?

A normal flora in the gastrointestinal tract
B presence of acid in sweat
C cilia lining the airway
D formation of galls

Adapted from VCAA 2013 Section A Q14

Question 11 (1 MARK)

In humans, the presence of lysozymes in tears is an example of

A a physical barrier.
B a chemical barrier.
C an adaptive barrier.
D a microbiological barrier.

Adapted from VCAA 2017 Sample Exam Section A Q21

Question 12 (1 MARK)

A microbiological barrier present in humans is

A the presence of normal flora in the small intestines.
B the production of mucus.
C a highly acidic vagina.
D a thick waxy cuticle.

Question 13 (1 MARK)

Which one of the following is an example of a barrier against a pathogen that is common to both plants and animals?

A secretion of lysozymes
B an intact outer covering
C formation of galls to wall off pathogens
D highly acidic portions of the gastrointestinal tract

Adapted from VCAA 2015 Section A Q17

Question 14 (1 MARK)

Which one of the following is an example of a barrier against pathogen invasion in plants?

A lysozyme production in galls
B cilia lining the surface of stems
C a waxy cuticle covering the surface of a leaf
D mucous secretions on the surface of stomata

Adapted from VCAA 2018 Section B Q3a

Question 15 (1 MARK)

There are many different innate defences against pathogens in the human body. Which of the following rows of innate defence examples is incorrect?

	Body system	Component	Type of barrier
A	gastrointestinal tract	stomach acid	chemical
B	respiratory tract	mucous secretions	chemical
C	genitourinary tract	healthy microbiota	microbiological
D	integumentary system	intact skin	physical

Multiple lessons

Question 16 (1 MARK)

Which one of the following statements about the first line of defence is incorrect?

A First line defences produce the same response irrespective of the type of antigen.
B Viruses and bacteria stimulate the same types of first line defences in humans.
C First line defences are capable of recognising specific pathogenic antigens.
D First line defences include chemical, physical, and microbiological barriers.

Question 17 (3 MARKS)

The first line of defence helps protect humans from invading pathogens such as bacteria and viruses.

a Identify one microbiological barrier that prevents pathogenic bacteria from entering the body. (1 MARK)
b Explain how the first line of defence could prevent a respiratory infection. (2 MARKS)

Question 18 (5 MARKS)

Salmonella is a bacterial pathogen that infects the gastrointestinal tract and causes food poisoning. In Australia, most *Salmonella* infections occur after eating contaminated food, but also sometimes after contact with an infected person. Symptoms include diarrhoea, vomiting, and fever, and usually last for two to seven days.

a Recent evidence suggests that normal flora in the gastrointestinal tract may provide protection against *Salmonella* infection. Describe how normal flora can protect against *Salmonella* infection. (1 MARK)
b *Salmonella* bacteria contain many enzymes crucial for metabolic function. One of these enzymes is ATPase, which catalyses the following reaction:

$ATP \rightarrow ADP + P_i$

i State the chemical barrier that *Salmonella* bacteria will encounter in the stomach. (1 MARK)
ii Explain the effect of this chemical barrier on the structure and function of ATPase, and the ultimate consequence this has for *Salmonella* bacteria. (3 MARKS)

Question 19 (5 MARKS)

In response to the growing need to provide specific and targeted therapies against specific pathogens to ensure their effectiveness, many new technologies have been developed. For example, radioactive primers, which can fluoresce under UV light, provide an effective method of identifying viral infections. Adenoviruses, which are a group of DNA viruses, can be detected using radioactive primers.

a Describe the structure of a virus. (1 MARK)

b Explain whether or not viruses can be cultured on standard nutrient agar. (1 MARK)

c Identify one physical barrier that prevents the invasion of a virus. (1 MARK)

d Suggest how radioactive primers could be used to detect an adenovirus infection. (2 MARKS)

Key science skills and ethical understanding

Question 20 (6 MARKS)

The beet caterpillar (*Spodoptera exigua*) is an insect pest of the tomato plant. When a beet caterpillar starts to eat a tomato plant, the plant responds by producing a chemical known as jasmonic acid. Jasmonic acid and its derivatives have a variety of odours.

Anne, a keen biology student, hypothesised that jasmonic acid inhibits a caterpillars' innate immunity to a particular lethal pathogen. She has isolated jasmonic acid in a spray, and has access to a large number of plants that do not produce jasmonic acid.

a Identify which kind of barrier the production of jasmonic acid is an example of. (1 MARK)

b Outline an experiment Anne could carry out to test her hypothesis, including three variables that must be controlled for. (3 MARKS)

c Describe the results that would support her hypothesis. (1 MARK)

d Describe how the bioethical concept of integrity may influence the actions of the researchers. (1 MARK)

Adapted from VCAA 2009 Exam 1 Section B Q4

7C THE SECOND LINE OF DEFENCE

Bruce, who inhabits our skin, is a devious strain of Staphylococcus aureus, *always looking for a way into our body. Unfortunately, as you were rushing to class, you fell down a flight of stairs and grazed your arm on the ground, providing Bruce with the perfect opportunity to bypass your first line of defence. Immediately after falling, you also noticed your arm became swollen, red, a bit hot, and was painful to touch. How will your body defend itself now that Bruce has broken through the first line of defence? And why is your arm so swollen, red, and hot to touch?*

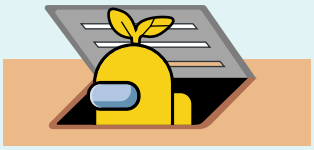


Image: Maybielater/Shutterstock.com

Lesson 7C

In this lesson you will learn about the key components of the second line of defence, as well as the processes involved in the inflammatory response.

◀ *Prerequisite knowledge*

Year 11

The phagocytosis of pathogens occurs via endocytosis, which involves the inward folding of the plasma membrane, the trapping of target molecules inside a vesicle, and the budding of the vesicle into the cell.

Chapter 3

The elevation of body temperature caused by a fever can denature pathogenic enzymes.

Future applications ▶

Lesson 7D

Macrophages and dendritic cells are antigen-presenting cells, which perform a crucial role in presenting pathogenic antigens to the adaptive immune system.

Study design dot point

- the innate immune response including the steps in an inflammatory response and the characteristics and roles of macrophages, neutrophils, dendritic cells, eosinophils, natural killer cells, mast cells, complement proteins, and interferons

Key knowledge units

Cells and components of the innate immune response	4.1.2.1
Steps in the inflammatory response	4.1.2.2

Cells and components of the innate immune response 4.1.2.1

OVERVIEW

The second line of defence is a component of the innate immune system, composed of a variety of cells and molecules that destroy pathogens which have entered the body, preventing the spread of infection.

THEORY DETAILS

Pathogens are sometimes able to slip past or breach the first line of defence. Fortunately, our bodies have a backup plan for when this happens – the **second line of defence**. The second line of defence is another component of the innate immune system, providing us with a form of non-specific and immediate protection against potential pathogens.

second line of defence a component of the innate immune system characterised by the non-specific and immediate response to injury and pathogens by a variety of cells and molecules

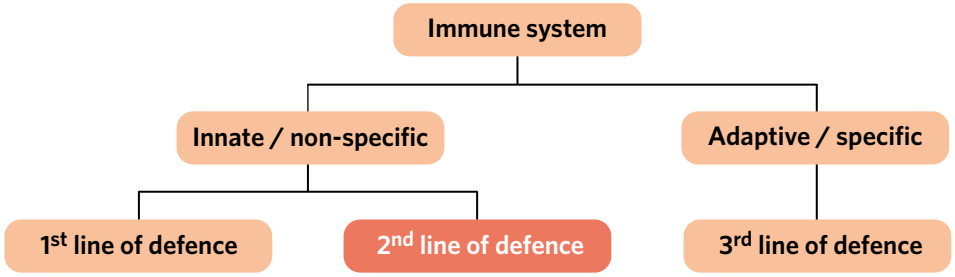


Figure 1 Breakdown of the immune system

There are two components of the second line of defence – cellular and non-cellular components. You'll take a closer look at these now.

Cellular components of the second line of defence

The second line of defence involves a variety of different cell types. All of the cells involved are called **leukocytes**, or white blood cells. Figure 2 shows the key innate immune cells you need to know.

leukocytes a group of blood cells responsible for protecting the body against pathogens and foreign material. Also known as **white blood cells**

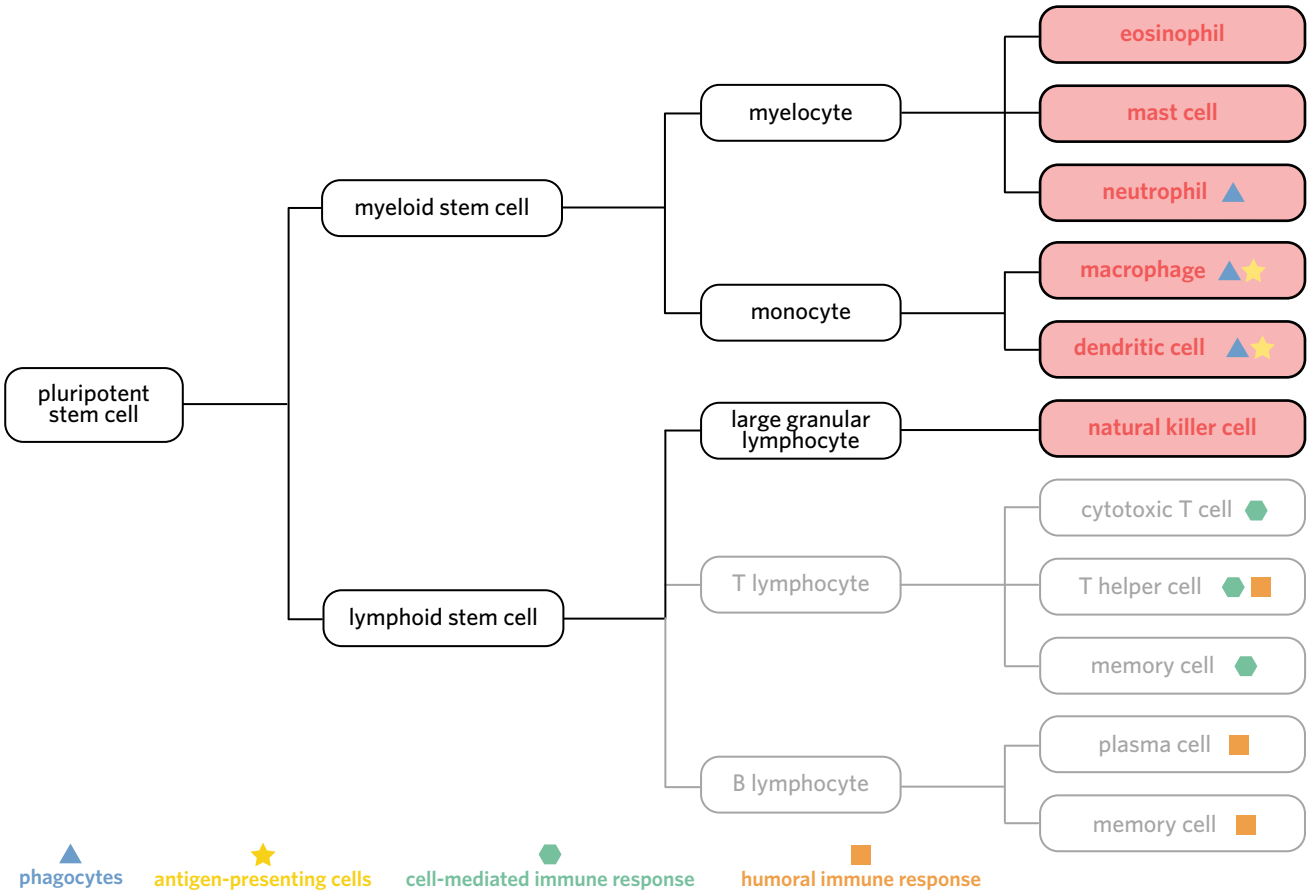


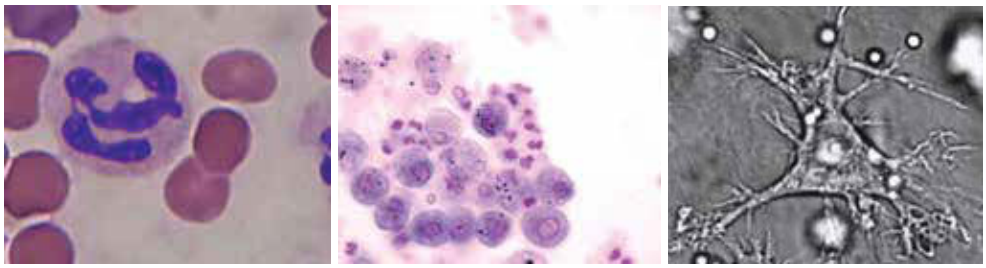
Figure 2 Highlighted are the cellular components of the innate immune system.

Phagocytes

Phagocytes are cells that engage in phagocytosis, a process in which they consume and destroy foreign or dead material present in the body by engulfing it through the process of endocytosis. Once engulfed, lysosomes containing lysozymes present in the cell destroy the foreign or dead material by fusing with the vesicles containing the engulfed material. The phagocytes you will need to know about include **neutrophils, macrophages**, and **dendritic cells**.

phagocyte a group of leukocytes responsible for the endocytosis and destruction of pathogens, foreign material, and cell debris

neutrophil the most common type of leukocyte in the body. Engages in phagocytosis of pathogens and foreign material, as well as the release of cytokines



neutrophils macrophages dendritic cells

Image: Jose Luis Calvo/Shutterstock.com

Figure 3 Neutrophils, macrophages, and dendritic cells

macrophage a type of leukocyte found throughout the body that engages in phagocytosis and antigen presentation

dendritic cell a type of leukocyte that engages in phagocytosis and antigen presentation

antigen-presenting cell a subgroup of phagocytes that display antigens from consumed pathogens on their surface and interact with the adaptive immune system

Additionally, two of these phagocytes – macrophages and dendritic cells – are also known as **antigen-presenting cells**. These cells not only consume and destroy foreign material, but they also present antigens from consumed material on their surface. In lesson 7A, you learned that while all nucleated cells of the body express MHC I on their surface, only specific cells in the body express MHC II. Antigen-presenting cells are the specific immune cells which also express MHC II, using them to present the consumed antigens on their surface. These cells will then use their MHC II markers with the presented antigen to interact with the adaptive immune system. This process will be covered in greater detail in the next lesson.

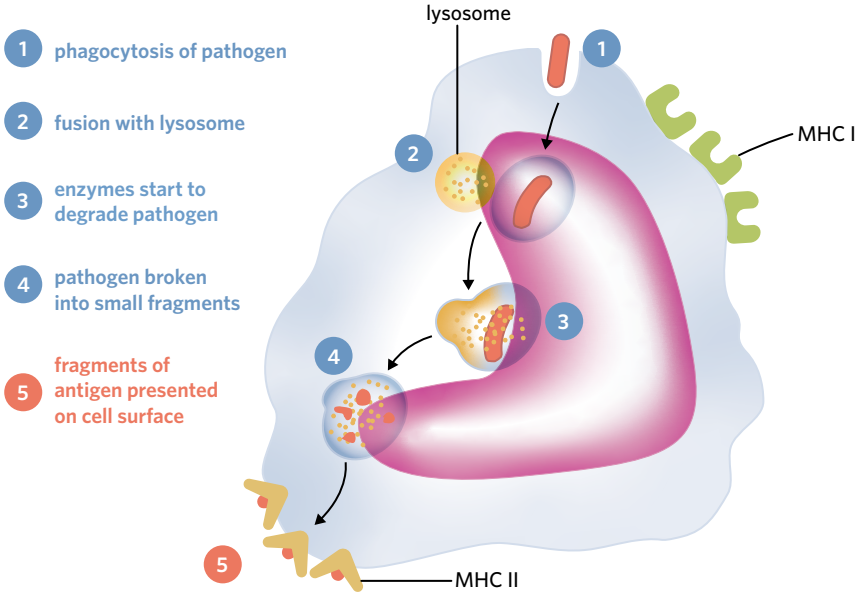


Figure 4 The process of phagocytosis and antigen presentation

To communicate within the immune system, phagocytes release a number of substances such as **cytokines**. Cytokines are important cell signalling molecules which help protect against pathogens and can help guide immune cells to the site of infection or injury, allowing them to function optimally.

cytokine a signalling molecule released by cells (typically in the immune system) which aids in communication between immune cells and helps protect against pathogens

natural killer (NK) cell a type of leukocyte responsible for the recognition and destruction of damaged and/or infected host cells

Natural killer (NK) cells

Natural killer cells are large granulated cells which target both abnormal and virally infected cells. This is achieved with the presence of two receptors – a killer inhibitory receptor and a killer activation receptor:

- killer inhibitory receptor – examines the surface of cells for MHC I markers
- killer activation receptor – binds to certain molecules which appear on cells undergoing cellular stress (e.g. infected or cancerous cells).

The presence of MHC I markers can be altered due to a number of different disease processes. For example, MHC I markers may be absent due to the presence of a viral infection, which can either destroy or suppress the production of MHC I markers. Additionally, the gene expression of MHC I markers may be affected in cancer cells, also leading to the absence of MHC I markers.

If the killer inhibitory receptor detects a sufficient number of MHC I markers, then it overrides the killer activation signal, preventing cell death. Cell death is only initiated in infected or abnormal cells with missing MHC I markers – that is, when the killer activation receptor is activated and the killer inhibitory receptor is unable to bind to a sufficient number of MHC I markers.

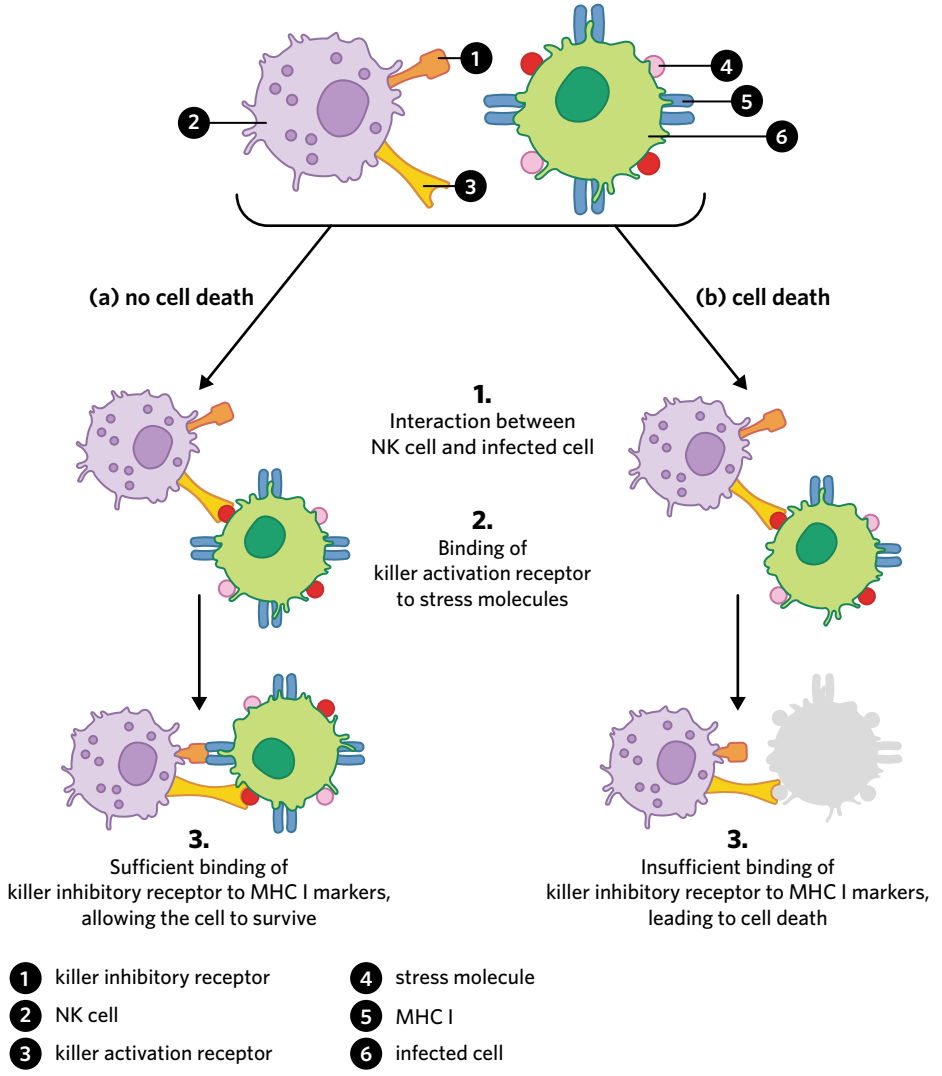


Figure 5 The interaction between a natural killer cell with an infected cell **(a)** without stimulating cell death and **(b)** stimulation leading to cell death.

Mast cells

Mast cells reside in connective tissues throughout the body. When they detect injury to surrounding cells or are stimulated by antigens or allergens, they become activated and **degranulate**, releasing **histamine**. Histamine has a number of effects on the body and is particularly important in the **inflammatory response**.

Eosinophils

Eosinophils are large granulated cells containing various toxic chemical mediators such as DNases, RNases, and proteases, which help destroy invading pathogens. They typically target pathogens which are too large to be phagocytosed by degranulating on contact with them and releasing the chemical mediators contained within their granules.

Non-cellular components of the second line of defence

In addition to leukocytes, there are other key molecules and processes that play an important role in the second line of defence. These include **interferons**, **complement proteins**, and the initiation of a fever.

Lesson link

DNases, RNases, and proteases are all enzymes that catalyse the breakdown of DNA, RNA, and proteins respectively. Flick back to ***lesson 3A*** to catalyse your memory on enzymes.

mast cell a type of leukocyte responsible for releasing histamine during allergic and inflammatory responses

degranulation the release of granule contents from a cell

histamine a molecule released by mast cells that plays a key role in inflammation

inflammatory response a series of biochemical events that occur in the body as a result of infection and/or trauma. Characterised by swelling, redness, pain, and heat in the affected tissue

eosinophil a large granular leukocyte responsible for the release of toxic chemical mediators

interferon a cytokine released by virally infected cells that increases the viral resistance of neighbouring uninfected cells

complement proteins a number of different types of proteins found in the blood that opsonise, cause lysis, and attract phagocytes to invading pathogens

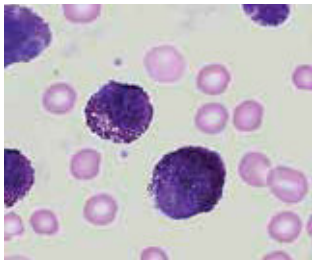


Figure 6 Mast cells containing dark-staining histamine

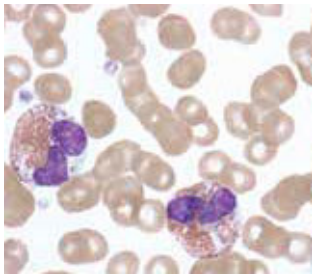


Figure 7 Eosinophils

Interferons

When a cell is infected with a virus, it releases a type of cytokine called interferons. These interferons interact with receptors on neighbouring cells, causing them to undergo a number of changes that make them less susceptible to viral infection. This helps prevent the virus from spreading between cells.

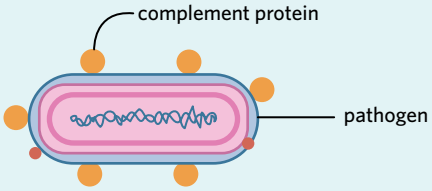
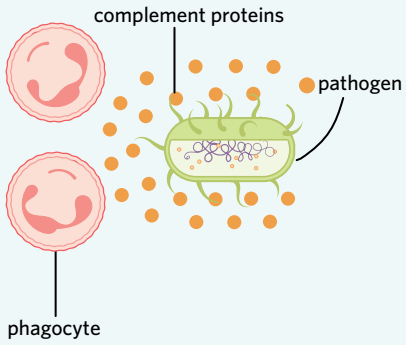
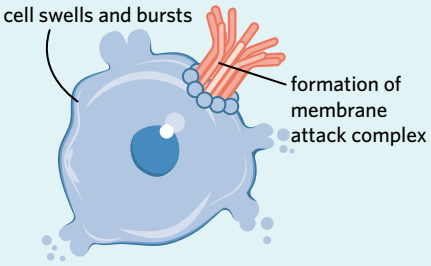
Complement proteins

Within the blood, there are a number of different complement proteins that together form the complement system. In the presence of certain pathogens, these proteins begin reacting with each other in a series of reactions called the **complement cascade**. The three major outcomes of this cascade are summarised in Table 1.

complement cascade a complex sequence of events which occurs after the activation of complement proteins

opsonisation the mechanism by which complement proteins attach to the surface of pathogens, making them easier to phagocytose

Table 1 Outcomes of the complement cascade

Outcome	Description	Diagram
Opsonisation	Complement proteins stick on the outside surface of pathogens and make it easier for cells of the immune system, such as phagocytes, to recognise them as foreign.	 Figure 8 Opsonisation of a pathogen by complement proteins
Chemotaxis	Complement proteins gather near a pathogen and attract phagocytes to it, making it more likely to be destroyed.	 Figure 9 Chemotaxis of phagocytes towards a pathogen
Lysis	Complement proteins can join together on the surface of pathogens, forming a **membrane attack complex (MAC)**, which creates pores in their membrane. This destroys the pathogen by causing lysis via the sudden influx of fluid into the pathogen, causing it to burst.	 Figure 10 Formation of a membrane attack complex

Fever

A fever is a temporary increase in body temperature. A complex series of responses can raise the set temperature point of the body during a fever. In response, the body initiates a number of countermeasures that increase core body temperature to reach this new setpoint, including shivering and heat-conserving behaviours such as putting on a jumper. This is an innate response to potential infection, as many pathogens cannot survive at the elevated temperatures created by a fever. Fevers are also thought to help the immune system by activating certain proteins in the body that bolster the strength of the body's defences.

However, it is important to note that prolonged fevers can be detrimental to the body due to the additional stress placed on our cells, which are no longer functioning at their optimal temperature.

chemotaxis the attraction of phagocytes towards a pathogen

lysis the disintegration or rupturing of a cell

membrane attack complex (MAC) a pore formed by complement proteins in the cell membranes of a pathogen, disrupting the membrane and leading to the pathogen's destruction

Examiners' tip

The immune system is extremely complicated and there are many components such as defensins and perforins that you may have heard of which haven't been covered in this lesson. The VCAA, however, have only specified that you need to understand the role and characteristics of the components covered in this lesson.

Lesson link

Fevers typically work by causing proteins and enzymes in pathogens to denature. As you learned in ***Chapter 3***, this results in degradation of the 3D shape of the protein and subsequent loss of function.

Steps in the inflammatory response 4.1.2.2

OVERVIEW

The process of inflammation increases blood flow to an injured area, bringing a greater number of immune cells and components to help clear debris and fight pathogens that may have entered the body. This increase in blood and fluid to the affected tissue causes swelling, pain, heat, and redness, which are the characteristic signs of inflammation.

THEORY DETAILS

The inflammatory response is designed to eliminate the effects of an injury, defend against potential pathogens, clear out cells that may have been damaged or destroyed, and initiate repair. It is a complex, non-specific process that always occurs in the same way regardless of the pathogen present or the injury that has occurred. Below, you'll look at an example of an injury and the body's inflammatory response to it.

Process of inflammation

There are three main aspects of the inflammatory response – initiation, **vasodilation**, and migration.

vasodilation the widening of blood vessels

Initiation

Imagine you've been chopping up some wood for the fire. You go to pick up a piece, and you get a splinter in your finger. The splinter pierces the skin, damaging cells and introducing pathogens such as bacteria into the body. In response to this injury, both immune cells located in the tissue and damaged cells release cytokines. Additionally, mast cells degranulate, releasing histamine.

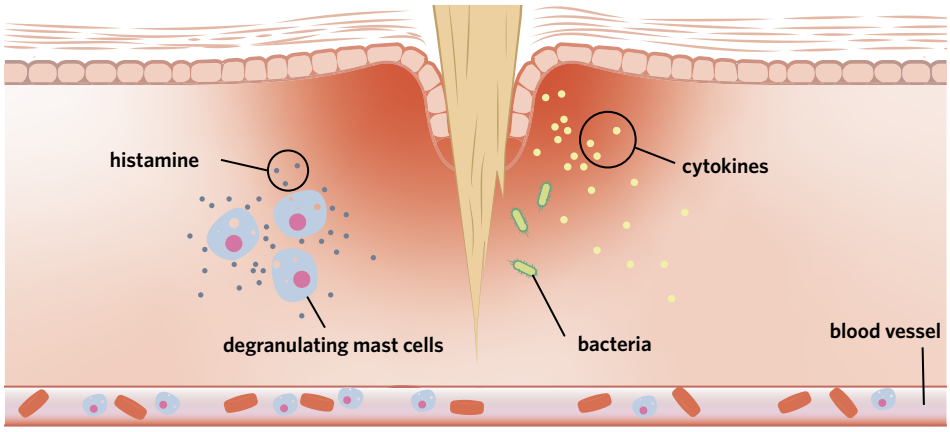


Figure 11 Initiation of inflammation involves cytokines being secreted from damaged cells and activation of mast cells, causing the release of histamine.

Vasodilation

The histamine released from mast cells travels to nearby blood vessels and binds to specific receptors, causing vasodilation. This causes blood vessels to widen, increasing blood flow to the injury site, and this is the reason behind the swelling, redness, and warmth we often associate with inflammation. Additionally, the formation of gaps in the vessel wall increases its permeability to cells of the immune system.

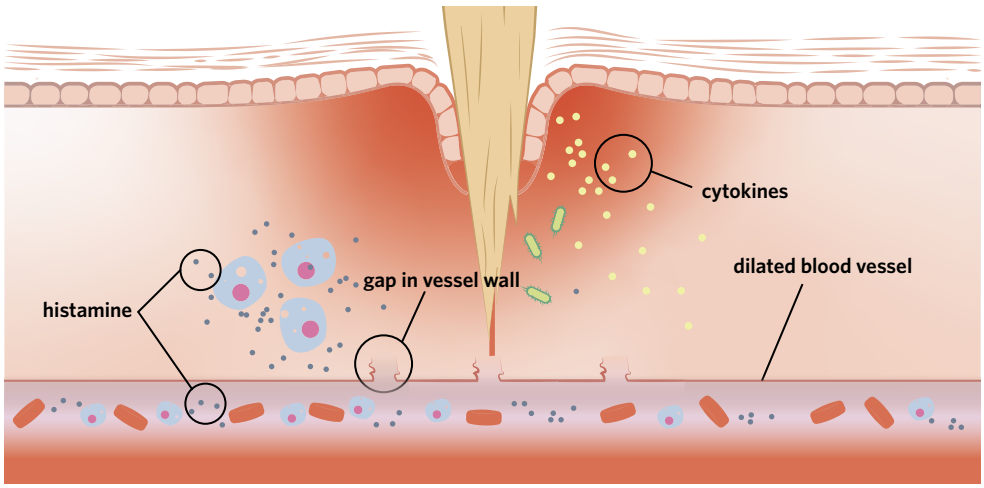


Figure 12 Histamine has caused the blood vessel to dilate and become 'leaky'.

Migration

Vasodilation and the increased leakiness of blood vessels allow for a number of innate immune system components to leave the bloodstream and enter the site of injury. These components include:

- Phagocytes are guided by the cytokines secreted by damaged cells to the site of injury, where they phagocytose pathogens.
- Complement proteins are attracted to pathogens and make it easier for phagocytes to destroy them.

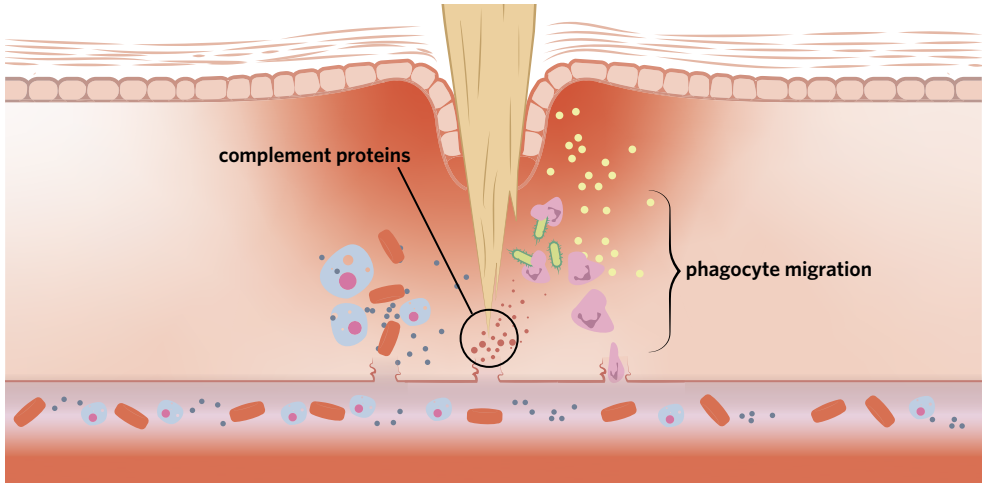


Figure 13 Various components of the innate immune system are able to access the site and destroy pathogens and remove debris.

The pus that comes out of an injured area is caused by this increase in blood flow and immune cell activity. Pus contains a large amount of dead immune cells and pathogens – think about that next time you're popping a pimple!

The response continues until the site has been cleared of pathogens and debris, and the site of injury has been healed and will eventually return to normal.

Theory summary

The second line of defence consists of a number of non-specific cells and molecules that work quickly to limit the spread of injury or infection. An important example of the ways in which these components interact with each other is the inflammatory response.

Table 2 Components of the second line of defence and their roles

Cellular components	
Neutrophil	Phagocytosis of pathogens
Macrophage	Phagocytosis of pathogens and antigen presentation within the adaptive immune system
Dendritic cell	
Natural killer cell	Destroys infected or abnormal cells with insufficient MHC I markers
Mast cell	Causes inflammation through the release of histamine
Eosinophil	Releases toxic chemical mediators to destroy invading pathogens
Non-cellular components	
Interferons	Released by virally-infected cells and causes changes to neighbouring cells that make them less susceptible to infection
Complement proteins	React with each other and aid in the destruction of pathogens via opsonisation, attraction of phagocytes to pathogens, and the formation of membrane attack complexes (MAC)
Fever	An abnormally high body temperature used by the body to kill pathogens

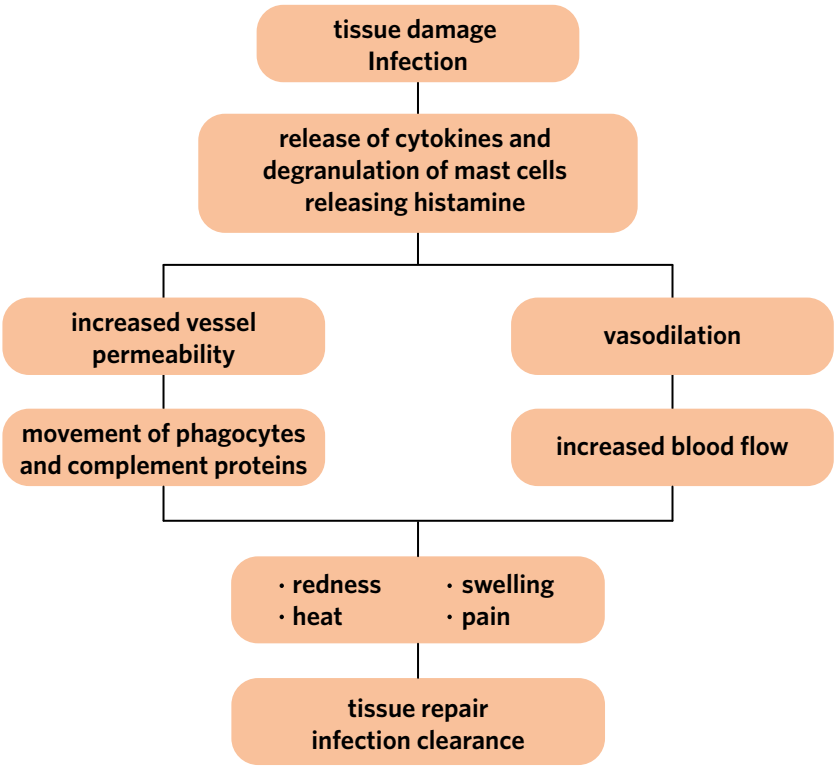


Figure 14 Summary of the stages in the inflammatory response

Fortunately, even though Bruce broke through the first line of defence, we have the second line of defence to help defend against him. The second line of defence provides a series of cellular and non-cellular defences which form a part of our innate immune system. Additionally, the swollen, red, and hot nature of your arm originates from the inflammatory response, which caused the vasodilation of blood vessels, increasing blood flow to the injured area. However, will this be enough to prevent Bruce from replicating and progressing further into our body?

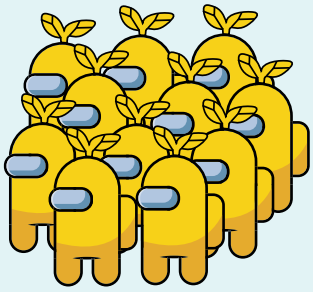


Image: Maybielater/Shutterstock.com

7C QUESTIONS

Theory review questions

Question 1

Which one of the following statements about the second line of defence is false?

A The second line of defence is non-specific.
B Neutrophils are a type of antigen-presenting cell.
C Inflammation results in increased blood flow to the site of injury.
D The second line of defence includes the destruction of pathogens by phagocytosis.

Question 2

Match the innate immune cell to its description.

Innate immune cell	Description
• mast cell	**I** _____ presents antigens to the adaptive immune system
• neutrophil	**II** _____ detects cells with insufficient MHC I markers
• eosinophil	**III** _____ cell that only phagocytoses pathogens
• dendritic cell	**IV** _____ releases toxic chemical mediators
• natural killer cell	**V** _____ releases histamine

Question 3

Match the innate immune molecule to its description.

Innate immune molecule	Description
• complement protein	**I** _____ broad group of molecules which facilitate communication between immune cells
• histamine	**II** _____ increases the resistance of uninfected cells to viral infection
• interferon	**III** _____ opsonises pathogens
• cytokine	**IV** _____ causes vasodilation

Question 4

The inflammatory response

A involves cytokines released by damaged cells which attract leukocytes to the site of infection.
B involves the degranulation of mast cells which release toxic chemicals against specific viruses.
C stimulates natural killer cells to destroy bacteria by phagocytosis.
D results in decreased blood flow and bleeding at the site of injury.

Question 5

Fill in the blanks with the following terms.

- complement proteins
- opsonisation
- phagocytes
- mast cells

As part of the inflammatory response, _____ release histamine, causing vasodilation. _____ migrate to the site of infection or injury, and consume pathogens/debris. _____ assist these cells in destroying pathogens in a number of different ways, including _____.

SAC skills questions

Case study analysis

Use the following information to answer Questions 6–10.

Allergens are non-pathogenic antigens recognised by the immune system as foreign and result in an inappropriate immune response called an allergic reaction. Not only is this immune response unnecessary, but it also causes the unpleasant symptoms we know as allergies. These vary widely depending on the allergen and the body system affected but can include an itchy rash, runny nose, sneezing, shortness of breath, and swelling.

The development of an allergy involves two stages – sensitisation and re-exposure. Sensitisation involves the initial exposure of an individual to an allergen, where antibodies, a component of the adaptive immune system, bind to mast cells, sensitising them. Subsequently, on re-exposure, the allergen binds to the antibodies located on the mast cells, stimulating them to degranulate and release histamine, which initiates the inflammatory response. When this occurs, our airways constrict, our blood vessels vasodilate, and mucous secretion is increased. While allergic reactions to some substances such as pollen can be mild and annoying, they can also be serious and deadly, with the potential to cause an anaphylactic shock.

To detect the presence of an allergy, doctors can perform a skin-prick test. This test involves the application of several different potential allergens (ranging from peanuts to pollen) on the arm and pricking the skin to allow the potential allergen to seep into the skin, causing it to react with any sensitised mast cells that are present. Doctors can determine whether an individual is allergic to the allergen by observing the skin's reaction. If an individual is allergic to the substance applied, an inflammatory response will have been generated, resulting in redness and swelling of the skin. The more allergic an individual is to a particular allergen, the greater the inflammatory response will be. The following images depict an example of a skin-prick test as well as a doctor's drawing of the results from a skin-prick test.

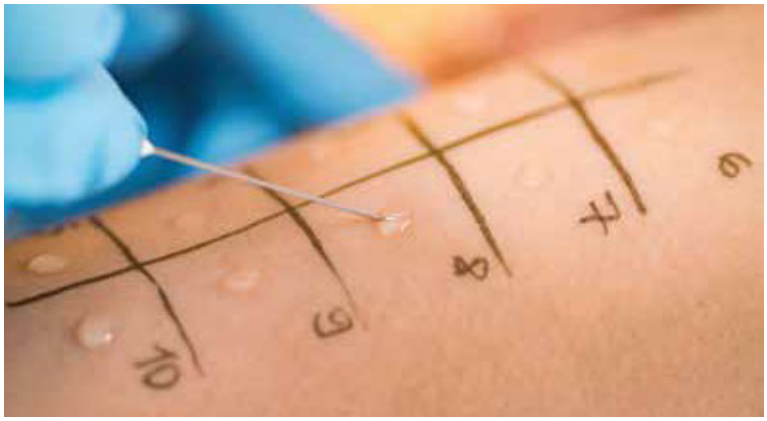
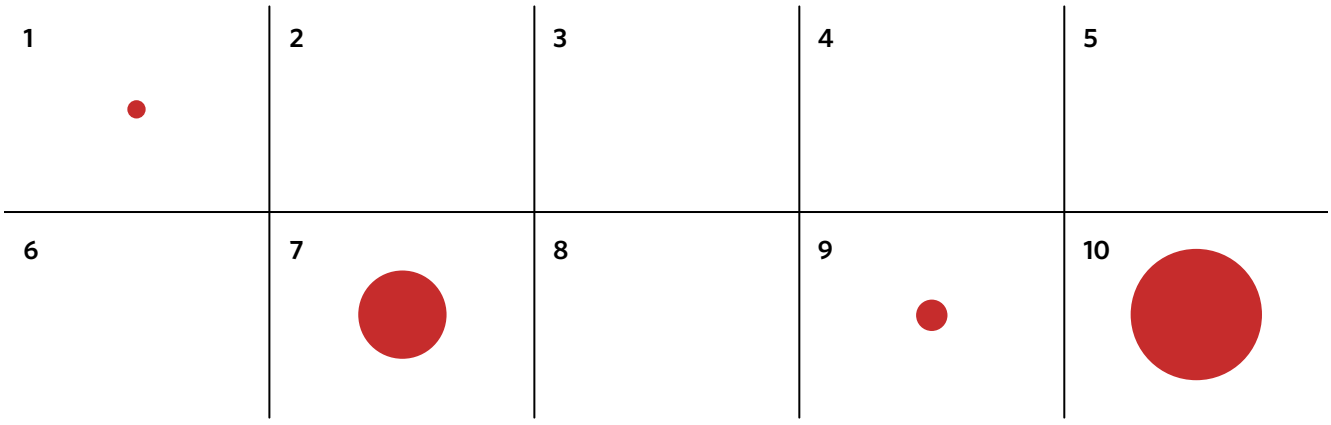


Image: Microgen/Shutterstock.com



Allergens tested in the skin-prick test			
1	Cat	6	Chicken
2	Dog	7	Peanut
3	Dust	8	Milk
4	Tuna	9	Pollen
5	Peas	10	Egg

Question 6

During sensitisation,

A antibodies bind to mast cells.

B mast cells release histamine.

Question 7

Antibodies are a component of the

A innate immune response.

B adaptive immune response.

Question 8

In response to allergens, swelling and redness are caused by the

A vasoconstriction of blood vessels near the surface of the skin.

B presence of normal flora on the surface of skin.

C release of histamine from mast cells.

D infection of the skin by a pathogen.

Question 9

Based on the results of the skin-prick test, the individual tested is allergic to

A peanuts, pollen, cats, and eggs.

B peanuts, cats, dogs, and dust.

C eggs, cats, dogs, and pollen.

D peas, milk, pollen, and eggs.

Question 10

Based on the results of the skin-prick test, the individual produced the greatest allergic reaction to the

A peanut.

B pollen.

C egg.

D cat.

Exam-style questions

Within lesson

Use the following information to answer Questions 11–13.

The inflammatory response is a complex mechanism which serves to protect organisms from infection and injury.

Question 11 (1 MARK)

The inflammatory response

A is specific to particular antigens.

B is part of the first line of defence.

C involves the degranulation of mast cells.

D involves the release of interferons to increase blood flow.

Question 12 (1 MARK)

The inflammatory response helps to prevent infection by

A making blood vessels less permeable.

B promoting the phagocytosis of pathogens.

C activating natural killer (NK) cells to kill bacteria.

D releasing interferons to activate the complement cascade.

Question 13 (1 MARK)

A girl is carrying a piece of wood. A splinter breaks off and becomes embedded in her finger. The next day, she notices her finger is swollen.

In the region around the small piece of wood embedded in her finger,

A antigens from the foreign material would be presented on the surface of neutrophils to cells of the adaptive immune system.

B mast cells would be leaving the blood vessels and phagocytosing foreign material.

C cytokines from damaged cells would be attracting leukocytes to the site of injury.

D phagocytes would be releasing histamine to cause vasodilation.

Adapted from VCAA 2015 Section A Q18

Question 14 (1 MARK)

As part of the second line of defence in the human immune system, cells that present antigens to cells of the adaptive immune system include

A mast cells.

B neutrophils.

C dendritic cells.

D natural killer cells.

Adapted from VCAA 2013 Exam 1 Section A Q14

Question 15 (1 MARK)

Defence mechanisms against viral pathogens include

A neutralisation by cytokines.

B interferons that protect uninfected cells from viral attack.

C degranulation of mast cells releasing complement proteins.

D destruction of viral particles in the bloodstream by natural killer cells.

Adapted from VCAA 2013 Section A Q15

Question 16 (1 MARK)

Cytokines are chemicals that

A attract phagocytes to the site of injury.

B stimulate the degranulation of mast cells.

C cause blood vessels to vasodilate and become leaky.

D kill bacteria by producing holes in the bacterial cell membrane.

Adapted from VCAA 2012 Exam 1 Section A Q23

Question 17 (11 MARKS)

Meningitis is a disease which causes the inflammation of tissue surrounding the brain and spinal cord in humans.
There are many different pathogens which can cause meningitis. For example, a bacterium known as *Neisseria meningitidis*, which produces a variety of different bacterial enzymes, as well as a family of viruses known as coxsackieviruses can both cause meningitis.

a Outline three changes that would occur during the inflammatory response in the tissue surrounding the brain. (3 MARKS)

b When infected with *Neisseria meningitidis*, a fever is often initiated. On a cellular level, explain how a fever can help combat bacterial meningitis. (3 MARKS)

c Antigen-presenting cells form an important component of the body's defence against pathogens.

- **i** Identify two cell types belonging to the second line of defence that serve as antigen-presenting cells. (2 MARKS)
- **ii** State the two roles of antigen-presenting cells in the innate immune system. (2 MARKS)

d In cells infected with coxsackieviruses, the gene expression of MHC I markers is often affected. Identify the cell type belonging to the innate immune system which is primarily responsible for the elimination of these virally infected cells. (1 MARK)

Adapted from VCAA 2011 Exam 1 Section B Q5

Multiple lessons

Question 18 (1 MARK)

Some components of the second line of defence destroy pathogens by disrupting the pathogen's cell wall. Which one of the following pathogens would be affected by this process?

A viruses
B worms
C prions
D fungi

Question 19 (1 MARK)

An example of an innate response by the human immune system to a protein coat-bound pathogen is the

A release of interferons by infected cells.
B release of complement proteins by mast cells.
C opsonisation of the pathogen by macrophages.
D phagocytosis of the pathogen by natural killer cells.

Question 20 (3 MARKS)

During foetal development, many peptides and chemicals from the innate immune system can be found in the placenta and can help provide a form of protection against potential pathogens in newborns. Additionally, many of these chemicals can be passed on from the mother to her child via breastfeeding.

a Based on the information provided, state and describe two possible chemicals of the innate immune systems that scientists could expect to find in the milk. (2 MARKS)

b State one reason why these compounds are considered part of the innate immune system. (1 MARK)

Key science skills and ethical understanding

Question 21 (5 MARKS)

Scientists wanted to explore the response of the innate immune system to different concentrations of a pathogen in a population of mice. To do so, a group of 10 mice were used. Each mouse was infected with a different concentration of pathogenic bacteria under their skin. One mouse received no bacteria. After a few hours, the scientists then measured the levels of neutrophils and histamine present in the skin of the mice. This procedure was conducted once.

a State a reasonable hypothesis that the scientists could be testing with this experiment. (1 MARK)

b State whether a control was used in the experiment. Identify what the control is/should be. (1 MARK)

c Explain whether this experiment enables the scientists to reliably draw conclusions about the response of the innate immune system to different concentrations of pathogen. (1 MARK)

d Outline how neutrophils and histamine protect the mouse's body once a bacterial pathogen has gained entry to the internal environment. (2 MARKS)

7D THE THIRD LINE OF DEFENCE

Unfortunately, due to the speed and persistence of Bruce's attack on our body, the second line of defence has been unable to contain him. Indeed, our macrophages, neutrophils, and dendritic cells have all been valiantly phagocytosing Bruce, but he is simply replicating too quickly. What other defences does our body have against Bruce? Is there a more developed and specific response than our innate immune system?

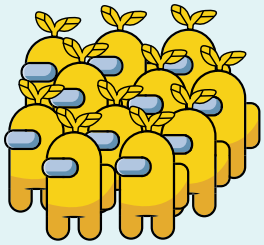


Image: Maybielater/Shutterstock.com

Lesson 7D

In this lesson you will learn about the cells and processes that make up the adaptive immune system.

◀ *Prerequisite knowledge*

Chapter 2
The sequence of amino acids, and the bonds and interactions that form between them, influence the antigen-binding site of antibodies, which are composed of protein.

Lesson 7C
Macrophages and dendritic cells are antigen-presenting cells that are involved in the phagocytosis and presentation of pathogenic antigens to the adaptive immune system.

Future applications ▶

Lesson 7E
The migration of antigen-presenting cells and the processes of antigen presentation, clonal selection, and clonal expansion occur within the lymphatic system.

Lesson 8A
Vaccinations stimulate the adaptive immune system to generate immunological memory without causing disease.

Lesson 8D
Modern therapies against cancer include the designing and production of monoclonal antibodies, which are capable of targeting specific antigens in the body.

Study design dot points

- initiation of an immune response, including antigen presentation, the distinction between self-antigens and non-self antigens, cellular and non-cellular pathogens, and allergens
- the characteristics and roles of the components of the adaptive immune response against both extracellular and intracellular threats, including the actions of B lymphocytes and their antibodies, helper T, and cytotoxic T cells

Key knowledge units

Initiation of the third line of defence	4.1.3.2
Humoral immunity	4.1.5.1
Cell-mediated immunity	4.1.5.2
Immunological memory	4.1.5.3

Initiation of the third line of defence 4.1.3.2

OVERVIEW

The third line of defence, also known as the adaptive immune system or the specific immune response, is initiated by the presentation of non-self antigens to specific immune cells of the adaptive immune system.

THEORY DETAILS

The **third line of defence** is a key component of the immune system in humans. Like the second line of defence, it is designed to combat and destroy pathogens that have breached the first line of defence. However, there are two unique features of the adaptive immune system which separate it from the second line of defence:

- specificity – the adaptive immune system responds to each distinct pathogen in a unique and tailored manner
- **immunological memory** – the adaptive immune system results in the production of cells that allow the body to respond to future re-infections by a previously encountered pathogen quickly and effectively.

third line of defence a subset of the immune system within vertebrates that is composed of the humoral and cell-mediated responses which create a specific immune response and form immunological memory. Also known as the **adaptive immune system** or **specific immune response**

immunological memory the ability of the immune system to quickly and aggressively combat a previously encountered pathogen due to the presence of T and B memory cells

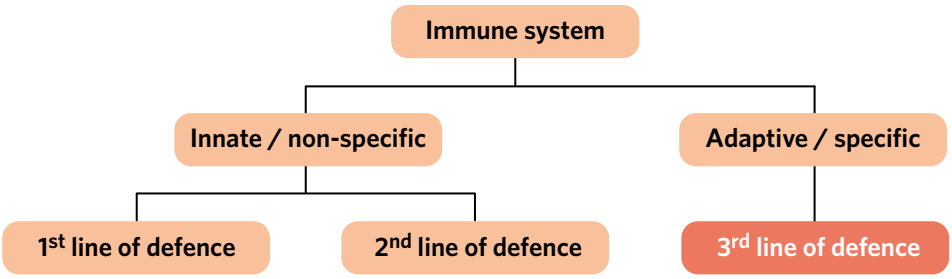


Figure 1 Breakdown of the immune system

There are a number of different cells that make up the adaptive immune system. These are highlighted in Figure 2 and will all be explored in this lesson.

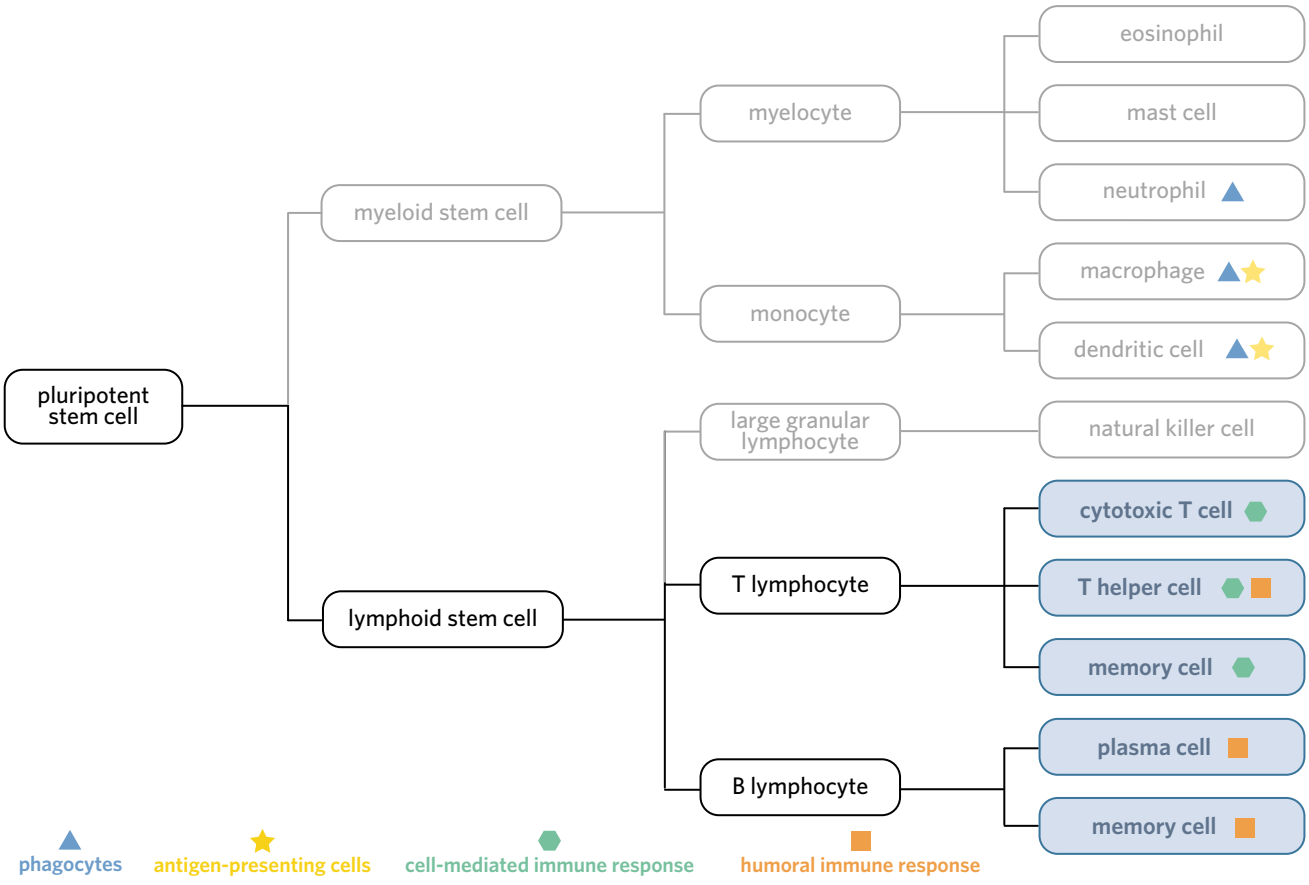


Figure 2 Highlighted are the cellular components of the adaptive immune system

Antigen presentation

A key process in the initiation of the adaptive immune response involves the selection of a type of **T lymphocyte** called a **T helper cell** via a process called antigen presentation (Figure 3).

T lymphocyte a type of lymphocyte that plays an important role in cell-mediated immunity. It differentiates into cytotoxic T cells, T memory cells, and T helper cells

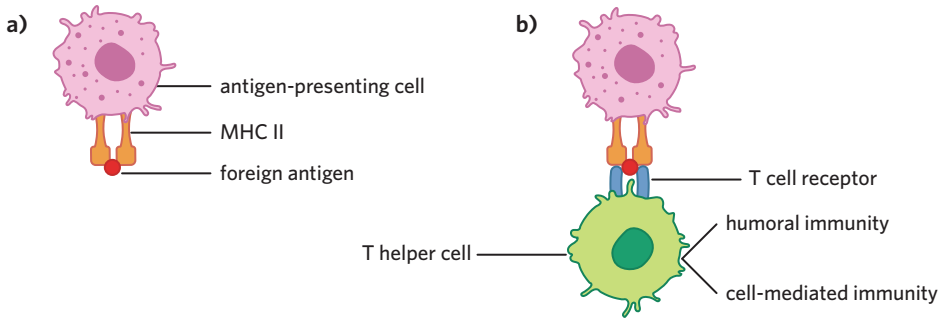


Figure 3 **(a)** An antigen-presenting cell displaying a foreign antigen via MHC II. **(b)** An antigen-presenting cell presenting an antigen to a complementary T cell receptor on a T helper cell, consequently activating it.

From lesson 7C, you should remember that **antigen-presenting cells** (APCs) engulf and digest pathogens via phagocytosis, displaying pathogenic antigens on their MHC II markers. After this process, they travel via the **lymphatic system** to **lymph nodes** to present foreign antigens on their surface using MHC II proteins. These then interact with complementary T cell receptors on the surface of T helper cells. Importantly, each T helper cell has a unique set of T cell receptors for a single antigen on its surface, facilitating the specificity of the adaptive immune response.

When this interaction occurs, the T helper cell becomes activated and is said to be 'selected'. The activated T helper cell can then help initiate the adaptive immune response through either the **humoral** or **cell-mediated immune responses**.

> ***Lesson link***
>
> The lymphatic system, which facilitates the transportation of antigen-presenting cells for antigen recognition and initiation of the adaptive immune response, is explored in ***lesson 7E***.

Humoral immunity 4.1.5.1

OVERVIEW

Humoral immunity involves the neutralisation and destruction of extracellular pathogens via the production and secretion of antibodies.

THEORY DETAILS

B lymphocytes, which are a type of white blood cell, are the key mediators of humoral immunity. Their surfaces are covered in B cell receptors, also known as **antibodies**. They travel around the body in the bloodstream and reside in high numbers within lymph nodes. The activation of these B lymphocytes occurs through their interaction with pathogenic antigens and T helper cells. Below, the humoral immune response is broken up into key individual stages.

1. A pathogen with an antigen that is complementary in shape to the antigen-binding site on the receptor of a B cell interacts with that B cell. When this occurs, the B cell is said to have been 'selected'.
2. Once a B cell has been selected, a T helper cell selected through antigen presentation, which also has a complementary receptor to the antigen, will recognise the selected B cell and secrete a number of different **cytokines**. These cytokines cause the B cell to undergo **clonal expansion**, through which many copies of the selected B cell are produced. The process of selecting the specific T helper cell and B cell is termed **clonal selection**.
3. In addition to cloning, the T helper cell also stimulates the selected B cell via cytokines to undergo the process of **differentiation**, in which the clones of the selected B cell are driven to differentiate into two different types of B cells – **B memory cells** and **plasma cells**.
4. Plasma cells are differentiated clones of the selected B cell. After differentiating, they secrete antibodies into the blood in order to defend against the selected pathogen.

T helper cell (T_h) a type of differentiated T lymphocyte that supports the functioning of a number of different immune cells, including the cloning and differentiation of selected T and B cells

antigen-presenting cell a subgroup of phagocytes that display the antigens from consumed pathogens on their surface and interact with the adaptive immune system

lymphatic system a large network of vessels and tissues throughout the body that form an important component of both the circulatory and immune systems

lymph node a small secondary lymphoid tissue of the lymphatic system where antigen-presenting cells activate the adaptive immune system

humoral immunity an adaptive immune response in which extracellular pathogens are targeted by specific antibodies produced by plasma cells. Also known as **B cell immunity**

cell-mediated immunity an adaptive immune response in which infected or abnormal cells are destroyed by cytotoxic T cells. Also known as **T cell immunity**

B lymphocyte a type of lymphocyte that plays an important role in humoral immunity and differentiates into plasma cells and B memory cells

antibody a protein produced by plasma cells during the adaptive immune response that is specific to an antigen and combats pathogens in a variety of ways. Also known as **immunoglobulin**

cytokine a signalling molecule released by cells (typically in the immune system) which aids in communication between immune cells and helps protect against pathogens

clonal expansion the process in which many copies of a lymphocyte are generated

clonal selection the process in which B and T cells encounter an antigen that matches their antigen-binding site, and then generate many copies of themselves

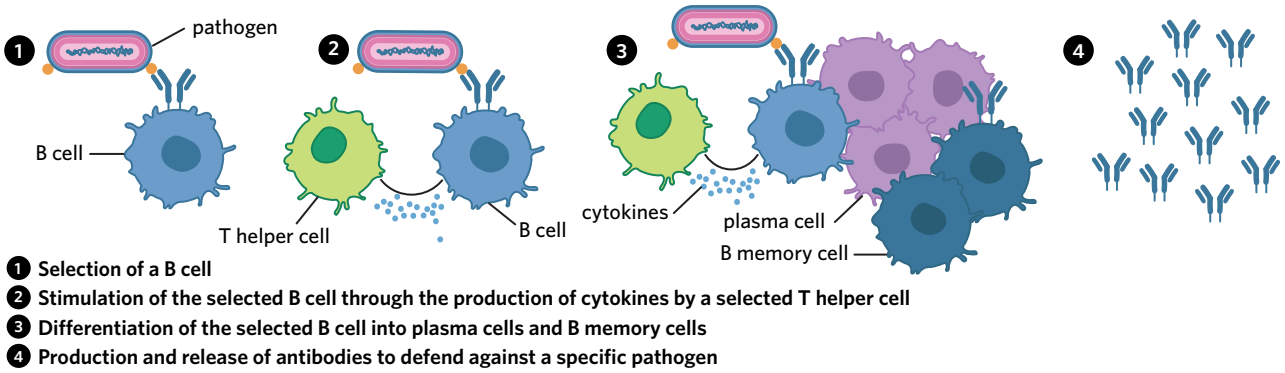


Figure 4 A summary of the humoral immune response

Additionally, the other product of B cell differentiation – B memory cells – are clones of the selected B cell that reside in the body for a prolonged period of time and are responsible for immunological memory. These will be examined more thoroughly later in this lesson.

differentiation the process in which cells develop specialised characteristics, typically transforming them from one cell type to another more specialised cell type

B memory cell a differentiated B lymphocyte that is responsible for providing long-lasting immunological memory of an antigen

plasma cell a differentiated B lymphocyte that is responsible for the generation and secretion of antibodies during the humoral response

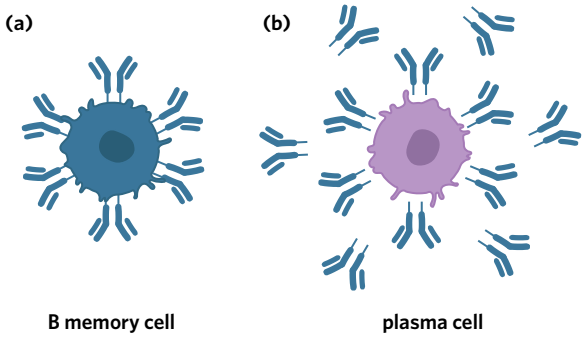


Figure 5 A B cell can differentiate into **(a)** B memory cells and **(b)** plasma cells which secrete antibodies.

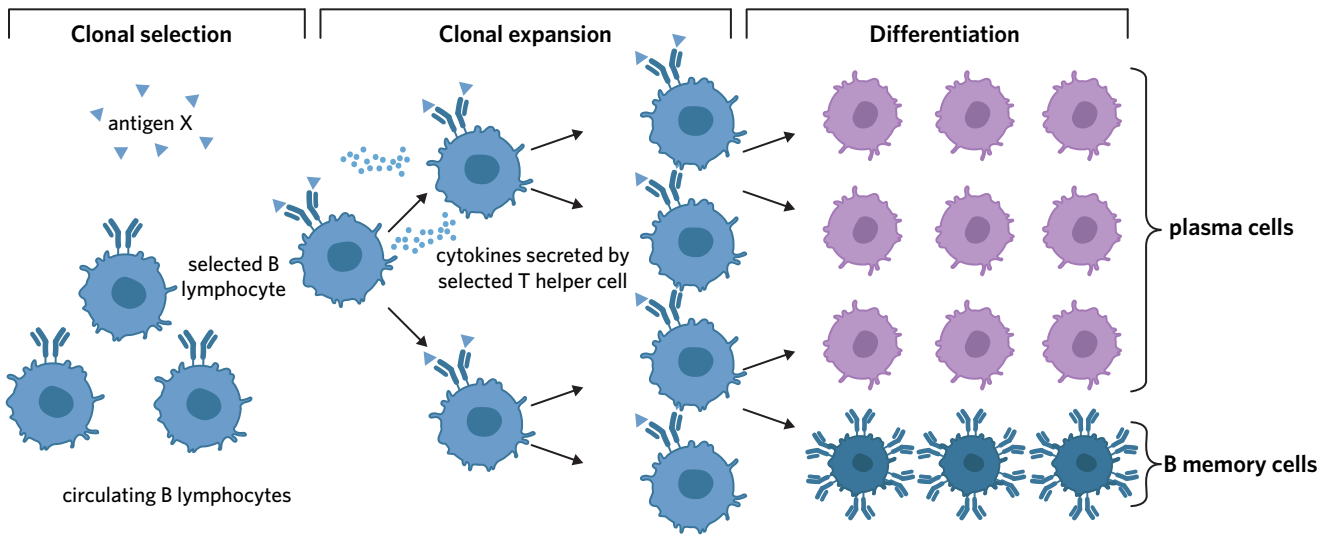


Figure 6 The processes of B cell clonal selection, expansion, and differentiation

Antibodies

Antibodies released by plasma cells are proteins. They are composed of four polypeptide chains, including two heavy chains and two light chains (Figure 7). The two heavy chains are joined by a **disulphide bond**. Each antibody also has a constant region and a variable region. These regions come together to form two identical antigen-binding sites that allow antibodies to perform their role – bind with antigens on the surface of pathogens. As there are two antigen-binding sites present, an antibody can bind with two pathogens at once.

disulphide bond a strong covalent bond occurring between two sulphur atoms

Additionally, like T cells, each B cell produces unique receptors that are complementary in shape to a specific antigen. There are five types of antibodies – IgA, IgD, IgE, IgG, IgM – with each serving a slightly different function. Importantly, the different types of antibodies are also secreted at different times in the immune response. A summary of the antibody types can be found in Table 1.

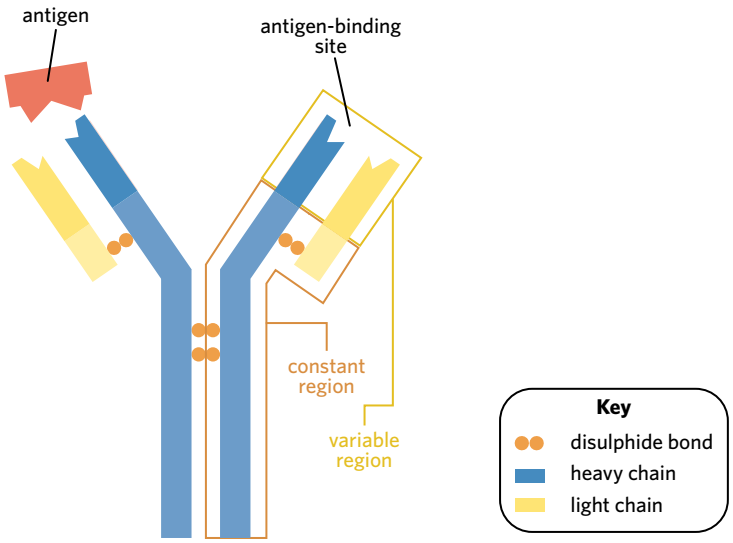


Figure 7 The structure of an antibody. Note the complementary structures of the antigen-binding site and the specific antigen.

Table 1 Types of antibodies

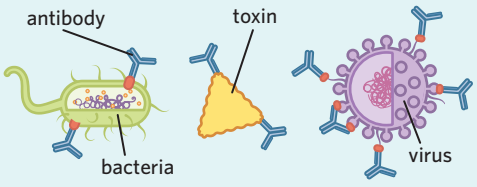
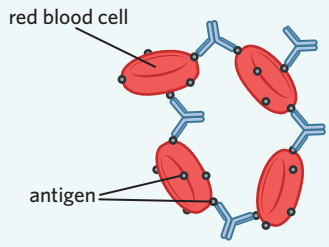
Type	Characteristic
IgA	Found in mucus, breast milk, and saliva.
IgD	Important for the activation of other immune cells.
IgE	Protects against parasitic worms. Also responsible for allergic reactions.
IgG	Most common antibody found in the body. Able to cross the placenta and travel to the foetus.
IgM	The first type of antibody produced by plasma cells in response to an infection.

Antibodies that have been secreted into the blood will eventually come into contact with the pathogen that was originally presented to the selected B cell. Due to the process of clonal selection, these antibodies are specific and have an antigen-binding site that is complementary to the antigens located on the pathogen. Antibodies interact with pathogenic antigens in a number of key ways in the humoral immune response (Table 2).

Examiners' tip

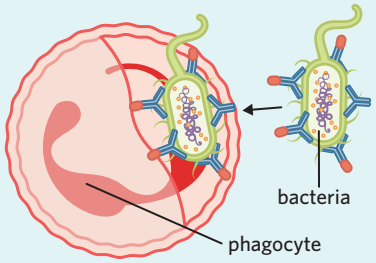
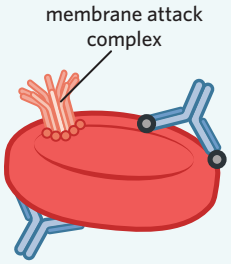
The VCAA probably won't expect you to know in detail what each type of antibody does. You are, however, expected to know about the general structure of antibodies (Figure 7).

Table 2 The key functions of antibodies

Function	Description	Diagram
Neutralisation	Antibodies can block the sites of pathogens that are used to attack host cells (e.g. the site used by a virus to enter a cell) and can block the active sites of toxins.	
Agglutination	Antibodies can bind together with antigens on two separate pathogens, forming large **antigen-antibody complexes**. This makes it easier for phagocytes to recognise the pathogens as foreign bodies and destroy them.	
Immobilisation	Antibodies can also restrict the movement of pathogens around the body through the formation of large antigen-antibody complexes.	

cont'd

Table 2 Continued

Function	Description	Diagram
Opsonisation	Antibodies can bind directly to the surface of a pathogen to make it easier to phagocytose.	
Activation of complement proteins	Antibodies attached to the surface of pathogens can facilitate the actions of complement proteins, including the formation of **membrane attack complexes (MACs)**.	

Theory in context

RED BLOOD CELLS (PART 2)

In lesson 7A, you learned that the antigens on the surface of red blood cells (RBCs) are the basis of how we determine blood types. For example, a person with type A blood displays the A antigen on the surface of their RBCs.

Just like any antigen, RBC antigens are recognised by the immune system and stimulate the production of antibodies. An individual with type A antigens will have anti-B antibodies. If they are transfused blood that is type B or AB, the anti-B antibodies in their blood will agglutinate the introduced type B antigens on the RBCs. This is why patients who are transfused the wrong blood type become extremely sick so quickly – their preformed antibodies quickly react to the foreign antigen presented on the transfused blood and launch an immune response against it.

The following diagram shows the antigens and antibodies present in each blood type.

	Type O	Type A	Type B	Type AB
Red blood cell type				
Antibodies in plasma	 anti-A + anti-B	 anti-B	 anti-A	none
Antigens on red blood cells	none	 antigen A	 antigen B	 antigen A + antigen B

Figure 8 Antigens and antibodies present in each blood type

In addition to the ABO grouping system, another blood grouping system is based on the presence or absence of another common antigen – the **Rhesus antigen**. This antigen is either present (Rhesus positive, R+) or absent (Rhesus negative, R-) on the surface of a person's RBCs. Blood types are usually classified using these two systems (although other rarer blood type systems do exist) giving eight possible blood types:

- A+
- A-
- B+
- B-
- O+
- O-
- AB+

agglutination the clumping of particles together. In the immune system, antibodies can help clump pathogens together

antigen-antibody complex a structure formed by the complementary binding between antigen and antibody molecules

membrane attack complex (MAC) a pore formed by complement proteins in the cell membranes of a pathogen, disrupting the membrane and leading to the pathogen's death

Rhesus antigen an antigen on the surface of red blood cells that can cause an immune response if not matched correctly between donor and receiver

Theory in action

Check out scientific investigation 7.1 to put this into action!

Theory in context

HAEMOLYTIC DISEASE OF THE NEWBORN

Haemolytic disease of the newborn (HDN) is an example of an antibody-mediated disease.

Normally a foetus' blood and its mother's blood remain separate. During birth, however, as the placenta separates from the wall of the uterus, some of the baby's blood can mix with the mother's circulating blood. This normally does not cause any issues. However, if the mother is Rhesus negative (e.g. A-) but the baby is Rhesus positive (e.g. A+), then the mother's immune system will recognise the Rhesus positive antigen as foreign and produce antibodies against it (these are called anti-D or anti-Rh antibodies).

Fortunately, these anti-D antibodies don't affect the foetus as it is usually born by the time they're produced. If, however, the same woman becomes pregnant in the future with another Rhesus positive foetus, then these anti-D antibodies can cross the placenta and destroy the RBCs of the second foetus. If this occurs the foetus can develop anaemia or, in severe cases, die.

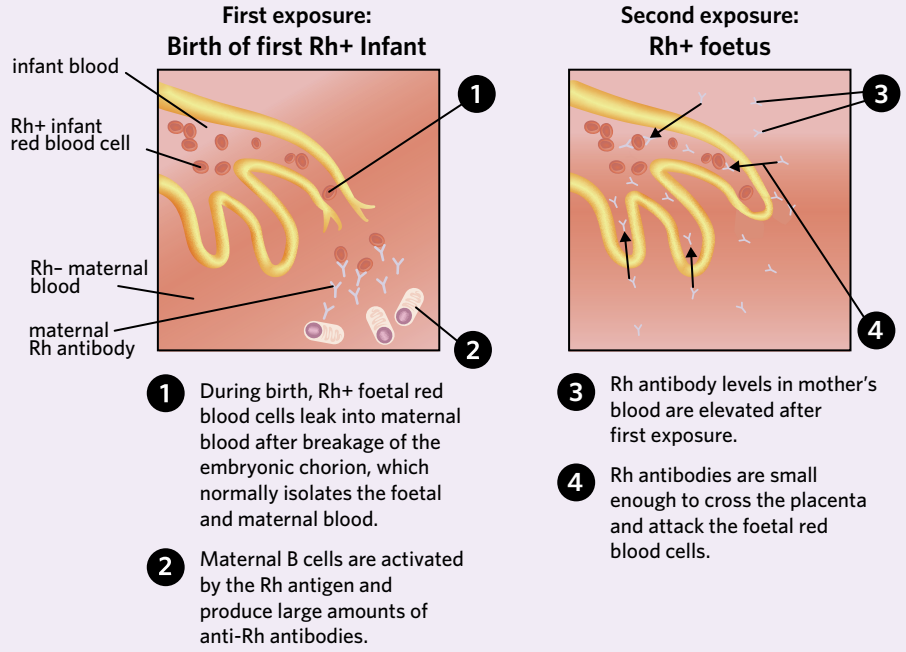


Figure 9 Haemolytic disease of the newborn (HDN)

Cell-mediated immunity 4.1.5.2

OVERVIEW

Cell-mediated immunity involves the destruction of infected or abnormal cells via the clonal selection of a cytotoxic T cell.

THEORY DETAILS

Cytotoxic T cells, which are a type of T lymphocyte, are the key players of cell-mediated immunity. They primarily carry out their role by assessing the MHC I marker of infected cells. From lesson 7A, you should remember that all nucleated cells display MHC I. In addition to their role of self-recognition, MHC I can also display antigens that have been broken down in a cell on its surface. Therefore, in a cell that has been infected with a virus, their MHC I may present foreign viral antigens on its surface, which can be detected by cytotoxic T cells. Below, the cell-mediated immune response is broken up into key individual steps.

cytotoxic T cell (T_c) a differentiated T lymphocyte that is responsible for the destruction of infected or abnormal cells

1 At the same time as the selection of T helper cells, antigen-presenting cells eventually come upon a naive T cell with a T cell receptor that matches the antigen being presented, initiating the process of clonal selection (Figure 10a). When this occurs, the naive T cell becomes selected and is stimulated by cytokines released by the selected T helper cell to undergo the processes of clonal expansion and differentiation (Figure 10b).

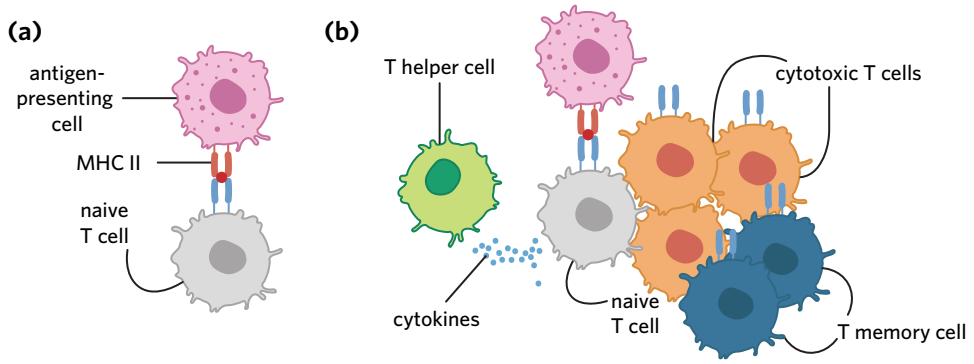


Figure 10 **(a)** Antigen-presenting cell presenting an antigen to a complementary T cell receptor on a naive T cell, consequently selecting it. **(b)** Cloning and differentiation of a selected T cell into cytotoxic T cells and T memory cells via the secretion of cytokines from a selected T helper cell.

2 The clones of the selected T cell differentiate into two types of T cells – cytotoxic T cells and **T memory cells**. T memory cells, like B memory cells, are copies of the originally selected T cell that reside in the body for extended periods of time and help form immunological memory. The majority of selected T cells differentiate into cytotoxic T cells, which leave the lymph node and travel throughout the body, eventually reaching the site of infection.

3 Due to the process of clonal selection, the cytotoxic T cells that arrive at the site of infection all have T cell receptors that are specific to the foreign antigen selected for. Once the cytotoxic T cell has found an abnormal cell that is presenting complementary foreign antigens on its MHC I complex, it binds to it via interactions between its T cell receptor and the antigen-MHC I complex (Figure 11a). Chemicals, such as perforin, are then secreted by the cytotoxic T cell to induce **apoptosis** in the cell (Figure 11b).

T memory cell a differentiated T lymphocyte that is responsible for providing long-lasting immunological memory

apoptosis the controlled death of cells in the body. Also known as **programmed cell death**

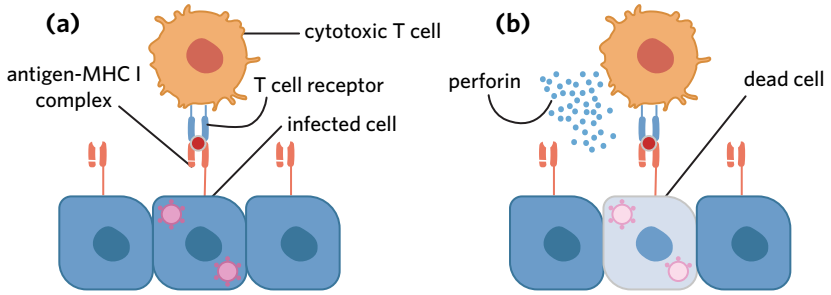


Figure 11 **(a)** A cytotoxic T cell recognising an infected cell via interaction with its MHC I receptor. **(b)** A cytotoxic T cell killing an infected cell by releasing chemicals that induce apoptosis.

Examiners' tip

Humoral immunity primarily acts against extracellular pathogens, whereas cell-mediated immunity primarily acts against intracellular pathogens. Cell-mediated immunity is also responsible for destroying cells that have become abnormal (e.g. cancer cells) and is the primary source of organ rejection (it recognises the non-self organ as foreign and creates cytotoxic T cells that attack the transplanted organ).

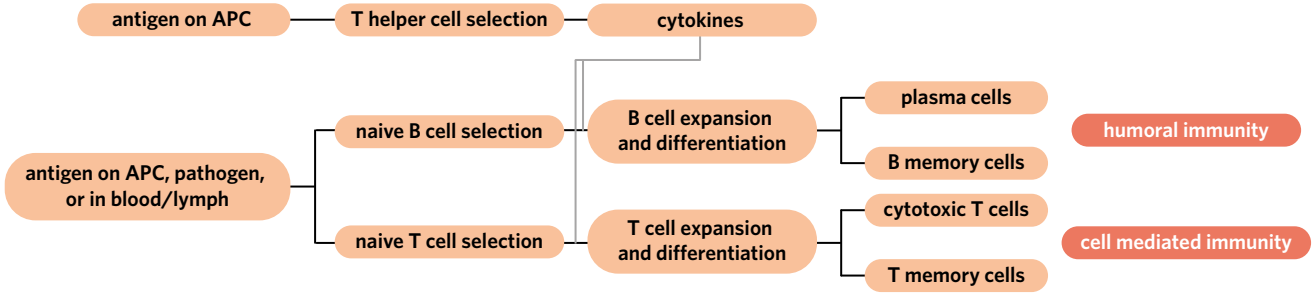


Figure 12 A flowchart depicting the third line of defence

Immunological memory 4.1.5.3

OVERVIEW

B memory cells and T memory cells formed during the adaptive immune response remain in the blood for an extended period of time, allowing the body to respond to pathogens it has previously encountered quickly and effectively.

THEORY DETAILS

A key component of both the humoral and cell-mediated adaptive immune responses is the creation of B and T memory cells, respectively. Each of these cells confers the body with long-lasting immunological memory:

- B memory cells contribute to immunological memory by rapidly dividing and forming new antibody-producing plasma cells when they encounter an antigen that matches their receptor.
- T memory cells proliferate rapidly into T helper cells and cytotoxic T cells upon stimulation by an antigen-presenting cell that is presenting a previously encountered antigen.

B memory cells also create immunological memory by constantly secreting low amounts of their antibody. In this way, a person who is immune to a pathogen will always have trace amounts of the antibody against that pathogen in their blood.

Immunological memory has a number of advantages, including the creation of a more rapid and effective immune response upon re-infection, as antibodies are produced at a greater rate and cytotoxic T cells are created more rapidly to kill any infected cells. Therefore, immunological memory can help prevent the formation of disease in those re-exposed to a previously encountered pathogen, as that pathogen can no longer replicate fast enough to cause disease.

Lesson link

The generation of memory cells and the formation of immunological cells forms the basis of vaccinations, which are explored in ***lesson 8A***.

Theory in context

BYE BYE BOTOX!

Botox injections are a common cosmetic treatment used to reduce the appearance of people's wrinkles. But did you know that the substance injected into patients is actually a toxin produced by the bacterium *Clostridium botulinum*? This toxin paralyses muscle fibres by interfering with the nerves that control them, preventing the muscle from contracting. These 'relaxed' muscles no longer pull on the overlying skin, seemingly making wrinkles disappear (Figure 13)!

In some people, the effectiveness of Botox treatment decreases over time. This is due to immunological memory. In these people, the immune system has recognised Botox as a non-self antigen and has initiated an adaptive immune response, including the production of antibodies and memory B cells. These memory B cells reside in the body for an extended period of time. When they encounter a Botox antigen again, they are able to quickly produce a large number of antibodies to neutralise the toxin, thereby reducing its effect.

Image: TanyaLovus/Shutterstock.com

Figure 13 Before and after Botox treatment

Theory summary

The adaptive immune system is composed of two responses. The humoral response results in the destruction of pathogens via the release of antibodies from plasma cells. The cell-mediated response results in the killing of infected or abnormal cells by cytotoxic T cells. Both the humoral and cell-mediated responses result in the creation of immunological memory which allows the body to respond more quickly and effectively when it re-encounters a pathogen.

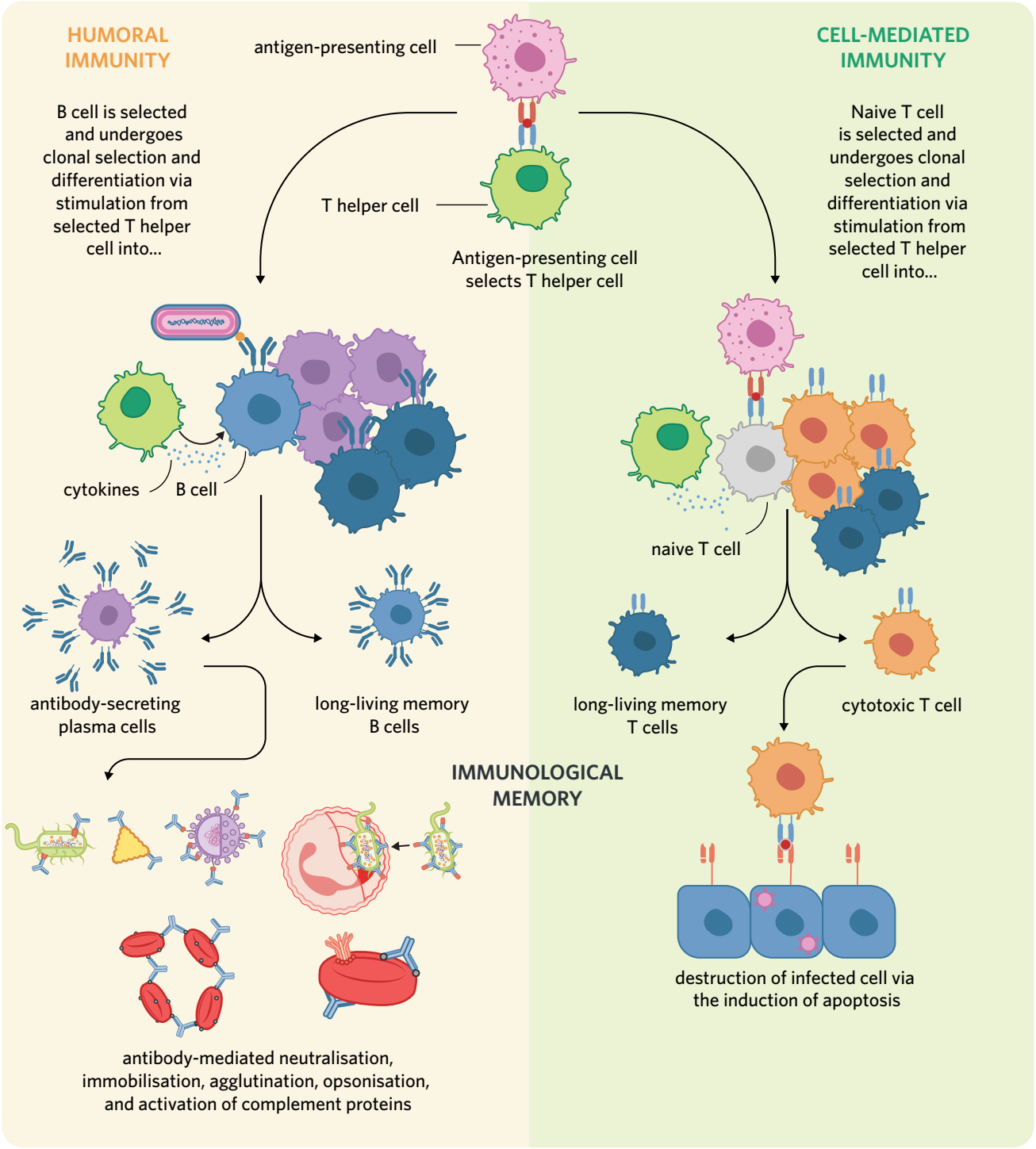


Figure 14 Summary of the third line of defence

Bruce was able to penetrate our first line of defence and then overcome the second line of defence. As soon as his antigen was presented by antigen-presenting cells to T helper cells and the complementary B cell was selected, however, he stood no chance. Our immune system's third line of defence is a formidable force, with each plasma cell secreting antibodies at an astonishing rate of approximately 2 000 antibodies per second.

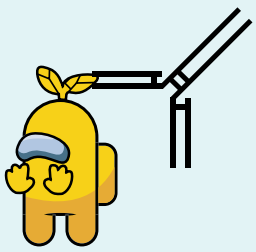


Image: Maybielater/Shutterstock.com

7D QUESTIONS

Theory review questions

Question 1

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- adaptive immune system
- innate immune system
- complement proteins
- T helper cell
- cytokines
- B cell
- T cell

The _____ is composed of humoral immunity and cell-mediated immunity. Activation of either of these pathways begins with the presentation of a pathogenic antigen by an antigen-presenting cell to a complementary _____. After selection, a T helper cell secretes _____ to activate the selected B or T cell.

Question 2

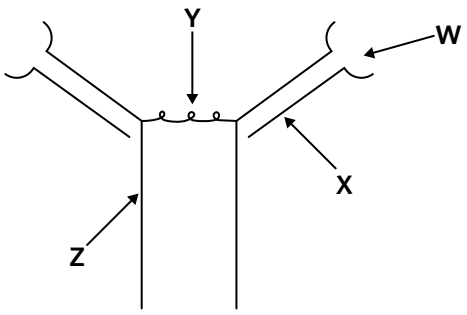
Order the steps to correctly describe the humoral immune response.

I Extracellular pathogens are phagocytosed by antigen-presenting cells.
II T helper cells produce cytokines to stimulate selected B cells.
III B cells differentiate into plasma cells and B memory cells.
IV Antibodies travel to the pathogen and bind to the antigen.
V Antigen-presenting cells interact with T helper cells.

Question 3

Label the parts of the antibody from the list of terms.

- antigen-binding site
- disulphide bridge
- heavy chain
- light chain



Question 4

Match the different antibody interactions to their descriptions.

Antibody interaction	Description
• neutralisation	**I** _____ the formation of large antigen-antibody complexes to facilitate phagocytosis
• agglutination	**II** _____ binds to the surface of pathogens to make it easier to phagocytose
• opsonisation	**III** _____ blocks sites of pathogens used to attack host cells
• immobilisation	**IV** _____ restricts the movement of pathogens

Question 5

Match the cells of the adaptive immune response to their descriptions.

Cell	Description	
• plasma cell	**I** _____	produces antibodies that help defend against pathogens
• memory cell	**II** _____	helps prevent future infections through its rapid proliferation upon re-exposure
• T helper cell	**III** _____	induces apoptosis in infected or abnormal cells through the secretion of chemicals
• cytotoxic T cell	**IV** _____	forms the bridge between the innate and adaptive immune systems by interacting with antigen-presenting cells

SAC skills questions

Scientific methodology comparison

Use the following information to answer Questions 6–10.

Tuberculosis is a disease caused by the bacterium *Mycobacterium tuberculosis*. Unlike other bacteria, *M. tuberculosis* is known as an extremely successful pathogen due to its ability to adapt and survive within its host. Transmission of *M. tuberculosis* occurs through airborne droplets formed through sneezing and coughing, eventually entering the lungs, which is where *M. tuberculosis* primarily inhabits.

Once inside the lungs, it is phagocytosed by macrophages. However, macrophages are incapable of digesting *M. tuberculosis* due to the composition of its cell wall, which prevents the fusion of a lysosome with the vesicle containing the bacteria. Therefore, *M. tuberculosis* is able to safely replicate inside the macrophage. *M. tuberculosis* has also been reported to interfere with the process of antigen-presentation, thereby helping prevent the initiation of the adaptive immune response. In an effort to investigate the response of the human immune system against *M. tuberculosis*, two scientists set up separate experiments.

- Scientist A recruited a large population of mice, creating two groups. Group X was infected with *M. tuberculosis* and Group Y was left uninfected. Each day after the initial infection, the scientist measured the levels of macrophages, dendritic cells, B cells, and antibodies in each of the mice.
- Scientist B visited a hospital and recruited a small population of admitted patients with confirmed *M. tuberculosis* infections. This scientist used data from previous blood tests conducted at the hospital, which also included information about the levels of various white blood cells.

Question 6

Mycobacterium tuberculosis is capable of

A evading the release of lysozymes.

B preventing phagocytosis.

Question 7

The humoral immune response against *M. tuberculosis* would be best measured by the level of

A cytotoxic T cells.

B macrophages.

C T helper cells.

D plasma cells.

Question 8

Before accessing patient records, Scientist B must gain consent from patients. The most relevant bioethical concept which Scientist B must comply with is

A non-maleficence.

B beneficence.

C respect.

D justice.

Question 9

Which scientist is likely to have produced the most precise results?

A Scientist A

B Scientist B

Question 10

Which scientist is likely to have produced the most accurate results?

A Scientist A

B Scientist B

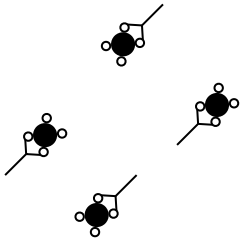
Exam-style questions

Within lesson

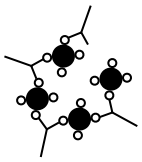
Question 11 (1 MARK)

Which of the following correctly depicts an antigen-antibody complex?

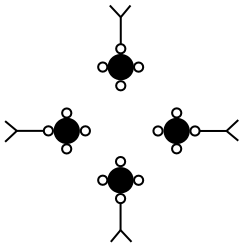
A



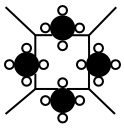
B



C



D



Question 12 (1 MARK)

Chronic lymphocytic leukaemia is a type of cancer that affects the production of lymphocytes in the bone marrow. Often, B cells multiply uncontrollably and are unable to differentiate, leaving the patient vulnerable to recurring infections from normally non-pathogenic bacteria.

Based on the information provided, patients with chronic lymphocytic leukaemia

A won't be able to form memory B cells.

B won't be able to form cytotoxic T cells.

C will have non-functioning T helper cells.

D will have more antibodies in their system.

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q2

Question 13 (1 MARK)

The third line of defence against pathogens includes the

A killing of infected cells by T memory cells.

B formation of antigen-antibody complexes.

C secretion of antibodies by cytotoxic T cells.

D presentation of antigens to lymphocytes by neutrophils.

Adapted from VCAA 2014 Section A Q15

Question 14 (1 MARK)

Botox injections are a cosmetic treatment that reduces facial wrinkles by paralysing the muscles connected to nerve cells. Botox injections contain small amounts of weakened botulinum toxin. The muscle paralysis from the initial injections lasts for about four months. Muscle paralysis from subsequent injections lasts for shorter periods of time.

The production of which of the following cells could be responsible for the decreasing effectiveness of the Botox injections over time?

A antigen-presenting cells
B cytotoxic T cells
C memory B cells
D T helper cells

Adapted from VCAA 2018 Section B Q4

Question 15 (6 MARKS)

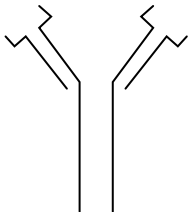
Rotaviruses are a group of RNA viruses and are a common cause of diarrhoea among infants and young children. Usually, most people recover from a rotavirus infection after 3–7 days, however, in some cases, severe dehydration and death can sometimes occur.

a Outline how the body would eliminate cells infected by rotavirus. (4 MARKS)
b Explain whether antibodies could be produced in response to a rotavirus infection. (2 MARKS)

Multiple lessons

Question 16 (10 MARKS)

Antibodies form a crucial component of the humoral immune response against extracellular pathogens. Their structure is composed of two heavy chains and two light chains. The following diagram presents an antibody.



a Draw a generalised pathogen against which this antibody would be effective. Include four surface antigens in your drawing and label one of them. (2 MARKS)
b State three ways antibodies provide protection against pathogens. (3 MARKS)
c Outline the body's response to an extracellular pathogen. (3 MARKS)
d Antibodies are proteins composed of a sequence of amino acid monomers, eventually forming a large complex structure. Explain what 'tertiary' structure is and what is responsible for its formation. (2 MARKS)

Adapted from VCAA 2014 Section B Q4 and VCAA 2016 Section B Q1

Question 17 (6 MARKS)

Both the adaptive and innate immune systems in vertebrates feature phagocytes.

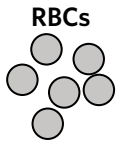
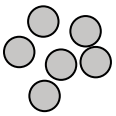
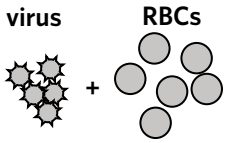
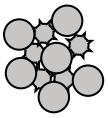
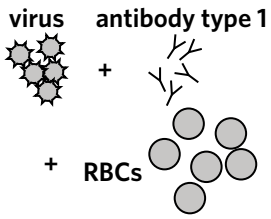
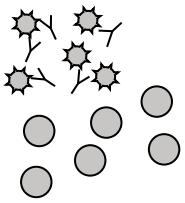
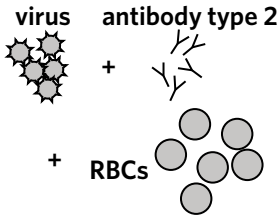
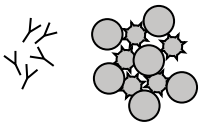
a Compare the innate and adaptive immune responses. (2 MARKS)
b A phagocytic cell found in the body has MHC II proteins displayed on its surface and plays an important role in the adaptive immune response. Name this cell type and explain how MHC II assists this cell in performing its function. (2 MARKS)
c One type of phagocytic cell is not involved in the activation of the adaptive immune response. Identify this cell type, and explain why it is not involved in activating the adaptive immune response. (2 MARKS)

Adapted from VCAA 2015 Section B Q5

Key science skills and ethical understanding

Question 18 (8 MARKS)

Scientists performed an experiment in which they observed the interaction of red blood cells (RBCs), the influenza virus, and antibodies extracted from different mice *in vitro*. Two different mixtures of antibodies were used. The first mixture of antibodies (antibody type 1) was extracted from a mouse that was infected with influenza whilst the second mixture (antibody type 2) was extracted from a mouse that had never been exposed to the virus. The results are shown in the table.

	Components added to the well	Interaction	Observation
Well A			no clumping of red blood cells
Well B			clumping of red blood cells
Well C			no clumping of red blood cells
Well D			clumping of red blood cells

a State a hypothesis that the scientists could be testing with this experiment. (1 MARK)

b Identify a well that served as a control group. Explain your reasoning. (2 MARKS)

c Identify the dependent and independent variables in this experiment. (2 MARKS)

d Explain one mechanism by which the antibodies prevent the clumping of red blood cells. (1 MARK)

e Describe how the bioethical concept of non-maleficence could be applied to this experiment. (2 MARKS)

Adapted from VCAA 2017 Northern Hemisphere Exam Section A Q17

7E THE LYMPHATIC SYSTEM

Fortunately, Bruce is now being destroyed via the action of antibodies as part of our humoral immune response and we should return to our healthy self in a few days. The delay in the activation of our adaptive immune system, however, has caused us much pain and suffering as Bruce was allowed to roam free throughout our body unchecked. Why did it take so long for the adaptive immune system to initiate? Why was Bruce given the chance to wreak havoc inside our body?

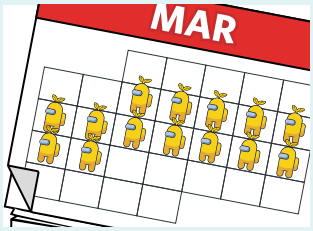


Image: Maybielater/Shutterstock.com

Lesson 7E

In this lesson you will learn how the lymphatic system serves as a transport system for antigen-presenting cells to facilitate antigen recognition.

◀ *Prerequisite knowledge*

Lesson 7C
Macrophages and dendritic cells are two types of antigen-presenting cells that are critical in the second line of defence.

Lesson 7D
The lymphatic system is where the initiation of the adaptive immune system takes place through antigen-presenting cells. These cells present pathogenic antigens to cells of the adaptive immune system. It also serves as the location of leukocyte production and maturation.

Future applications ▶

Lesson 8A
As the site of antigen transportation and the activation of the adaptive immune system, the lymphatic system serves as a crucial component of acquiring immunity in the immunisation process.

Study design dot point

- the role of the lymphatic system in the immune response as a transport network and the role of lymph nodes as sites for antigen recognition by T and B lymphocytes

Key knowledge units

Introduction to the lymphatic system	4.1.4.1
The lymphatic system as a transport network	4.1.4.2

Introduction to the lymphatic system 4.1.4.1

OVERVIEW

The lymphatic system is a large network of vessels throughout the body through which lymph flows. It has two primary functions – to act as a transport system for antigen-presenting cells and pathogens and to serve as the location of clonal selection.

THEORY DETAILS

Everyone's heard of the 'rockstar' systems of the body – the **circulatory system**, the digestive system, and the nervous system. The **lymphatic system**, however, is the Luke Hemsworth of body systems – it is just as important as the others (just ask Mrs Hemsworth!), yet it doesn't get anywhere near as much attention. Well, all that's about to change!

circulatory system a collection of tissues and organs involved in the transportation of substances around the body. Composed of the lymphatic and cardiovascular systems

lymphatic system a large network of vessels and tissues throughout the body that form an important component of both the circulatory and immune systems

Functions of the lymphatic system

The lymphatic system is a core component of the body's immune system. It has a number of major functions:

- transportation of **antigen-presenting cells** to **secondary lymphoid tissues** for antigen recognition and initiation of the adaptive immune response
- production of leukocytes, including lymphocytes in **primary lymphoid tissues**
- removal of fluid from tissues around the body
- absorption of fatty acids from the digestive system.

While the lymphatic system has a variety of roles within the body, the VCAA only requires you to know in detail about its relevance to the immune system and how it serves as the initiation site of the adaptive immune response. But before we delve into how the lymphatic system carries out these functions, we will explore the various components that make up the lymphatic system.

antigen-presenting cell a subgroup of phagocytes that display the antigens from consumed pathogens on their surface and interact with the adaptive immune system

secondary lymphoid tissue components of the lymphatic system that are responsible for the maintenance of mature lymphocytes and the activation of the adaptive immune response. Includes lymph nodes and the spleen

primary lymphoid tissue components of the lymphatic system that are responsible for the production and maturation of lymphocytes. Includes bone marrow and the thymus

lymph a pale fluid that flows through the lymphatic system and has a high concentration of leukocytes

Components of the lymphatic system

The lymphatic system is comprised of a series of lymphatic vessels throughout the body that function to transport **lymph** to a number of primary and secondary lymphoid tissues.

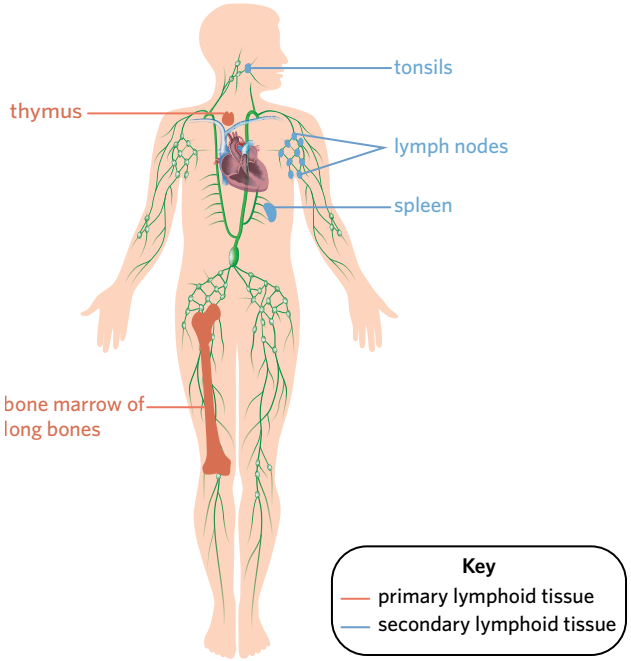


Image: Alila Medical Media/Shutterstock.com

Figure 1 Overview of the lymphatic system

Primary lymphoid tissues

Primary lymphoid tissues are responsible for the creation and maturation of lymphocytes. The main primary lymphoid tissues include the **bone marrow** and the **thymus** (Figure 1).

The production of B and T lymphocytes occurs in the bone marrow, which is primarily found inside long bones such as the femur and humerus. While B lymphocytes remain in the bone marrow to mature further, T lymphocytes travel to the thymus to mature. Therefore, depending on where a lymphocyte matures, it is possible to determine what type of lymphocyte it will be.

Secondary lymphoid tissues

Secondary lymphoid tissues are responsible for maintaining mature lymphocytes and initiating the adaptive immune response. The main secondary lymphoid tissues include the **lymph nodes** (e.g. **tonsils**) and the **spleen**.

In these tissues, mature lymphocytes are clustered together and 'scan' passing lymph for the presence of any pathogens or antigen-presenting cells. If a foreign antigen matches the receptors of specific lymphocytes, these lymphocytes then undergo **clonal selection** and differentiation. This results in a large number of B and T cells being created within these tissues, resulting in the characteristic swelling of lymph nodes when you're sick (Figure 3)!

bone marrow semi-solid tissue found within bones. Serves as the primary site of the creation of red blood cells and leukocytes

thymus a primary lymphoid organ located in the chest. Serves as the site of T cell maturation

lymph node a small secondary lymphoid tissue found throughout the body where antigen-presenting cells activate the adaptive immune system

tonsils the name given to the two lymph nodes that reside at the back of the throat

spleen an organ located in the upper abdomen that serves a variety of functions in the immune system and the regulation of red blood cells

clonal selection the process in which B and T cells encounter an antigen that matches their antigen-binding site, and then generate many copies of themselves

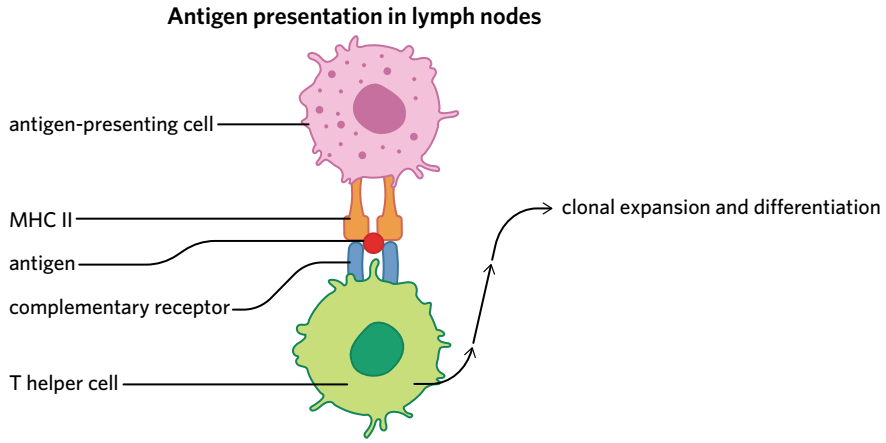


Figure 2 Antigen presentation occurs between an antigen-presenting cell and a T helper cell

Image: SingjaiStocker/Shutterstock.com

Figure 3 A person with an enlarged lymph node in their neck, indicating the presence of a pathogen in the mouth and/or throat

The lymphatic system as a transport network 4.1.4.2

OVERVIEW

One of the primary functions of the lymphatic system is to serve as a transport network for the transportation of antigen-presenting cells to lymph nodes for antigen presentation and the initiation of the adaptive immune response.

THEORY DETAILS

Now that you know the functions and components of the lymphatic system, it's time to dive in and see how everything comes together! Figure 4 summarises how the lymphatic system works, including the three stages – lymphatic drainage, lymphatic flow, and lymphatic surveillance.

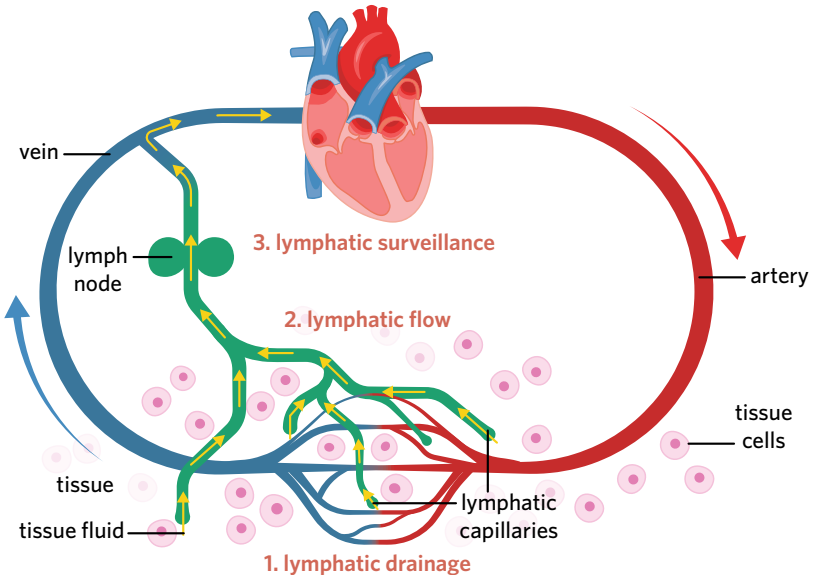


Figure 4 How the lymphatic system works, in three stages

Examiners' tip

In the past, the VCAA has not assessed the minute details of how the lymphatic system works. Extra detail has been added here to provide you with the foundations on which to understand what you are expected to know. The VCAA focuses on the immune-related purposes of the lymphatic system, including how it acts as a transport network for antigen-presenting cells and that it serves as the site for initiating the adaptive immune response. For example, in the 2020 exam, students were questioned about the events which would occur inside a secondary lymphoid tissue in relation to the initiation of the adaptive immune response.

Lymphatic drainage

Fluid from blood vessels constantly leaks into the tissues of the body. From lesson 7C, you should recall that this leakage is increased during an inflammatory response to allow for the movement of leukocytes into tissues. However, if fluid is constantly leaking into tissues, then why don't we swell up like a water balloon? The answer – the lymphatic system!

Lymphatic capillaries are extremely small vessels that exist throughout the tissues of the body, collecting fluid in tissues as well as any pathogens that might be present. Once this clear fluid enters the lymphatic capillaries, it is known as lymph and is carried away into the lymphatic system, where it eventually arrives at a lymph node.

lymphatic capillaries the smallest form of lymphatic vessel. Located in the spaces between cells

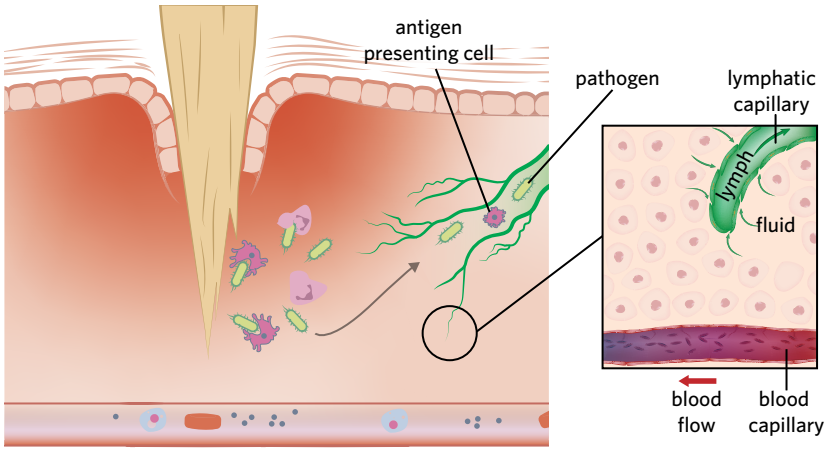


Image: Alila Medical Media/Shutterstock.com

Figure 5 Lymphatic drainage of an injured site. Fluid from tissues is drained through lymphatic capillaries into the lymphatic system for antigen surveillance.

Figure 5 should look familiar – it's a diagram from lesson 7C illustrating the inflammatory response. This time, however, we have included the lymphatic vessels that are present in the tissues that have been affected by the splinter. Note that pathogens from the site of injury, as well as antigen-presenting cells that have consumed pathogens, have been drained from the tissue and are now in the lymphatic system.

Lymphatic flow

The small lymphatic capillaries throughout the body gradually join together to form larger vessels that contain an increasing amount of lymph. These vessels have thin walls and rely on surrounding muscle movements to squeeze lymph fluid through the system. Therefore, it is important to note that the heart is not responsible for pumping lymph.

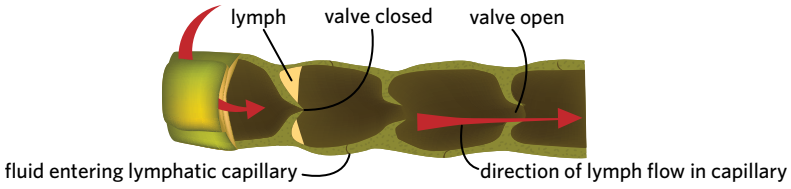


Image: Sakurra/Shutterstock.com

Figure 6 One-way flow of lymph fluid via valves prevents the backflow of lymph

Additionally, lymph vessels feature a number of one-way valves (Figure 6). These ensure that as muscle movements pump lymph, the fluid moves in one direction only – away from the tissues and towards the lymph nodes.

Lymphatic surveillance

Eventually, the fluid drained from tissues arrives at lymph nodes via **afferent lymphatic vessels**. It is here that lymph travels through clusters of B and T cells. As it drains through these clusters, antigen-presenting cells and pathogens are most likely to meet with a lymphocyte that has a matching antigen-binding site and stimulate the process of clonal selection.

afferent lymphatic vessel thin-walled structures that collect lymph from the tissues of the body and deliver it to lymph nodes

efferent lymphatic vessels thin-walled structures that collect lymph that has drained through lymph nodes, returning it back to circulation

Subsequently, if an adaptive immune response is initiated, antibodies and activated cytotoxic T cells will be transported in the lymph away from the lymph nodes via **efferent lymphatic vessels.** This lymph is then returned into circulation near the heart, where the lymphatic vessels join with the large veins returning blood back to the heart, to be pumped around the body.

Additionally, this lengthy process of transportation within the lymphatic system, antigen-presentation, and clonal selection and expansion explain the delay in launching the adaptive immune response. Therefore, unlike the innate immune system, which provides an immediate source of protection, the adaptive immune system is much slower to activate.

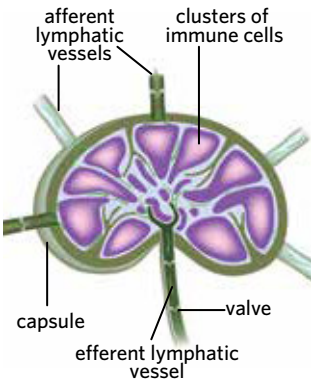


Image: Alila Medical Media/Shutterstock.com

Figure 7 Structure of a lymph node

Memory device

Imagine you have just slipped off the monkey bars and you are worried you have broken your arm. What do you do next? Well, you could run up to all the people who are nearby asking if any of them are doctors, or you could phone an ambulance and get it to take you to the hospital where there are plenty of doctors standing around waiting for someone to look after. Clearly, the second option makes much more sense. The lymphatic system thinks so too. Rather than just leave an antigen-presenting cell floating around the body in the vague hope that it will find a matching B or T cell, it ferries it along to the lymph node where a huge number of B and T cells (doctors) are waiting to find their perfect match.

Memory device

To help remember the flow of lymph through the lymphatic system, simply remember that it's a one-way system, with lymph **a**rriving at lymph nodes via **a**fferent lymphatic vessels and **e**xiting via **e**fferent lymphatic vessels.

Lesson link

If you're unsure about clonal selection and the processes involved in the adaptive immune response, it might be a good idea to revise the material in ***lesson 7D.***

Theory summary

The lymphatic system is a series of vessels and organs that link the different components of the immune system. It transports antigen-presenting cells around the body, specifically from the site of infection to a lymph node, which is the site of clonal selection. The key structures involved in the lymphatic system are summarised in Table 1.

Table 1 Summary of lymphoid tissues

	Structure	Functions
Primary lymphoid tissues	• Bone marrow	• Production of immature B and T cells • Maturation of B cells
	• Thymus	• Maturation of T cells
Secondary lymphoid tissues	• Lymph nodes	• Site where APCs meet lymphocytes
	• Spleen	• Location of clonal selection and expansion of T and B cells, and initiation of the adaptive immune response

Due to the lengthy processes of antigen-presentation and clonal selection and expansion, the adaptive immune system takes a while to activate. Therefore, Bruce was unfortunately given time to roam around our body and cause disease. Luckily for us, however, the production of memory cells will ensure that Bruce won't be able to cause disease in the future.

Image: Maybielater/Shutterstock.com

7E QUESTIONS

Theory review questions

Question 1

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- antigen-presenting cells
- bone marrow
- lymph nodes
- thymus

The lymphatic system is responsible for the transportation of _____ to _____for antigen recognition and the production of leukocytes. Additionally, while both B and T cells are produced in the _____, B cells mature in the _____ and T cells mature in the _____.

Question 2

Categorise the following as **primary lymphoid tissues** or **secondary lymphoid tissues**.

I bone marrow _____
II lymph nodes _____
III thymus _____
IV spleen _____
V tonsils _____

Question 3

In the lymphatic system

A lymph is pumped via the contractions of the heart.
B lymphatic capillaries drain fluid from between cells.
C clonal selection and expansion occur in the bone marrow.
D pathogens travel through the lymphatic system to lymph nodes where they are destroyed by helper T cells.

Question 4

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- clusters of immune cells
- phagocytosis
- clonal selection
- afferent
- efferent

Lymph arrives at the lymph node via _____ lymphatic vessels. From there, it is filtered through _____, which may cause _____ to occur. The lymph is then drained via _____ lymphatic vessels back into circulation.

SAC skills questions

Data analysis

Use the following information to answer Questions 5–9.

Non-Hodgkin's lymphoma is an inclusive term for various subtypes of cancer that affect the production and formation of lymphocytes. The subtypes of cancer can generally be categorised into either B cell lymphomas or T cell lymphomas depending on which lymphocyte is affected. Cases of non-Hodgkin's lymphoma arise when changes in particular genes cause lymphocytes to uncontrollably multiply, forming tumours. When this occurs, the function of the affected lymphocytes is impaired, severely weakening the immune system. For example, if an individual develops B cell non-Hodgkin's lymphoma, then their B cells may no longer be able to differentiate into plasma B cells, preventing the production of antibodies against invading pathogens.

Unfortunately, the exact cause of non-Hodgkin's lymphoma still remains unknown. However, a few risk factors which contribute to its formation include the use of immunosuppressant medication, infection with particular viruses, and exposure to certain chemicals. Symptoms of non-Hodgkin's lymphoma include prolonged fever, sudden weight loss, excessive sweating, and persistent fatigue.

Diagnosis of non-Hodgkin's lymphoma requires various tests such as a full blood examination (FBE) and lymph node biopsies. It is also important to remember that B cells mature in bone marrow while T cells mature in the thymus. In a full blood examination, the levels of various cells found within the blood are measured. In those suffering from non-Hodgkin's lymphoma, the affected lymphocyte count is generally elevated and the red blood cell count is reduced. The following table includes the results of a full blood examination from four different individuals.

	Healthy range	**Individual A**	**Individual B**	**Individual C**	**Individual D**
Haemoglobin (g/L)	115–165	150	116	110	95
Red blood cell count ($x10^{12}$/L)	3.8–5.8	3.9	5.9	3.7	3.5
Haematocrit (L/L)	0.36–0.47	0.40	0.35	0.55	0.37
White blood cell count ($x10^9$/L)	4.0–11.0	3.3	9.4	14.11	11.87
Lymphocytes ($x10^9$/L)	1.0–4.0	2.5	3.6	6.5	4.5
T helper cells ($x10^9$/L)	0.64–1.18	1.15	1.11	1.13	2.75
Neutrophils ($x10^9$/L)	2.0–7.5	0.5	5.5	7.4	7.5
Basophils ($x10^9$/L)	0.0–0.2	0.1	0.0	0.1	0.22
Eosinophils ($x10^9$/L)	0.0–0.4	0.2	0.3	0.11	0.04
Platelets ($x10^9$/L)	150–400	200	333	555	254

Question 5

The immune system of an individual affected by B cell non-Hodgkin's lymphoma will

A no longer be able to produce plasma B cells.

B continue to produce B memory cells.

C have decreased levels of B cells.

Question 6

A patient with non-Hodgkin's lymphoma suffering from a prolonged fever has most likely been

A infected with a particular virus.

B exposure to certain chemicals.

C using immunosuppressant medication.

Question 7

The individual with lower levels of antigen-presentation in lymph nodes is most likely to be

A individual A.

B individual B.

C individual C.

D individual D.

Question 8

The individual suffering from B cell non-Hodgkin's lymphoma is most likely to be

A individual A.

B individual B.

C individual C.

D individual D.

Question 9

The individual with an enlarged thymus is most likely to be

A individual A.
B individual B.
C individual C.
D individual D.

Exam-style questions

Within lesson

Question 10 (1 MARK)

In the lymphatic system

A T cells differentiate into plasma cells.
B transportation of antigen-presenting cells occurs.
C clonal selection occurs via the interaction of antigens and antibodies.
D lymph vessels collect red blood cells and return them to the body's circulation.

Adapted from VCAA 2013 Section A Q20

Question 11 (3 MARKS)

The human lymphatic system consists of primary and secondary lymphoid tissues. The following diagram shows a lymph node.

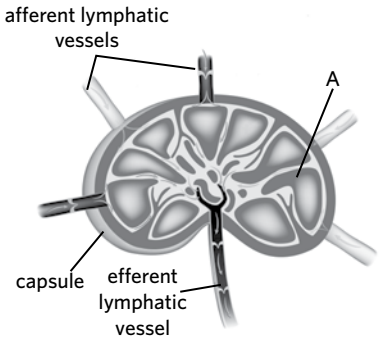


Image: Alila Medical Media/Shutterstock.com

a Explain the role of lymph nodes in the lymphatic system. (2 MARKS)
b Describe the role of the afferent lymphatic vessels. (1 MARK)

Multiple lessons

Question 12 (1 MARK)

It is reasonable to infer that an infection has occurred if

A inflammation has occurred.
B mast cells have degranulated.
C bacteria are found on the surface of the skin.
D a person's lymph nodes have become swollen.

Adapted from VCAA 2011 Exam 1 Section A Q6

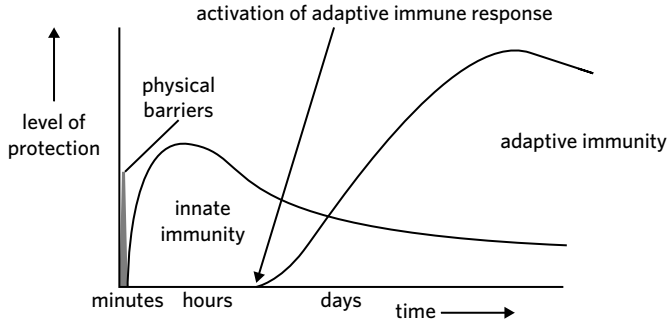
Question 13 (1 MARK)

The lymphatic system

A contains the site of B cell clonal selection.
B is a component of the first line of defence.
C initiates the inflammatory response.
D produces red blood cells.

Question 14 (5 MARKS)

After an individual is exposed to a microbial infection, the immune system increases its activities. The following graph summarises the level of these activities over time.



a Explain the delay seen in the activation of the adaptive immune response. (1 MARK)

b Describe the events occurring in the lymph nodes of the infected individual. (2 MARKS)

c One month later, the individual was re-exposed to the same microbe. Explain whether the microbe was able to cause disease in the individual. (2 MARKS)

Adapted from VCAA 2009 Exam 1 Section A Q21

Question 15 (6 MARKS)

Macrophages are one type of cell circulating within the lymph and lymph nodes of humans which help to bridge the gap between the innate and the adaptive immune response.

a Describe how macrophages perform their role within the human lymphatic system. (2 MARKS)

b Acute lymphoblastic leukaemia (ALL) is a type of cancer. In patients with ALL, the precursor cell to B and T lymphocytes, called a lymphoid blast, is overproduced and does not further differentiate into B and T lymphocytes. The patient can develop frequent and recurring infections as a result.

i Explain why an ALL patient's immune system would find it difficult to eliminate an infection caused by a virus. (2 MARKS)

ii Explain the consequences for a patient diagnosed with ALL who becomes infected with a bacterial pathogen. (2 MARKS)

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q2

Key science skills and ethical understanding

Question 16 (9 MARKS)

Elephantiasis is a disease in humans caused by the presence of a particular parasitic worm, *Wuchereria bancrofti* in the lymphatic system. The adult worms block the lymphatic vessels, resulting in the accumulation of lymph and swelling in the surrounding tissues. Adult female worms produce larvae that enter the human bloodstream. Additionally, it has been documented that *W. bancrofti* is capable of evading the immune system through the production of chemical mediators which downregulate the immune system.

In an effort to understand the effects of *W. bancrofti* on the immune system, scientists set up an experiment using three groups of mice. The mice in Group 1 were infected with *W. bancrofti*, the mice in Group 2 were infected with *Clostridium perfringens* (a known pathogen which produces a well-understood immune response), and the mice in Group 3 were not infected.

a Name two cell types that function as antigen-presenting cells. (1 MARK)

b After several days, the levels of antibodies and certain immune cells including T helper cells, cytotoxic T cells, and antigen-presenting cells were measured in the mice.

i Identify the independent and dependent variables in this experiment. (2 MARKS)

ii Explain which group(s) of mice served as a control in this experiment. (2 MARKS)

iii In one of the mice, decreased levels of T helper cells were observed. Describe the impact of this on the mice's immune response to *W. bancrofti*. (2 MARKS)

iv Describe two roles of the lymphatic system. (2 MARKS)

Adapted from VCAA 2005 Exam 1 Section B Q2

CHAPTER 7 SUMMARY

Innate immunity

7B – First line of defence

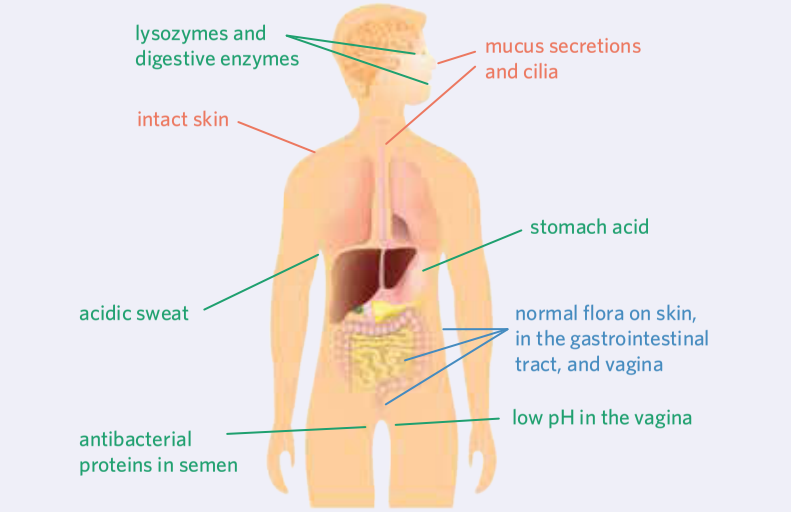
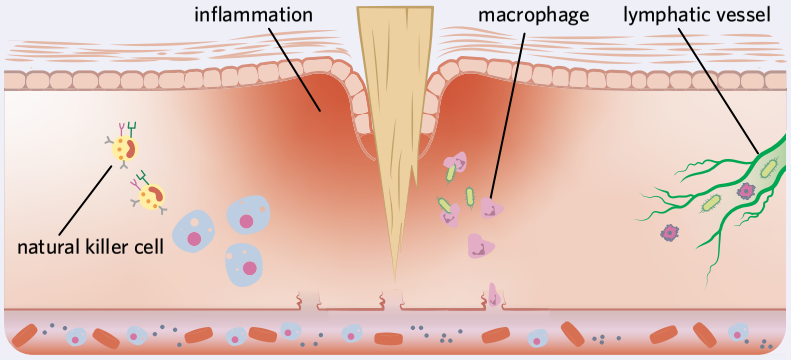


Image: Designua/Shutterstock.com

The first line of defence provides a non-specific and immediate form of protection against pathogens (lesson 7A). It is composed of physical, chemical, and microbiological barriers.

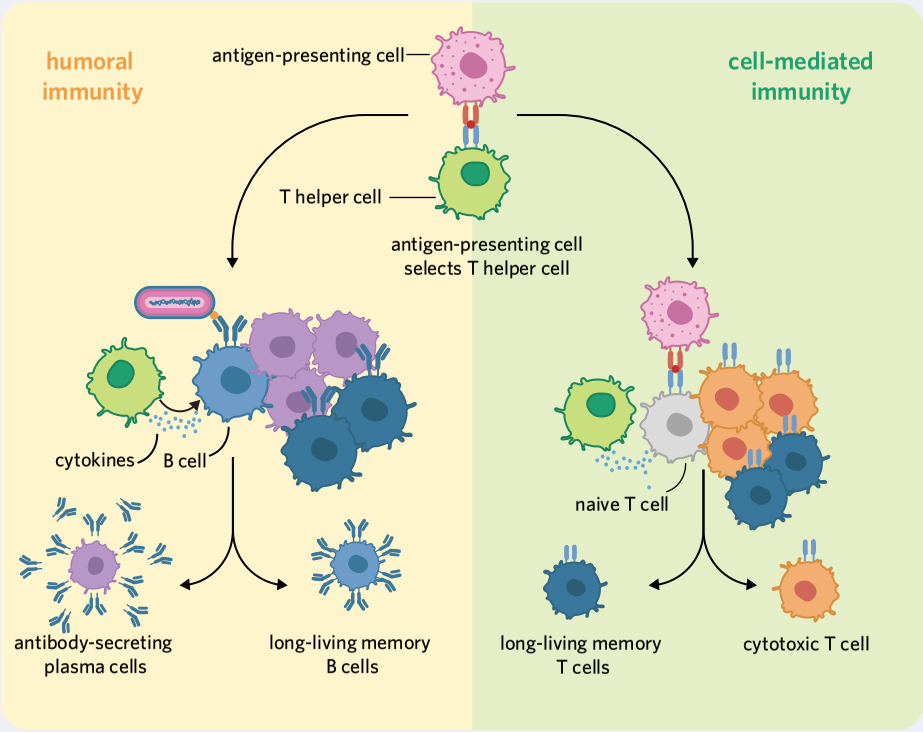
7C – Second line of defence



The second line of defence provides a non-specific cellular response to quickly destroy pathogens that have gained access to the interior of the body. It also includes non-cellular responses such as inflammation, fever, complement proteins, and interferons.

Adaptive immunity

7D – Third line of defence



The third line of defence provides a specific response tailored to individual pathogens and is activated via the interaction between an antigen-presenting cell and a T helper cell. Through clonal selection, T cells and B cells can differentiate into cytotoxic T cells and plasma cells respectively.

The lymphatic system (lesson 7E) is a series of vessels throughout the body which drain fluid from tissues, serving as a transport system for antigen-presenting cells and pathogens. Within lymph nodes, there exist clusters of immune cells which are involved in the activation of the adaptive immune response.

CHAPTER 7 SAC PRACTICE

SAC skills covered in this section:

✓ Case study analysis ✓ Data analysis ✓ Bioethical deep dive

WHEN THE IMMUNE SYSTEM ISN'T ENOUGH (22 MARKS)

Immunodeficiency disorders

Immune deficiency (or immunodeficiency) is a state in which an individual's immune system is unable to combat pathogens effectively. As a result, people with immunodeficiency are more susceptible to infection. Often, they develop opportunistic infections, which are infections involving microorganisms that are not normally capable of causing disease. When an individual has a compromised immune system, however, these microorganisms are able to grow and thrive, becoming pathogenic.

There are two types of immunodeficiency – primary and secondary immunodeficiency. Primary immunodeficiencies originate from within the immune system and include genetic conditions which affect its functioning. For example, chronic granulomatous disease is caused by genetic defects in the gene encoding for the production of chemical mediators involved in phagocytosis.

Secondary immunodeficiencies, also known as acquired immunodeficiencies, occur when an external factor impacts the effectiveness of the immune system. For example, people receiving medical treatments such as chemotherapy may develop an immune deficiency as a side effect of their treatment due to the suppression of their immune system. Secondary immunodeficiencies can also be caused by immune deficiency diseases, often caused by particular pathogens.

1 State one consequence of immunodeficiency. (1 MARK)

2 Describe how the body prevents pathogenic agents from entering. (1 MARK)

3 Explain whether normal flora is typically considered pathogenic. (1 MARK)

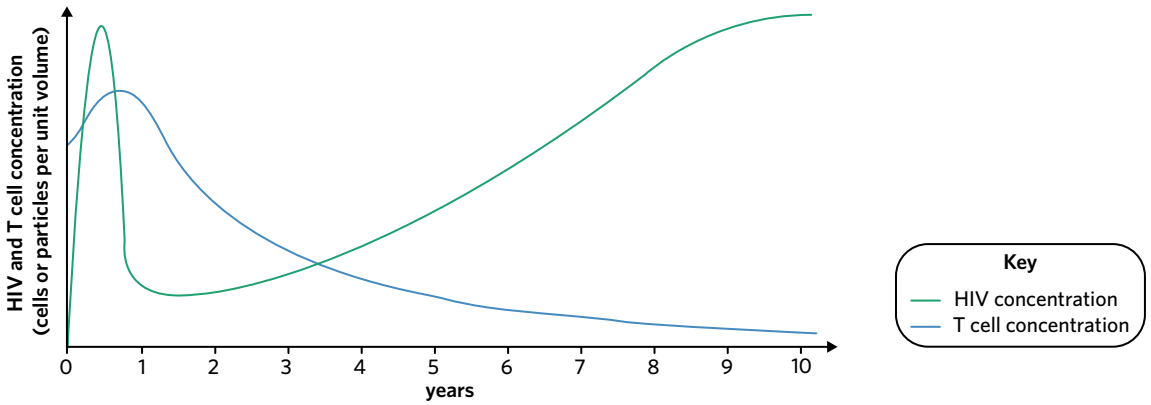
4 Identify one chemical mediator typically released inside phagocytes. (1 MARK)

5 Malnutrition can often lead to immunodeficiency. Identify whether immunodeficiency caused by malnutrition would be categorised as a primary or secondary immunodeficiency. Justify your response. (1 MARK)

Acquired immunodeficiency syndrome

The human immunodeficiency virus (HIV) is an RNA virus that causes HIV infection and, if left untreated, can cause the development of acquired immunodeficiency syndrome (AIDS). HIV infects macrophages and T helper cells, destroying them via a number of different mechanisms including the initiation of cell death in infected T helper cells, direct viral killing of infected T helper cells, and cytotoxic T cell-mediated destruction of T helper cells. Over time, the number of T helper cells in the body decreases and the body becomes vulnerable to infection. Symptoms of HIV infections include recurring fevers, chronic diarrhoea, swollen lymph nodes, weakness, and persistent fatigue.

HIV can be transmitted via the sharing of bodily fluids including blood, semen, vaginal secretions, and breast milk. It can also be transmitted from mother to child during pregnancy. While there is no current cure for HIV, treatments such as antiretroviral drugs which prevent HIV from replicating have been developed. There are many different classes of retroviral drugs that can be used in treating HIV, including protease inhibitors, which act to prevent the maturation of the HIV virus, and nucleotide reverse transcriptase inhibitors, which prevent the conversion of RNA into DNA for transcription. Ultimately, these classes of drugs attempt to halt the destruction of T helper cells, thus preventing the progression of HIV to AIDS. The following graph depicts HIV and T cell concentrations in an individual monitored for several years following infection with HIV.



6 Describe the trends in the graph occurring during the first year of HIV infection. (1 MARK)

7 One receptor involved in the binding and entering of HIV into a cell is called CCR5. Explain why viruses must enter cells to replicate. (2 MARKS)

8 Describe the function of macrophages. (2 MARKS)

9 Explain why individuals diagnosed with HIV struggle to combat infection. (3 MARKS)

10 Explain why individuals diagnosed with HIV often present initially with swollen lymph nodes. (1 MARK)

While the transmission and spread of HIV is relatively controlled in Australia, some developing countries have a high prevalence of HIV/AIDS. For example, in Australia, it was estimated that there were around 29 045 individuals living with HIV in 2019, equivalent to just 0.11% of the total population. In comparison, Eswatini, a developing nation found in Southern Africa, had an estimated 200 000 adults and 15 000 children aged between 0–14 diagnosed with HIV in 2019, translating into 27.2% of the total population being diagnosed with HIV.

11 Suggest why the prevalence of HIV/AIDS is significantly higher in Eswatini when compared to Australia. (1 MARK)

12 Suggest one reason for the high rates of HIV in Eswatini children. (1 MARK)

Treating patients with AIDS

There are many different bioethical issues that can arise regarding the delivery of healthcare. Consider the following bioethical scenario surrounding the treatment of an individual with an advanced HIV/AIDS infection.

Mark is a 45-year-old male diagnosed with acquired immunodeficiency syndrome (AIDS) due to an untreated HIV infection. Unfortunately, due to his weakened immune system, he has developed several infections in his body, leaving him extremely weak and bed-ridden. When questioned about receiving further medical treatment, he strongly declines any form of treatment which would unnecessarily prolong his life.

Overnight, Mark becomes unconscious and is placed on a ventilator. Knowing that he is unlikely to regain consciousness and is likely in a great deal of pain, doctors wish to remove him from the ventilator, which would allow him to pass away. However, his wife strongly opposes the decision to remove Mark from the ventilator, demanding that further treatment be administered.

13 Discuss the relevance of the bioethical concept of beneficence to the scenario and whether Mark should be removed from the ventilator. (2 MARKS)

14 Discuss the relevance of the bioethical concept of non-maleficence to the scenario. (2 MARKS)

15 Discuss the relevance of the bioethical concept of justice to the scenario. (2 MARKS)

CHAPTER 7 EXAM PRACTICE

Section A (12 MARKS)

Question 1 (1 MARK)

Spongiform encephalopathy involves a pathogen capable of inducing the abnormal folding of proteins in humans and animals. This pathogen is most likely a

A virus.
B prion.
C protozoa.
D bacterium.

Question 2 (1 MARK)

Vaccinations involve the stimulation of the immune system with pathogenic antigens to produce memory cells. In doing so, these memory cells can help generate a rapid immune response upon subsequent exposure to the pathogen. Which one of the following antigens could scientists use in the preparation of a vaccine against malaria?

A a complement protein
B an antibody to malaria
C a protein on the surface of the malaria parasite
D a protein that attaches to the surface of the malaria parasite

Adapted from VCAA 2016 Section A Q24

Question 3 (1 MARK)

An example of a plant defence against a pathogen is

A open stomata.
B mast cells that play a key role in inflammation.
C cytotoxic T cells that attack and kill pathogens.
D production of defensins that are toxic to microbes.

Adapted from VCAA 2015 Section A Q17

Question 4 (1 MARK)

Which of the following matches a molecule correctly with its role in the immune response?

	Molecule	Role
A	complement protein	released from mast cells during the inflammatory response
B	histamine	chaperones intracellular peptides for presentation to T cell receptors
C	interferon	a cytokine that is important for immunity against viruses
D	MHC	gets activated as part of a cascade that results in the stimulation of phagocytes

Adapted from VCAA 2017 Section A Q22

Question 5 (1 MARK)

There are many different types of immune cells found in the lymph nodes. One of these cells has a large nucleus and extensive endoplasmic reticulum, and plays an important role in the adaptive immune response. Which one of the following cells fits this description?

A a mast cell
B a plasma cell
C a memory cell
D a macrophage

Adapted from VCAA 2015 Section B Q5c

Use the following information to answer Questions 6 and 7.

In preparation for organ transplantation, various tests including blood typing and tissue typing must be conducted to ensure compatibility between the donor and recipient. These tests assess for similarities between the MHC markers belonging to the donor and recipient, and if a high degree of similarity is present, then the transplantation can proceed. This helps decrease the risk of organ rejection following transplantation.

Question 6 (1 MARK)

Which one of the following methods could not be used to prevent organ rejection?

A transplantation of organs from a genetically identical twin
B use of medications that suppress the immune system
C growth of organs from the recipient's own cells
D inhibition of histamine production

Question 7 (1 MARK)

The immune cells directly responsible for attacking the cells of a rejected organ transplant are

A cytotoxic T cells.
B memory B cells.
C T helper cells.
D mast cells.

Adapted from VCAA 2014 Section A Q19

Question 8 (1 MARK)

In the lymphatic system

A lysozyme is produced.
B mucus is generated to trap bacteria.
C antigen-presenting cells are transported to lymph nodes.
D mast cells release histamine to increase vessel permeability.

Question 9 (1 MARK)

Which one of the following cells provides an immediate response to invading pathogens?

A plasma cells
B T helper cells
C macrophages
D cytotoxic T cells

Question 10 (1 MARK)

Consider the following diagram of four pathogens and three antibodies.

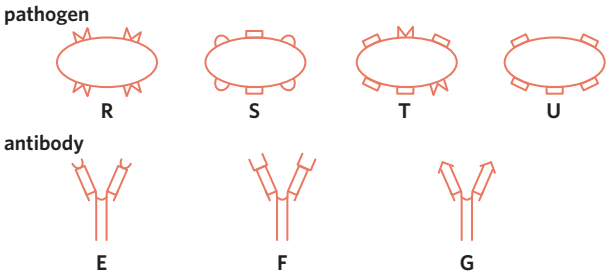
Which one of the following statements is incorrect?

A Antibody E is only effective against pathogen S.

B Antibody F would be effective against pathogen T.

C Antibody G would be effective against pathogen R and pathogen T.

D Antibody F would not be effective against both pathogen U and pathogen S.



Adapted from VCAA 2015 Section A Q16

Question 11 (1 MARK)

Necrosis is a form of unregulated cell death primarily initiated by significant damage such as physical trauma or exposure to chemicals, causing cells to swell, burst, and release their contents into the surrounding environment. Due to the release of cellular contents, certain chemical mediators released can trigger the inflammatory response.

Which one of the following events would not immediately occur after the death of cells via necrosis?

A plasma cells rapidly producing antibodies

B blood vessels increasing in permeability

C phagocytes engulfing pathogens

D mast cells releasing histamine

Question 12 (1 MARK)

Antigen-presenting cells serve a crucial role in the activation of the adaptive immune response. Following the activation of T helper cells via antigen-presenting cells, you would expect

A the activation of dendritic cells.

B the production of complement proteins.

C an increase in the number of mast cells.

D the differentiation of B cells into plasma cells.

Adapted from VCAA 2018 Section A Q17

Section B (28 MARKS)

Question 13 (14 MARKS)

Meningitis occurs when the membranes that cover the spinal cord and brain, known as the meninges, become inflamed. This often occurs due to the presence of a viral or bacterial infection in the cerebrospinal fluid, which is the fluid that surrounds the brain and spinal cord. When this occurs, a lumbar puncture is often performed to extract a sample of the cerebrospinal fluid in order to test it for the presence of a pathogen.

a Explain what is meant by the term 'pathogen'. (1 MARK)

Adapted from VCAA 2014 Section B Q4a

b *Haemophilus meningitis* is a type of bacteria known to cause meningitis.

i Is *Haemophilus meningitis* considered cellular or non-cellular? Justify your response. (1 MARK)

ii Describe how complement proteins can be used to combat *Haemophilus meningitis*. (2 MARKS)

iii Describe how an adaptive immune response would be initiated against *Haemophilus meningitis* (2 MARKS)

iv Explain whether *Haemophilus meningitis* would be able to cause meningitis in an individual previously infected with *Haemophilus meningitis*. (2 MARKS)

c People with meningitis often develop a fever.

i Explain how a fever helps defend the body against pathogens. (2 MARKS)

ii Describe two changes that occur during the inflammatory response and their significance. (4 MARKS)

Question 14 (5 MARKS)

Plants have many important chemical and physical methods of defence against pathogens and insects.

a Describe one physical defence present in plants. (1 MARK)

Adapted from VCAA 2017 Section B Q2a

b Identify two mechanisms through which chemical barriers in plants help protect against pathogens. (2 MARKS)

Adapted from VCAA 2018 Section B Q3a

c Explain why the innate immune system is so important in plants. (2 MARKS)

Question 15 (9 MARKS)

Varicella zoster is a type of virus responsible for causing chickenpox in children and adolescents. There are many different strains of varicella zoster that exist around the world, each with differing infectious rates and characteristics. To investigate the differences in immune responses against each strain, scientists designed an experiment as follows.

Seven groups of 50 mice were used. In Groups 1–6, each group was infected with a different strain of varicella zoster. In Group 7, the mice were not infected with anything. The mice were left for several days, after which scientists measured the levels of antigen-presenting cells and cytotoxic T cells in the mice.

a State the independent and dependent variables in the experiment. (2 MARKS)

b Identify and describe the purpose of the control group in this experiment. (2 MARKS)

c Describe the role of cytotoxic T cells in the immune response. (1 MARK)

d Antigen-presenting cells serve a crucial component in the activation of the adaptive immune response.

i Describe the purpose of MHC I and MHC II markers. (2 MARKS)

ii Describe the functions of the lymphatic system in aiding the immune response. (2 MARKS)

CHAPTER

8

Immunity

Key knowledge

- the difference between natural and artificial immunity and active and passive strategies for acquiring immunity
- vaccination programs and their role in maintaining herd immunity for a specific disease in a human population
- the emergence of new pathogens and re-emergence of known pathogens in a globally connected world, including the impact of European arrival on Aboriginal and Torres Strait Islander peoples
- scientific and social strategies employed to identify and control the spread of pathogens, including identification of the pathogen and host, modes of transmission, and measures to control transmission
- the development of immunotherapy strategies, including the use of monoclonal antibodies for the treatment of autoimmune diseases and cancer

Image: Yurchanka Siarhei/Shutterstock.com

8A ACQUIRING IMMUNITY

As the preeminent biologist of our time Jay-Z once said, 'I got 99 problems but, thanks to a comprehensive vaccination schedule, dying from poliomyelitis ain't one'. In fact, poliomyelitis, the deadly infectious disease caused by the poliovirus, has almost disappeared from the surface of the Earth – in 2018 there were just 33 cases of wild polio compared to 350 000 wild cases in 1988. This drastic drop in disease prevalence is due to the herd immunity created by the polio vaccine – but what exactly is a vaccine, and how do they work?

If you're reading this, yell out 'Immunity!' as loud as you can. Congratulations – you and everyone around you now have heard immunity!

Image: puhhha/Shutterstock.com

Lesson 8A

In this lesson you will learn about the two different types of immunity and the strategies that can be used to generate them.

◀ *Prerequisite knowledge* *Future applications* ▶

Years 7–10
The body responds to infection from microorganisms in a number of ways.

Chapter 7
The development of immunity and the functioning of vaccinations relies on the ability of the adaptive immune response to generate immunological memory, thereby preventing disease upon subsequent exposure.

Lesson 8C
Immunisation programs and the creation of herd immunity are crucial to the containment of pathogens and disease.

Lesson 9F
Flu vaccines must be readministered each year due to mutations in the influenza virus that enable it to evade previously generated memory cells.

Study design dot points

- the difference between natural and artificial immunity and active and passive strategies for acquiring immunity
- vaccination programs and their role in maintaining herd immunity for a specific disease in a human population

Key knowledge units

Natural immunity	4.1.6.1
Artificial immunity	4.1.6.2
Herd immunity	4.1.9.1

Natural immunity 4.1.6.1

OVERVIEW

Natural immunity is immunity to a disease that has developed without any medical intervention.

natural immunity protection against a disease formed without medical intervention

artificial immunity protection against a disease formed as a result of medical intervention. Also known as **induced immunity**

THEORY DETAILS

A person's immunity to a disease can be classified according to how it was generated. **Natural immunity** is immunity that has been developed without medical intervention, whereas **artificial immunity** is immunity that has been developed via a medical intervention of some type.

We can also further classify a person's immunity to a disease based on the strategy used to develop it (i.e. the source of their antibodies). In **active immunity**, a person's own adaptive immune system has developed antibodies and memory cells to a particular antigen, whereas in **passive immunity** a person's immunity to a disease is created by antibodies from an external source (Figure 1).

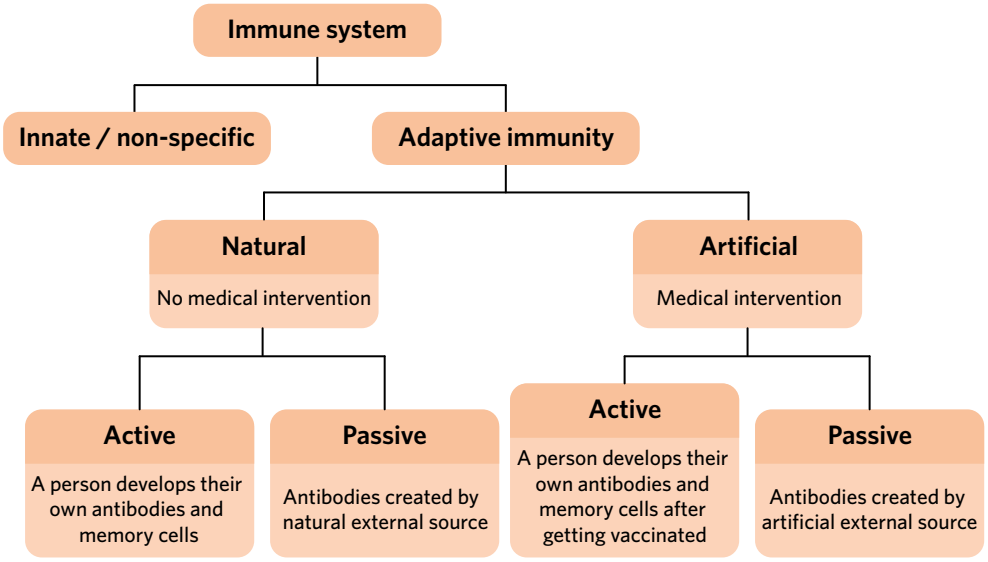


Figure 1 The different types of immunity

In this section, you will learn about natural immunity and the two strategies used to acquire it.

Natural active immunity

Natural active immunity is created when an individual's own immune system encounters a pathogen and mounts a response against it, creating antibodies and memory cells specific to that pathogen. This process is 'natural' because it occurs without medical intervention, and 'active' because a person's own immune system is creating the antibodies and memory cells (Figure 2). The next time the person encounters the same pathogen, it will be rapidly recognised by the appropriate memory cells, which will proliferate and differentiate so that the pathogen can be neutralised before it can cause disease within the body.

Natural passive immunity

Natural passive immunity is created when an individual acquires antibodies from a 'natural', non-medical external source. The two methods of acquiring natural passive immunity you need to be aware of are:

- breastfeeding – human breast milk contains many nutrients and proteins essential for healthy growth and development, including antibodies generated from the mother's own immune system. Once ingested, these antibodies are absorbed into the baby's bloodstream and protect them against pathogens. This is important, as babies have poorly developed adaptive immune systems and aren't able to fully protect themselves against pathogens for the first few months of life.
- placenta – during pregnancy, some antibodies produced by the mother are able to cross the placenta and enter the foetus' bloodstream via the umbilical cord (Figure 3). These confer the child protection during pregnancy and after it is born for a short period of time, helping to compensate for the baby's weak adaptive immune system.

Artificial immunity 4.1.6.2

OVERVIEW

Artificial immunity is immunity to a disease that has developed as a result of medical intervention. Artificially acquired active immunity is formed after vaccination. Artificially acquired passive immunity is formed when an individual receives an injection of preformed antibodies.

active immunity protection against a disease created by antibodies and memory cells formed by a person's own adaptive immune system

passive immunity protection against a disease created by antibodies from an external source

natural active immunity protection against a disease created by antibodies and memory cells produced by an individual's own immune system without medical intervention. Also known as **naturally acquired active immunity**

natural passive immunity protection against a disease created by antibodies from an external non-medical source. Also known as **naturally acquired passive immunity**

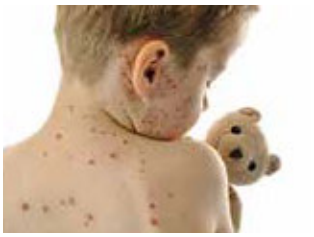


Image: Dan Race/Shutterstock.com

Figure 2 Once this guy's adaptive immune system gets rid of the varicella-zoster virus causing his chickenpox, he'll have naturally acquired active immunity against it!

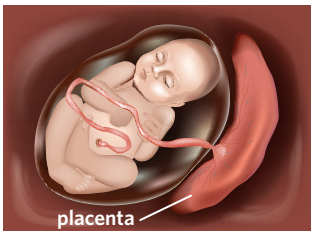


Image: Sakurra/Shutterstock.com

Figure 3 Natural passive immunity can occur after the transfer of antibodies from mother to child across the placenta.

Lesson link

If you've forgotten how the adaptive immune response works, head back to ***lesson 7D*** to jog your memory!

THEORY DETAILS

Artificial immunity is immunity against a disease that's generated as a result of medical intervention. Like natural immunity, there are both active and passive strategies for creating artificial immunity.

Artificial active immunity

Artificial active immunity is created when an individual's own adaptive immune system produces antibodies and memory cells due to a medical intervention. This is the basis of how **vaccines** work.

artificial active immunity protection against a disease created by antibodies and memory cells produced by an individual's own immune system after medical intervention. Also known as **artificially acquired active immunity**

vaccine a medical treatment typically containing antigens designed to stimulate a person's adaptive immune system to create immunity to a pathogen without actually causing disease

Vaccines

No one likes getting needles – well, normal people don't at least. But vaccines are truly one of modern medicine's greatest achievements, and well worth the little sting of pain they cause. Prior to vaccines, the only way to generate active immunity (including long-lasting immunity from memory cells) was to actually get sick. Nowadays, however, vaccines can generate active immunity in a person without them having to become unwell at all. So how do they work?

Vaccines are medical treatments that contain components that resemble a certain pathogen's antigens, but these components are not able to cause disease. These components can sometimes be attenuated (weakened) or inactivated (dead) pathogens, toxoids (toxins that have been altered so they can't cause disease), specific proteins from the surface of pathogens, or RNA that enters immune cells and causes them to make pathogen-like proteins. A person's adaptive immune system recognises these components as foreign and mounts a response to them. This means that when this individual encounters the actual pathogen in the future, their immune system has antibodies and memory cells that can target its antigens and rapidly attack it. Figure 4 outlines the relationship between vaccines and the concentration of antibodies for a particular antigen in a person's blood.

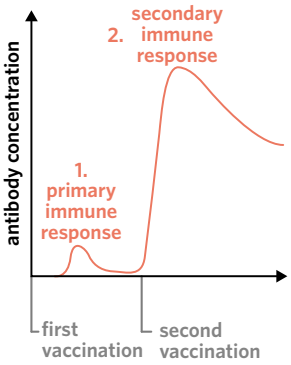


Figure 4 The formation of artificial active immunity via vaccination

> **Examiners' tip**
>
> The graph presented in Figure 4 has appeared in several VCE Biology exams – for example, in Section A of the 2017 exam, Question 26 required students to analyse a graph displaying the concentration of antibodies in a person's body after they had received an influenza vaccine. Make sure you understand the shape of this graph and, most importantly, why the amount of antibodies change over time/after being vaccinated.
>
> It's also important to note that this graph is also relevant to natural active immunity – after being exposed to a pathogen once, a primary immune response is initiated. When a person comes into contact with the same pathogen in the future, a larger secondary immune response occurs, quickly eliminating the pathogen typically before a person becomes unwell. For VCE Biology, however, the VCAA has only ever asked about primary and secondary immune responses in the context of vaccinations.

There are two main phases to forming artificial active immunity – the **primary immune response** and the **secondary immune response**.

1. After a person receives their first vaccination, there is a delay in the adaptive immune system's response. This is because the adaptive immune response is relatively slow – it takes time for antigen-presenting cells to find T and B cells complementary to the vaccine's antigen and for the process of clonal selection to occur. Eventually a primary immune response takes place in which a moderate number of antibodies and memory cells are formed, however these quickly diminish over time.
2. Upon receiving a second vaccination, the memory cells created by the first vaccine quickly recognise the antigen in the vaccine and mount a rapid, large secondary immune response. This results in the generation of a large number of antibodies and memory cells that go on to create long-lasting immunity.

primary immune response the reaction of the adaptive immune system to an antigen it has not previously been exposed to

secondary immune response the heightened reaction of the adaptive immune system to an antigen it has previously been exposed to

vaccination program a series of vaccinations designed to create long-term immunity to a disease. Also known as a **vaccination schedule**

Note that more than one vaccination typically needs to take place in order for immunity to be formed. **Vaccination programs** vary depending on the type of vaccine being used and the disease being prevented. Vaccination programs consisting of more than two separate vaccines work similarly to Figure 4, with subsequent immune responses each generating larger, more rapid responses until long-lasting immunity has been achieved.

Example

MEET *CLOSTRIDIUM TETANI*

Clostridium tetani is a bacterium with spores that are prevalent in soil around the world (Figure 5). If *C. tetani* breaches the first line of defence and gains access to the tissue of our body via a cut, it can grow and produce a toxin called tetanospasmin that interferes with the functioning of the nervous system in a substantial way, causing muscle spasms and damage. This condition, called tetanus, can even be life threatening if left untreated.

The good news – a vaccine for tetanospasmin was developed in 1924, and if you grew up in Australia you've probably had it already. The bad news – according to the current Australian guidelines, in order for a person to develop artificial active immunity to tetanospasmin they need to be vaccinated SIX times from the age of 2 months to 12 years of age.

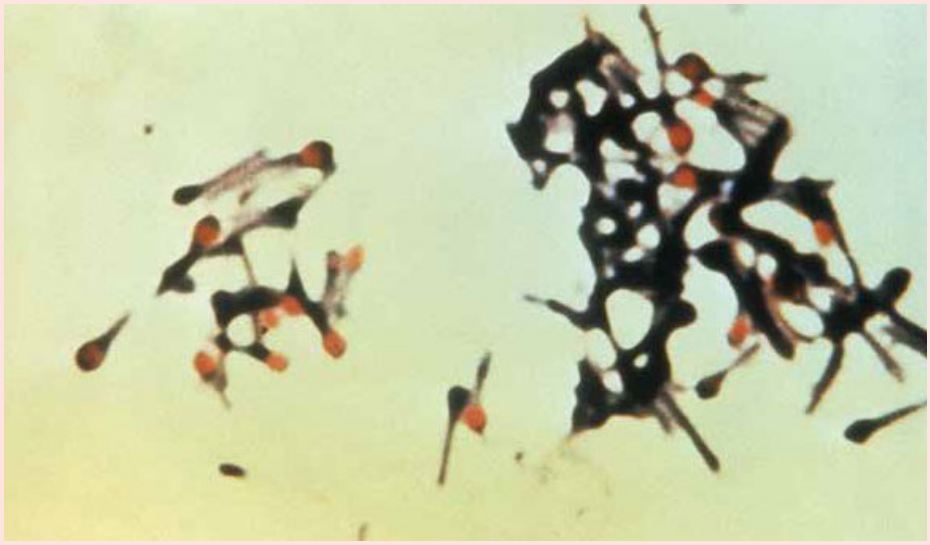


Figure 5 *Clostridium tetani* under a microscope

Examiners' tip

Vaccines can be administered via an injection or, in some cases, orally in the form of a liquid. The VCAA has only ever asked about injected vaccines, but regardless of the method of administration, the underlying principles and mechanisms of how vaccines create artificial active immunity are the same.

Booster vaccines

It is normal for memory T and B cells to die after a long period of time. This means that in individuals who were immunised against a disease many years ago, their immunity may start to wane and they may become susceptible to a disease they were previously immune to. In these individuals, we administer a **booster vaccine**, which is simply another injection of the vaccine they received earlier. By doing so, we stimulate any remaining memory cells to mount an immune response, generating more antibodies and memory cells to restore their immunity. Note that these booster shots are separate to vaccination programs that include multiple vaccinations in that they are given much later after the initial program has been completed.

booster vaccine a vaccination given to a person later in time after they have completed their initial vaccination program to enhance their existing immunity against a disease. Also known as a **booster shot**

Example

TRYING TIMES WITH TETANUS – PART ONE

Imagine you're walking along in your backyard, minding your own business, thinking about all the Biology homework you haven't done, when – OUCH – you step on a rusty nail (Figure 6). You think back to this lesson, and you realise that the very unclean nail you stepped on might be harbouring some nasty *C. tetani* bacteria on it, so you book an appointment at your G.P. ASAP.

At the G.P., the doctor gives you a booster shot of the tetanus vaccine to kick your immune system into gear making tetanospasmin antibodies and memory cells. Remembering chapter 7 of your VCE Edrolo Biology textbook, however, you turn to your doctor in horror – 'Hang on doc, doesn't the immune system take some time to respond to antigens in vaccines? How are we going to mop up any tetanospasmin that might be being secreted by *C. tetani* right now? Please, there's so much I haven't done yet, so many Edrolo videos I haven't seen! I'm too young to d---'

Image: drsnaut/Shutterstock.com

Figure 6 While the imminent pain and potential tetanus infection that are in store for this person are bad, neither are as terrible as their decision to wear socks with sandals.

Lesson link

Have you ever wondered why people need to have a new flu shot every year even though vaccines create long-lasting immunity? Great question – hold onto that thought, it will be answered in ***lesson 9F***!

Artificial passive immunity

Artificial passive immunity is created when an individual acquires antibodies from an external source via a medical intervention (either via an injection or an infusion). For example, people who have been bitten by a snake are given an **antivenom** which contains antibodies designed to neutralise the venom. Figure 7 outlines how the concentration of antibodies for a particular antigen in a person's blood changes over time after an antibody injection or infusion.

Antibody treatments immediately increase the number of antibodies in the blood, but over time these antibodies degrade until they've all disappeared and the immunity they created has gone. If someone is only given antibodies, they will not develop active immunity because the antibodies they receive will not trigger production of the memory cells responsible for immunological memory.

artificial passive immunity protection against a disease created by antibodies from an external medical source. Also known as **artificially acquired passive immunity**

antivenom a medical treatment containing antibodies specific to the toxins present in venomous bites or stings

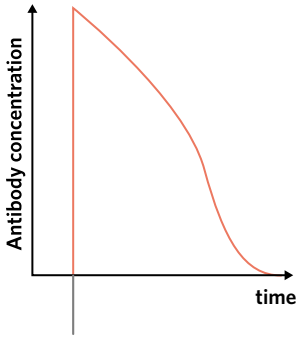


Figure 7 The formation of artificial passive immunity via antibody injection

Example

TRYING TIMES WITH TETANUS – PART TWO

Before you can finish your sentence, your doctor has STABBED you in the arm with another needle.

'That's a shot of tetanospasmin antibodies. They will circulate in your blood for a while and protect you until your own immune system starts to respond to the vaccination I gave you before and makes its own antibodies. Didn't you learn about the difference between artificial active and artificial passive immunity in chapter 8 of your Edrolo VCE Biology book?'

Image: Popel Arseniy/Shutterstock.com

Figure 8 'The doctor will see you meow...'

Theory in context

TREATING HAEMOLYTIC DISEASE OF THE NEWBORN

In lesson 7D, you learned about haemolytic disease of the newborn (HDN), a disease in which anti-D antibodies are created in a Rhesus-negative mother after exposure to the Rhesus-positive blood of her foetus. The anti-D antibodies and memory cells created by the mother's adaptive immune system reside in her body for extended periods of time and can attack a future Rhesus-positive foetus if she becomes pregnant again in the future.

The way we manage HDN nowadays is by preventing the formation of maternal anti-D antibodies in the first place. This is achieved by injecting the mother with artificially produced anti-D antibodies that circulate throughout the mother's blood and destroy any foetal Rhesus-positive blood cells before the mother's adaptive immune system can respond to them (Figure 9). In other words, we're creating artificial passive immunity in the mother to prevent her adaptive immune system from creating long-lasting natural active immunity that would affect future pregnancies.

The antibody used in anti-D injections is rare. In Australia, a man by the name of James Harrison was found many years ago to have this antibody naturally in his blood. Over the course of his life, he has donated blood over 1 100 times, helping protect more than two million babies from HDN in Australia. He gave his final donation in May 2018, having donated for over 60 years despite his fear of needles!

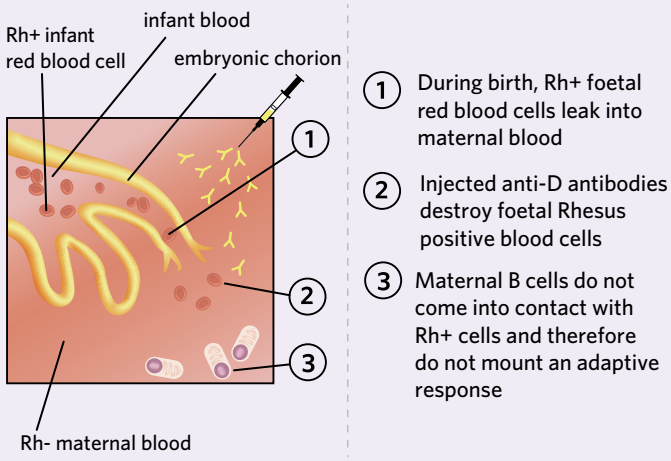


Figure 9 Injected anti-D antibodies destroy foetal Rhesus-positive red blood cells before they interact with the maternal adaptive immune system.

Herd immunity 4.1.9.1

OVERVIEW

Herd immunity is achieved when the majority of people in a community are immune to a particular pathogen, helping to prevent the spread of the pathogen to those who haven't been vaccinated or who haven't already been infected with the pathogen.

THEORY DETAILS

Herd immunity is immunity to an infectious disease at a population level. Diseases are transmitted in a population when pathogens are spread between individuals. But, if a sufficiently large proportion of people in a population are immune to a disease via vaccination (the exact number depends on the disease – more contagious diseases require more people to be immune), then the pathogen causing that disease cannot easily reproduce and spread throughout. This then protects the people who aren't immune – they are now highly unlikely to come into contact with a person harbouring the pathogen, and therefore won't become sick (Figure 10)!

herd immunity protection against a disease conferred to non-immune individuals when a high percentage of a population is immune to the same disease. Herd immunity is often achieved through high rates of vaccination

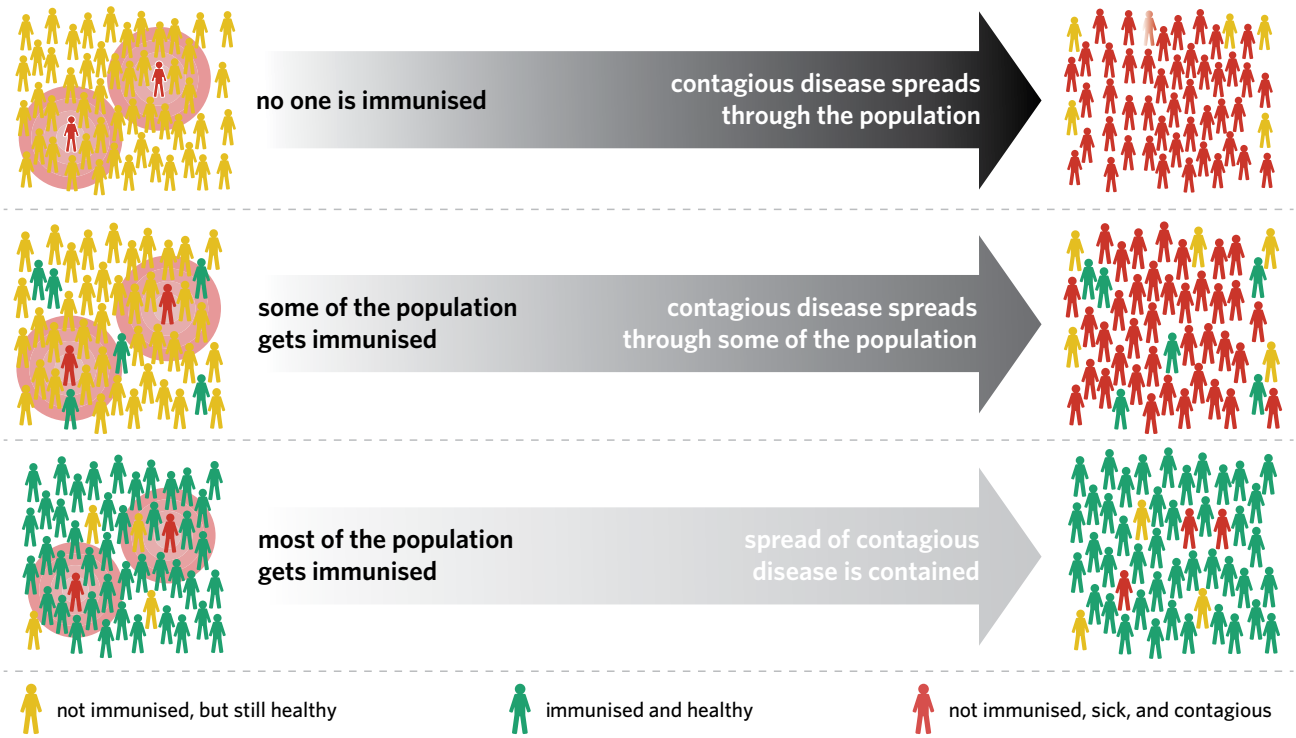


Figure 10 Herd immunity protects vulnerable people by reducing the number of potential pathogen hosts and containing the spread of disease.

Achieving herd immunity is an extremely important step in combating disease and community disease transmission. There will always be people who are not able to be vaccinated or who choose not to be. Even people who have been vaccinated may still become unwell if they are exposed to a pathogen they're immune to (albeit they typically experience a milder illness). The most effective way we can comprehensively protect everyone in a population from a disease is through herd immunity.

Theory in context

POLI-G-O AWAY

Poliomyelitis, commonly shortened to polio, is a disease caused by the poliovirus. In most infections, people experience no symptoms. In about 1% of cases, however, the virus can enter the central nervous system causing meningitis (inflammation of the membranes covering the brain and spinal cord). This can sometimes lead to paralysis, limb deformities, and even death.

Polio is a highly contagious disease – it's estimated that one person with polio can spread the disease to 5–7 people. Fortunately, we have two types of vaccines available to combat the poliovirus: one is delivered via injection, the other is consumed orally. Given that polio is highly contagious, achieving herd immunity requires a high proportion (80–86%) of the population to be immune. In Australia, we fortunately have a high polio vaccination rate – in 2017, 95% of one-year-olds were fully vaccinated against polio.

Image: Talukdar David/Shutterstock.com

Figure 11 A child receiving the oral poliovirus vaccine in India

Theory in context

THE VACCINE DEBATE

Various groups have raised concerns about vaccinations, either questioning their efficacy or, more commonly, their safety. While there are rare occurrences of individuals experiencing severe side effects to a vaccine, these complications are less common than the adverse risks associated with the disease the vaccine prevents. Vaccines must undergo rigorous safety and efficacy testing before being introduced to the public. To date, any study questioning the safety of approved vaccines has been proven unreliable, and all studies investigating the efficacy and safety of these vaccines have found that they are safe and incredibly effective at preventing people from becoming ill with serious, deadly diseases.

Unfortunately, anti-vaccination sentiments have led to some people refusing to get vaccinated or having their children or relatives vaccinated. This has led to outbreaks of diseases that were previously controlled by herd immunity. For example, unfounded speculation about the safety of the mumps-measles-rubella (MMR) vaccine led to a decrease in vaccinations in Wales in the 2000s, leading to an outbreak of measles in 2013.

Theory summary

There are four types of immunity that individuals can acquire: natural active, natural passive, artificial active, and artificial passive. Vaccinations are a medical intervention containing non-disease-causing antigens that result in the formation of artificial active immunity. If a sufficiently large proportion of a population is immune to a disease via vaccination or natural active immunity, herd immunity is generated and protects those who are unvaccinated.

Table 1 The types of immunity

	Medical intervention?	Source of antibodies	Example
Natural active immunity	✖	Individual's own immune system	Getting chickenpox and being immune to it afterwards
Natural passive immunity	✖	External	Antibodies passing from mother to child in breast milk or across the placenta
Artificial active immunity	✓	Individual's own immune system	Vaccines
Artificial passive immunity	✓	External	Antivenom

The polio vaccine introduced a non-disease-causing antigen into Jay-Z's body, stimulating his adaptive immune system to respond as if it were facing the real poliovirus. This created long-lasting immunity, so that if his body ever encounters the poliovirus for real he has preformed antibodies and memory cells ready to attack it before it can cause disease. So as the man himself said, 'Momma ain't raise no fool' – vaccines are safe and save lives.

8A QUESTIONS

Theory review questions

Question 1

Which of the following statements about immunity is false?

A Vaccinations result in natural active immunity.

B Antibodies crossing the placenta create natural passive immunity.

C Injection with antibodies against a pathogen creates artificial passive immunity.

Question 2

Which of the following statements about herd immunity is false?

A Booster shots support herd immunity.

B Herd immunity is created by injecting antibodies into unvaccinated people.

C Herd immunity can be achieved by vaccinating the majority of the population.

Question 3

Categorise the following as either a **passive strategy** or **active strategy** for developing immunity.

I vaccination _____

II antibody injection _____

III injection of booster shot _____

IV infant consumption of antibodies in breast milk _____

Question 4

Fill in the blanks in the following sentences.

_____ is created when a high proportion of the population is vaccinated against a disease and protects vulnerable people. Another way to protect vulnerable people is to give a _____ to people who have previously completed their vaccination schedule. This is needed, because the number of _____ in the body decreases over time. Vaccinations are different to injections with antibodies, which create a form of immunity known as _____.

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 5–10.

In 1998, Andrew Wakefield and a team of eleven co-authors published a paper in *The Lancet* in which they claimed that the measles, mumps, and rubella (MMR) vaccine caused colitis (inflammation of the large intestine) and autism spectrum disorders. In 2001 and 2002, Wakefield then published articles claiming that the measles virus had been found in tissue samples taken from children who had autism and bowel conditions. These articles led to widespread scepticism about the safety of the MMR vaccine, leading to a drop in public confidence in the vaccine and a decline in the number of children being given the vaccine from 92% of children being vaccinated in the UK before Wakefield's publications to less than 80% after them.

As it turns out, Wakefield's papers have since been completely discredited and proven false by a large number of studies. In 2004, it was discovered that Wakefield had not disclosed that he had received large sums of money prior to the publication of his research from a legal group seeking evidence to use against vaccine manufacturers. Additionally, it was also discovered that Wakefield and his co-authors altered data to fit with their 1998 study's conclusion, and that they acted unethically by conducting numerous invasive and unnecessary tests on the children in their study.

Question 5

Colitis is a condition that involves

A the development of autism spectrum disorder.

B inflammation of the large intestine.

Question 6

Public confidence in the MMR vaccine

A decreased to 80% after Wakefield's publications.

B increased to 92% after Wakefield's publications.

C decreased after Wakefield's publications.

Question 7

The decline in vaccination rate caused by Wakefield's incorrect suggestions that the MMR vaccine causes autism could lead to

A an increase in herd immunity to colitis and autism spectrum disorders.

B a decrease in herd immunity to the measles, mumps, and rubella viruses.

Question 8

By altering data used in his 1998 study, Wakefield was not adhering to the bioethical concept of

A justice.

B integrity.

C non-maleficence.

Question 9

The conducting of invasive and unnecessary tests on children performed by Wakefield and colleagues violates the bioethical principle of

A beneficence.

B respect.

C justice.

Question 10

Based on the information provided, which of the following is a justification for why authors need to be transparent about their sources of funding when conducting research?

A By receiving money from groups with particular interests, researchers can be persuaded to alter their results to fit with these interests.

B It is important for researchers to be honest about the sources of their funding so that groups that have funded the research can claim credit.

Exam-style questions

Within lesson

Question 11 (1 MARK)

Australian marsupials, such as wallabies, kangaroos, wombats, and koalas, give birth to very underdeveloped young called joeys. Joeys spend many weeks in the mother's pouch feeding on milk produced by mammary glands. This milk contains various antibodies.

The antibodies in the milk that are fed to the joey would be best described as

A artificially acquired, passive immunity.

B naturally acquired, passive immunity.

C artificially acquired, active immunity.

D naturally acquired, active immunity.

Adapted from VCAA 2017 Section B Q4c

Question 12 (7 MARKS)

Pertussis (whooping cough) is a highly contagious respiratory infection caused by the bacteria *Bordetella pertussis*. The pertussis vaccine is offered as part of an immunisation program for children at two months, four months, six months, four years, and in Year 10 of secondary school.

a State the type of immunity formed by the vaccination. Justify your answer. (2 MARKS)

Adapted from VCAA 2013 Section B Q4a

b Adults who have previously been vaccinated against pertussis are advised to receive an extra vaccination if they are planning on coming into contact with newborn babies. This is because babies aren't able to receive the whooping cough vaccine until they are two months old.

i State the name of this type of vaccine, and explain why they are necessary. (3 MARKS)

Adapted from VCAA 2013 Section B Q4c

ii By encouraging high vaccination rates and booster shots for pertussis every 10 years, governments are trying to achieve a specific type of immunity. Name this type of immunity and explain how it can protect babies who have not been vaccinated. (2 MARKS)

Adapted from VCAA 2018 Section B Q5b

Multiple lessons

Question 13 (1 MARK)

Vaccines usually take a number of weeks to become fully effective. This is because

A memory B cells are slow to respond.

B the inactivated pathogen requires time to multiply.

C it takes the innate immune system time to form memory B cells.

D the immune system needs time to produce enough antibodies to confer immunity.

Adapted from VCAA 2016 Section B Q5b

Question 14 (8 MARKS)

Why do people not vaccinate?

By Hal Willaby

Published in *The Conversation* March 27th 2014

The National Health Performance Authority's report on childhood vaccination coverage released this morning shows immunisation rates have slightly increased in 2011–2012. But there are still some areas where coverage is below the national target.

The good news is that Australia has one of the highest vaccination rates in the world with over 90% of children fully immunised by age five. But there are areas where only 80% of five-year-olds are protected against preventable contagious disease.

So why are some children not immunised? There are two broad influences on timely uptake of routine childhood vaccines – access and acceptance.

Access is partly a structural problem, linked to barriers such as a lack of transport, limited clinic opening times, home-boundedness and, beyond that, to poverty and social exclusion. Generally speaking, we can address access problems by minimising these barriers.

The other factor impacting vaccine uptake is acceptance. This is the psychological orientation to vaccines influencing uptake; it's about attitudes, beliefs and concerns regarding vaccines, parenting, medicine generally, and a host of related matters. An individual's vaccine acceptance is the result of a certain composition of these, like a metaphorical DNA.

The public tends to hear a lot more about acceptance factors than they do about access. It's an easy formula for mass media to pit vaccination opponents against proponents, and parade examples of non-vaccinating parents. It excites emotion, leading to high click rates in online articles and crowded comments pages.

Nevertheless, the attention given to such parents is out of proportion to their actual numbers, and the likelihood of changing their minds. Vaccine refusers are a very small proportion in Australia – about 2% of parents make a values-based choice to forego all vaccines for their children.

A more interesting group is the 12% of parents who are at least somewhat supportive of vaccination, but fear both vaccination and non-vaccination could have negative outcomes for their child. About half of that 12% vaccinate fully, and the other half may delay or avoid certain vaccines but will have others.

Any action taken at the community level starts with acknowledging that parents want the best for their children regardless of their access to and acceptance of vaccines. When otherwise well-intentioned messages criticise what these parents view as healthy skepticism, the result can be a further distancing from timely uptake.

Source: 'Why do people not vaccinate?' By Hal Willaby. Published in The Conversation March 27th 2014

a The article states that Australia has one of the highest vaccination rates in the world with over 90% of children fully immunised by age five. How would not completing a vaccination schedule impact the immunity of a child against a disease? (2 MARKS)

b Vaccines require the adaptive immune system to be stimulated to generate long-lasting immunity. Name one type of antigen-presenting cell responsible for initiating the adaptive immune response. (1 MARK)

c Which group of parents would be the best target of extra efforts to increase vaccination rates in Australian children? Use evidence from the article to justify your response. (2 MARKS)

d Based on the information in the article, suggest three broad strategies for increasing vaccination rates amongst Australian children. (3 MARKS)

Key science skills and ethical understanding

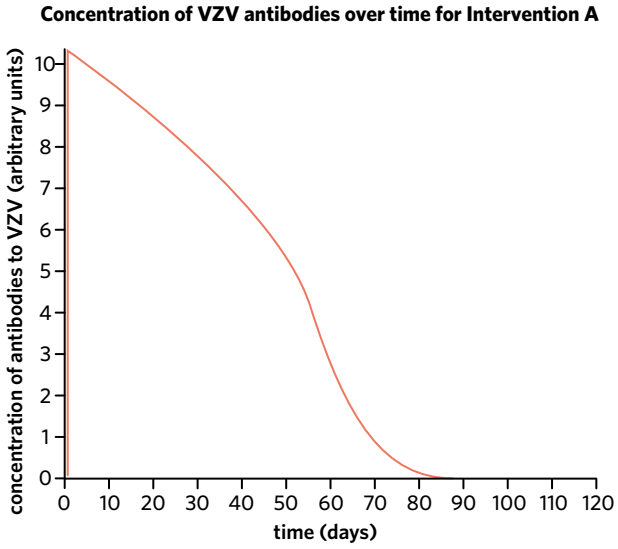
Question 15 (14 MARKS)

Chickenpox (also called varicella) is a highly contagious viral disease caused by the varicella-zoster virus (VZV). A vaccine against the disease became commercially available in 1984.

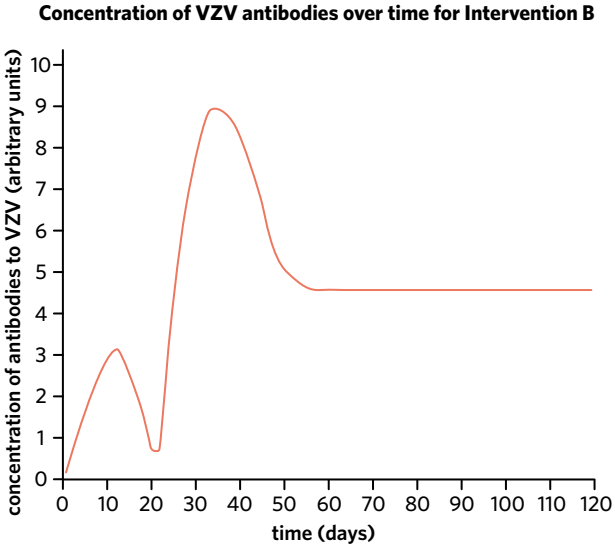
a What is a vaccine, and how can vaccines confer lifelong immunity? In your response, be sure to include information related to the humoral response. (4 MARKS)

Adapted from VCAA 2016 Section B Q5a

b A scientist wanted to compare the effects of two different injected medical interventions designed to prevent and/or treat chickenpox. To do so, she measured the antibody concentrations in blood samples from two patients, Patient 1 and Patient 2 over a 120-day period. Patient 1 received intervention A, whereas Patient 2 received intervention B. Both interventions began on day 1 of the experiment, and neither patient became ill during the 120 days. The two graphs show the scientist's findings over the 120-day period.



Adapted from VCAA 2015 Section A Q14



Adapted from VCAA 2017 Section A Q26

i Based on the information provided, identify the nature of each intervention that occurred on day 1 of the experiment and explain your response. (4 MARKS)

ii What occurred just after day 22 in intervention B, and why was this necessary? (2 MARKS)

Adapted from VCAA 2017 Section A Q26

iii Which type of immunity would be created by intervention A? (1 MARK)

c State whether a control was used in the experiment and identify what the control is/should be. (2 MARKS)

d An earlier version of this experiment involved the scientist infecting individuals with chickenpox, and then giving them each treatment and measuring its effectiveness, however an ethics board rejected this planned experimental design. How does this original experimental design fail to adhere to the bioethical concept of non-maleficence? (1 MARK)

8B EMERGENCE OF PATHOGENS

Life was great in October 2019 – radio stations were playing 'Dance Monkey' by Tones and I on repeat, and everyone was still processing the ending of 'Avengers: Endgame.' We'd never heard of the word 'COVID-19' – in fact, the SARS-CoV-2 virus that causes COVID-19 didn't even exist yet. Wind the clock forward six months, though, and this brand new virus had shut down the world. Where do pathogens such as SARS-CoV-2 come from?

Step aside, Thanos – there's a new big baddie in town!

Lesson 8B

In this lesson you will learn about where the pathogens that cause disease come from, and the impact of European arrival on Indigenous Australians.

◀ *Prerequisite knowledge* | *Future applications* ▶

Lesson 7A
Pathogens, which are disease-causing agents, can include a variety of different agents including viruses, bacteria, prions, fungi, and protozoa.

Lesson 8A
When novel pathogens appear, the lack of herd immunity within the population results in a large number of susceptible hosts, leading to significant numbers of infections.

Lesson 8C
A number of different techniques can be used by public health experts to control the spread of emerging and re-emerging pathogens.

Study design dot point

- the emergence of new pathogens and re-emergence of known pathogens in a globally connected world, including the impact of European arrival on Aboriginal and Torres Strait Islander peoples

Key knowledge units

Emergence and re-emergence of pathogens	4.1.7.1
Pathogens introduced by European arrival to Australia	4.1.7.2

Emergence and re-emergence of pathogens 4.1.7.1

OVERVIEW

Emerging and re-emerging diseases threaten to cause outbreaks of disease throughout the world. There are a range of factors that contribute to a pathogen's ability to enter the human population and spread between people.

THEORY DETAILS

Diseases can have many different causes. Some diseases, such as cystic fibrosis, are caused by abnormal genes, while others, such as cardiovascular disease, are largely caused by lifestyle factors such as diet and exercise. **Infectious diseases** are caused by **pathogens** that both harm their host (cause disease) and can be transmitted to others (as they're infectious).

infectious disease an illness caused by a pathogen that can be transmitted between individuals

pathogen an agent that causes disease

In lesson 7A, you learned about some different types of pathogens. In order to understand how a pathogen may impact a population, there are two things we need to know:

- how **contagious** the pathogen is – that is, how easily is it transmitted between people
- how **virulent** the pathogen is – that is, how severe is the disease the pathogen causes.

Pathogens that are highly contagious and highly virulent are of greatest concern, as they have the potential to infect large numbers of people and cause severe disease. We need to understand these two aspects of **emerging** and **re-emerging diseases** in order to effectively manage them.

contagious a property of a pathogen or disease meaning that it can be transmitted from one organism to another

virulence the potential of a pathogen or disease to cause serious illness or harm

emerging disease an infectious disease that is new to the human population, or that is rapidly increasing in incidence

re-emerging disease an infectious disease that was previously under control but that is now increasing in incidence

incidence the frequency of a disease in a population

What are emerging and re-emerging pathogens?

In addition to the pathogens that currently circulate among the human population and cause disease, there are always different pathogens threatening to infect us and cause emerging or re-emerging diseases:

- emerging diseases are diseases that have not occurred in humans before, have occurred previously but only affected particular populations in isolated places, or have occurred throughout history but have only recently been recognised as being caused by pathogens.
- re-emerging diseases are diseases that were once major public health problems and then declined dramatically in **incidence**, but are again becoming health problems for a large number of people.

In lesson 8C, you will learn how we combat emerging and re-emerging diseases. But, before we get there, we're left with a very important question – where do these emerging and re-emerging diseases come from?

Where do emerging and re-emerging diseases come from?

A number of factors contribute to the emergence and re-emergence of diseases. Some of these are summarised in Table 1.

Table 1 Factors contributing to the emergence and re-emergence of diseases

Factor	Contribution
Evolution of causative organism	The pathogens causing disease can evolve to either infect humans or, if previously capable of infecting humans, evolve to evade treatments by acquiring resistance.
Globalisation and travel	Due to our ability to quickly travel around the world, diseases that would otherwise have remained localised to a specific area can quickly spread to multiple countries (Figure 1).
Increased exposure of humans to animals	As the human population grows and climate change alters the environment, humans are more likely to come into contact with animals. A **zoonosis** is a disease caused by a pathogen that has been transmitted to humans from another species. An estimated 70% of all emerging diseases originate from some type of animal **reservoir** (Figure 2).
Increasing human population	Larger populations lead to increased population densities in cities, increasing the likelihood of a disease spreading and causing large scale health problems for a population.
Changing technology	Sometimes new technology can be responsible for the emergence or re-emergence of a disease. For example, Legionnaires' disease is caused by a pathogen that inhabits air conditioning systems.
Insufficient vaccination of the population	Previously managed diseases can re-emerge if the proportion of a population that is vaccinated against the disease decreases. This stems from the loss of herd immunity, with an increase in the number of susceptible individuals allowing the pathogen to more easily spread between individuals.

zoonosis an infectious disease that is caused by a pathogen that has transferred from an animal to a human

reservoir a population of animals or environment in which a pathogen normally lives

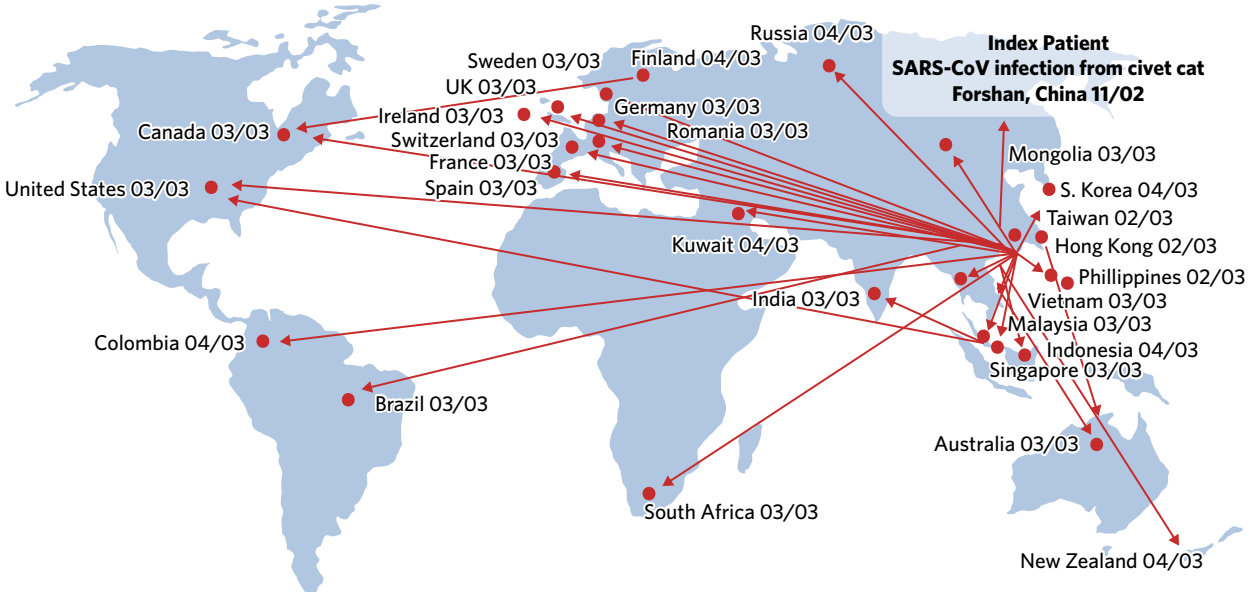


Figure 1 In 2002, an outbreak of the SARS-CoV-1 virus that causes severe acute respiratory syndrome (SARS) (not to be confused with the SARS-CoV-2 virus that causes COVID-19, a different respiratory disease) quickly spread to multiple countries around the world due to international air travel. The first known case of SARS-CoV-1 occurred in China in November 2002, and by the end of April 2003, it had spread to every continent on the globe.

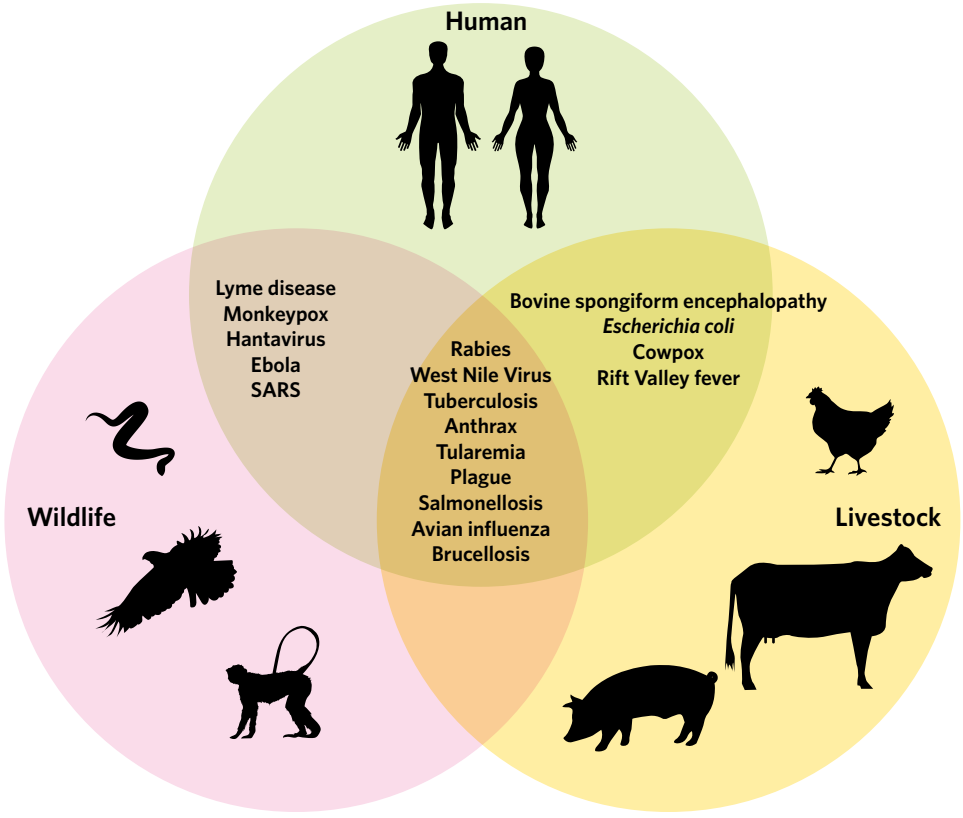


Figure 2 A number of diseases in humans can be traced back to their infectious origins in wildlife and livestock.

The factors contributing to emerging and re-emerging diseases are incredibly complex and differ depending on the causative organism. Table 2 outlines some examples of emerging and re-emerging diseases, as well as contributing factors that have led to their ability to cause widespread disease.

Lesson link

If you can't resist the urge to find out about how pathogens evolve resistance, flick forward to ***lesson 9F*** to learn more.

Lesson link

In ***lesson 8C*** you will learn about the ways authorities attempt to curb the spread of transmissible diseases to prevent epidemics and pandemics from occurring.

Table 2 Emerging and re-emerging diseases

Disease	Pathogen	Emerging/re-emerging	Contributing factors
Ebola haemorrhagic fever	Ebola virus	Re-emerging	Zoonosis – it is currently believed that bats are a natural reservoir of the virus and are responsible for many instances of human infection
Measles	Morbillivirus	Re-emerging	Reduction in vaccination coverage leading to outbreaks
Cholera	*Vibrio cholerae*	Re-emerging	Evolution of a new strain leading to increased virulence and survival in environment
Malaria	*Plasmodium*	Re-emerging	Evolution of drug resistance – changes in environmental conditions leading to an increase in mosquitoes that can transmit disease
Coronavirus disease 2019 (COVID-19)	SARS-CoV-2	Emerging	Suspected zoonosis – with global travel allowing it to quickly spread throughout the world
2009 Pandemic influenza	Swine-origin H1N1 influenza virus	Emerging	Zoonosis – transmission of the virus from pigs to humans
Acquired immunodeficiency syndrome (AIDS)	Human immunodeficiency virus (HIV)	Emerging	Zoonosis – believed to have originated in non-human primates, increased populations in cities, global travel, medical treatments including organ transplants and blood transfusions, drug use, and multiple sexual partners

It is important that authorities respond to emerging and re-emerging diseases quickly. If they do not, an unexpectedly large group of people may become infected, causing an **outbreak**. Disease outbreaks can be classified into one of two categories based on the geographic spread of the disease:

- **epidemics** involve a sudden, widespread increase in the occurrence of an infectious disease among a specific population in a specific location at a particular time
- **pandemics** involve epidemics that have spread to different countries and/or continents in different regions of the world. As such, pandemics typically affect a greater number of people when compared to epidemics and are much more difficult to control.

outbreak a sudden and unexpected increase in the occurrence of a disease

epidemic a dramatically increased occurrence of a disease in a particular community at a particular time

pandemic an epidemic that has spread across multiple countries and/or continents

Pathogens introduced by European arrival to Australia 4.1.7.2

OVERVIEW

Europeans arriving in Australia in the 18th century brought with them a number of diseases that quickly spread throughout the non-immune Aboriginal and Torres Strait Islander populations, infecting and killing thousands of Indigenous people.

THEORY DETAILS

Throughout history, the arrival of a colonising nation in a land occupied by an Indigenous population has resulted in the introduction of disease and mortality to that population. The European colonisation of Australia is no different. The arrival of the first convicts and settlers from England to Australia in 1788 brought about the introduction of disease and dispossession among the Indigenous population.

The major diseases in Europe during the late 18th century were smallpox, syphilis, tuberculosis, influenza, and measles. When colonists arrived in Australia, they brought these diseases with them, unleashing them on the local population and causing widespread disease and death (Figure 3). A number of factors contributed to Australia's Indigenous population being particularly susceptible to these diseases at this time.

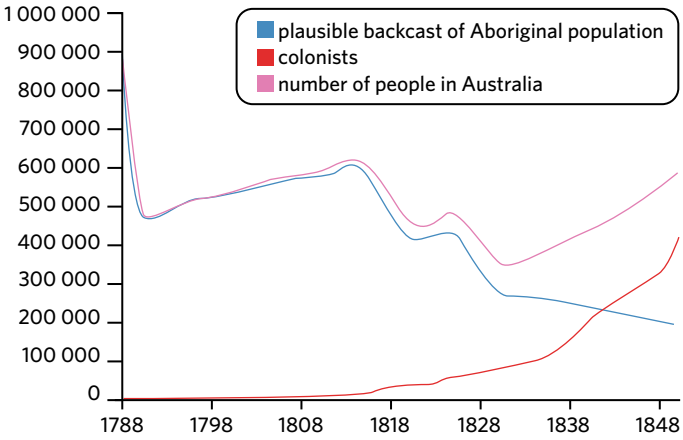


Figure 3 This graph illustrates the effect of European arrival on the Australian Indigenous population. Note that the Australian Aboriginal population numbers are estimates only.

A lack of immunity in the Indigenous population

Some diseases, such as measles, were frequently contracted during childhood by the Europeans. When contracted during childhood, the symptoms of measles are much less severe, and the immunity generated lasts into adulthood. For Indigenous populations, however, many individuals first encountered measles as adults, and as a consequence, experienced severe disease.

Additionally, other diseases brought by the Europeans such as influenza and smallpox were highly contagious and can be highly virulent regardless of age. The European arrivals to Australia would have encountered these diseases in childhood and, having survived them, had some form of **natural active immunity** to them. Unfortunately for the Indigenous Australian population, no such immunity for these diseases existed, meaning they were more likely to contract and experience severe symptoms from them.

natural active immunity protection against a disease created by antibodies and memory cells produced by an individual's own immune system without medical intervention. Also known as **naturally acquired active immunity**

A lack of knowledge and experience with European diseases

Indigenous populations had centuries of knowledge relating to the management and treatment of diseases present in their population. When Europeans arrived, however, and brought new diseases with them, Indigenous people had no knowledge about how to avoid or treat infections. Furthermore, their ability to practice Indigenous medicine was often prevented, meaning Indigenous people were left without any form of medical treatment to help them when infected.

The disruption caused by colonisation

Prior to colonisation, Indigenous people lived in societies that were well adapted to the local environment and were typically spread out and uncrowded. They also had a mixed diet rich in carbohydrates and protein, with most individuals being very healthy. However, after European arrival, their access to food and water was restricted and denied, medicine practices were disrupted, and their relationship with Country and culture was irrevocably changed. They were also forced into camps at the edges of towns, where the opportunities for infection was heightened due to increased population densities.

All of these conditions led to a general decrease in the health status of Aboriginal and Torres Strait Islander people, making them more susceptible to disease and death. However, it is difficult to accurately quantify the sheer impact that the Europeans had on the population and health status of Indigenous Australians.

In Victoria, it is thought that in the first 50 years of contact with European settlers, over 60% of the Indigenous population had succumbed to a series of epidemics. The decline of the Indigenous population in Australia continued into the 20th century, with the negative impacts of European arrival in Australia still being felt by many Indigenous Australians today.

Lesson link

If you've forgotten how immunity is generated, turn back to ***lesson 8A*** to receive a booster shot and refresh your memory.

Theory summary

A number of factors contribute to emerging and re-emerging diseases threatening to cause large-scale outbreaks of disease, such as epidemics or pandemics. The arrival of European settlers in Australia introduced a number of diseases to the susceptible Indigenous population, resulting in mass infections and death.

It is currently believed that the SARS-CoV-2 virus that causes COVID-19 originated in animals and somehow made the leap from animals to humans. As such, COVID-19 is thought to be an example of a zoonotic disease. It is important to understand where pathogens originate from so that we can take measures to decrease the risks of epidemics and pandemics occurring in the future.

8B QUESTIONS

Theory review questions

Question 1

Infectious diseases are caused by

A pathogens.

B lifestyle factors.

Question 2

Which of the following statements about emerging diseases is incorrect?

A Emerging diseases may have previously only occurred in a small, isolated population.

B Emerging diseases were once major public health issues and are increasingly becoming so again.

Question 3

Fill in the blanks in the following sentences.

There are two types of disease outbreaks. _____ are a sudden increase in the occurrence of an _____ disease amongst a specific population in a specific location. _____ are increases in the occurrence of a disease in many different countries and/or continents. Because of this, _____ are more difficult to control than _____ due to the number and geographical distribution of people affected.

Question 4

Which of the following factors contributed to the Australian Aboriginal and Torres Strait Islander population being highly susceptible to diseases brought by European colonists? (*Select all that apply*)

I changes to diet and lifestyle

II a lack of pre-existing immunity

III a lack of any form of medical knowledge

IV disruption to societies and living arrangements

SAC skills questions

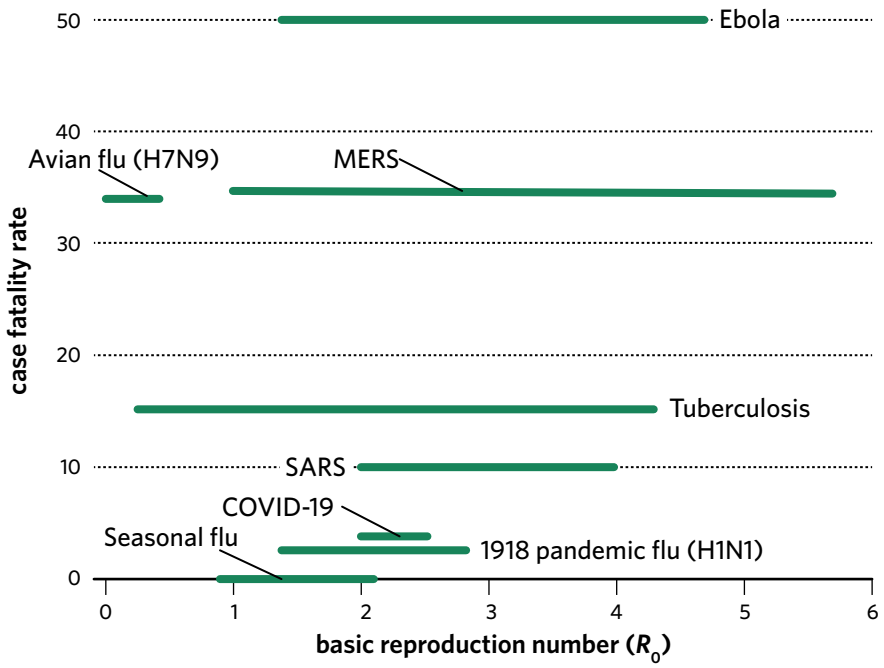
Data analysis

Use the following information to answer Questions 5–9.

Two of the things scientists are keen to understand when an emerging or re-emerging disease becomes known are its virulence and how contagious it is. A measure known as the case fatality rate (CFR) can be used to approximate how virulent a disease is. It represents the proportion of deaths from a certain disease out of the total number of people who were diagnosed with that disease during a period of time.

How contagious a disease is can be represented by another value, called the basic reproduction number (R_0). This value represents the expected number of cases directly caused by one case in a population where all other individuals are susceptible to infection.

The CFR and R_0 values differ for different diseases. The following diagram illustrates the two values for a wide variety of diseases.



Question 5

The case fatality rate of a disease can be used to estimate a disease's

A virulence.

B contagiousness.

Question 6

The R_0 value of a disease is an estimate of

A how many people will die from a certain disease.

B how many people one infected person will transmit the disease to.

Question 7

According to the graph, which disease is the most virulent?

A MERS

B Ebola

C Tuberculosis

D Seasonal flu

Question 8

According to the information presented, it can be reasonably concluded that

A an outbreak of Ebola would always contribute to more total deaths than an outbreak of tuberculosis.

B outbreaks of MERS and the H7N9 flu in an entirely susceptible population will lead to a similar number of deaths for each disease.

C a disease such as COVID-19 that can be fatal, but often presents without any symptoms, will likely have a CFR that is overestimated due to undiagnosed asymptomatic cases of the disease.

Question 9

Which of the following would you expect to not impact the basic reproduction number of a pathogen?

A the immunity status of a population

B the spread of a pathogen around the world

C how the pathogen spreads between individuals

Exam-style questions

Within lesson

Use the following information to answer Questions 10 and 11.

Zika fever is a rapidly emerging viral disease. It is most commonly transferred from one person to another by the *Aedes* genus of mosquito.

Zika fever in people was discovered in Uganda in 1947. It was thought that a bite from a mosquito had transferred the virus from monkeys to humans.

The symptoms of Zika fever are usually mild, and 80% of infected humans do not show symptoms. Infections of pregnant women, however, can cause severe defects in their babies.

Question 10 (1 MARK)

Which one of the following is not likely to have contributed to Zika becoming an emerging disease?

A A decrease in immunity to the virus that causes Zika fever.

B Environmental conditions suitable to the breeding of *Aedes* mosquitoes.

C Encroaching of the human population into the surrounding environment.

D Increased population density in urban settings where Zika fever is present.

Question 11 (1 MARK)

An outbreak of Zika fever occurred in 2015–2016, spreading from Brazil to neighbouring countries in the Americas.

Which one of the following is a correct statement about this outbreak of Zika fever?

A Unless controlled, this outbreak could spread to another country and cause a pandemic.

B Zoonosis is not likely a contributing factor to Zika fever.

C Zika fever is not an example of an infectious disease.

D This outbreak of Zika fever is an epidemic.

Multiple lessons

Question 12 (5 MARKS)

The disease COVID-19 is caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-2). The virus was first identified at the beginning of 2020, and has since gone on to cause a pandemic. It is currently believed that SARS-CoV-2 is an example of a zoonosis.

a What is a zoonosis? (1 MARK)

b What is meant by the term 'pandemic'? (1 MARK)

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q10a

c When cells are infected with viruses such as SARS-CoV-2 they can release interferons. Describe the role of interferons. (1 MARK)

Adapted from VCAA 2007 Exam 1 Section B Q8b

d Throughout 2020, much of the world's medical scientific community was devoted to developing a vaccine for SARS-CoV-2. To evaluate the effectiveness of new vaccines, both humoral and cell-mediated responses are measured in animal subjects. Identify the cell type that is involved in both responses, and explain its role in each. (2 MARKS)

Adapted from VCAA 2015 Section B Q4b

Question 13 (8 MARKS)

Yellow fever is a viral disease that is transmitted primarily by mosquitoes.

An outbreak of yellow fever was reported to have occurred in an area of Brazil in January 2017. This outbreak was reported to be spreading to other areas within Brazil.

a The outbreak of the disease was referred to by scientists as an epidemic rather than a pandemic. Why is the term 'epidemic' correct in this context? (1 MARK)

Adapted from VCAA 2017 Sample Exam Section B Q10a

b Yellow fever has been classified by some scientists as a re-emerging disease. Describe what this suggests about the history of the disease and its status in 2017. (2 MARKS)

c A vaccine for yellow fever first came into use in 1938.

i What type of immunity do vaccines generate? (1 MARK)

ii Explain how a vaccine could provide long-term immunity to yellow fever. (4 MARKS)

Adapted from VCAA 2015 Section B Q4a

Question 14 (3 MARKS)

When Europeans first arrived in Australia in the 18th century, diseases they brought with them quickly spread throughout the Indigenous populations of Australia, resulting in large proportions of these populations dying.

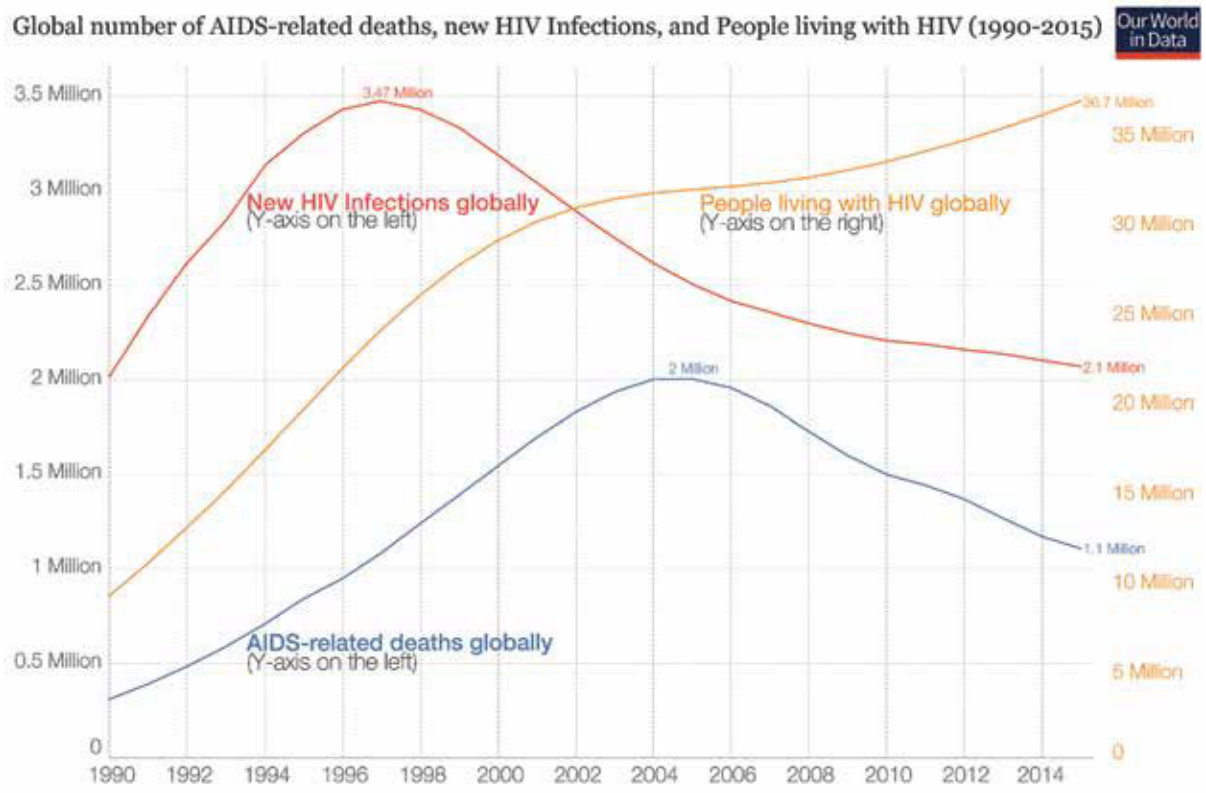
a Many Europeans experienced these diseases during childhood, and as a result had developed immunity to them. Identify the type of immunity developed by the European colonists during childhood to these diseases. (1 MARK)

b Identify two factors that led to Australia's Indigenous population being highly susceptible to the diseases brought by Europeans. (2 MARKS)

Key science skills and ethical understanding

Use the following information to answer Questions 15 and 16.

The graph shows the death rates from acquired immune deficiency syndrome (AIDS), an emerging disease. It also shows the number of people infected with the human immunodeficiency virus (HIV). Before 2004, many people infected with the HIV virus went on to develop AIDS, which led to their deaths.



Source: UN AIDS (2015), adapted by Ortiz-Ospina and Roser (2019)

Question 15 (1 MARK)

Based on the information in the graph, which of the following statements is true?

A The number of deaths peaked in 1997 at 3.47 million.

B The number of deaths in 2004 was approximately 20 million.

C The number of people living with HIV infection was highest in 2015 at 36.7 million.

D The number of people living with HIV infection increased at the fastest rate between 2002 and 2014.

Adapted from VCAA 2018 Section A Q29

Question 16 (1 MARK)

Medications involved in the management of AIDS can be very expensive. One reason for this is drug companies deliberately prolonging patents to drugs, preventing them from being sold for reduced prices.

According to the bioethical concept of respect, this behaviour is unethical because

A this actively causes harm to patients who cannot afford to pay for expensive treatments.

B it does not aim to maximise the benefits of treatments to patients.

C it does not give due regard to the welfare of patients.

D it results in dishonest reporting of findings.

8C CONTROLLING PATHOGEN SPREAD

When you think of what a 'doctor' looks like, you probably imagine someone wearing a stethoscope around their neck and a white lab coat. But in the Middle Ages, during the height of The Black Death, a.k.a. Bubonic Plague, a visit to your local G.P. would have been a bit more interesting – at least from a fashion standpoint. Whilst their terrifying outfit might have made them seem like they were on their way to a bird-based cosplay convention, these outfits actually helped protect the 'plague doctors' from Yersinia pestis*, the bacteria that causes bubonic plague. How did that work? Is* Yersinia pestis *afraid of birds (as we all should be), or was there some science behind their avian-themed get-up?*

Just because you're living in plague times doesn't mean you can't look stylish.

Image: illustrissima/Shutterstock.com

Lesson 8C

In this lesson you will learn about how pathogens are transmitted between hosts, as well as how scientists identify them and use this knowledge to help control disease transmission.

◀ *Prerequisite knowledge*

Lesson 7A
There are many different types of pathogens that can infect humans.

Lesson 8B
Pathogens are constantly evolving and can spread from animal hosts to humans, or can re-emerge after disappearing for a period of time.

Future applications ▶

Lesson 9F
The development of antimicrobial resistance threatens to severely limit our ability to prevent disease outbreaks.

Study design dot point

- scientific and social strategies employed to identify and control the spread of pathogens, including identification of the pathogen and host, modes of transmission, and measures to control transmission

Key knowledge units

Identifying pathogens	4.1.8.1
Modes of disease transmission	4.1.8.2
Controlling disease transmission	4.1.8.3

Identifying pathogens 4.1.8.1

OVERVIEW

Using a variety of physical, immunological, and molecular techniques, scientists are able to identify pathogens that cause disease.

THEORY DETAILS

As you learned in the previous lesson, **pathogens** are frequently evolving and changing. Sometimes a pathogen may change in such a way that it can infect humans, or a pathogen that already infects humans may change to become more **contagious** or more **virulent**. When this happens, we need to be able to quickly control its spread, otherwise we may face an **outbreak** that could potentially lead to an **epidemic** or **pandemic**.

pathogen an agent that causes disease

contagious a property of a pathogen or disease meaning that it can be transmitted from one organism to another

virulence the potential of a pathogen or disease to cause serious illness or harm

outbreak a sudden and unexpected increase in the occurrence of a disease

Before treating someone who is unwell, it is important for scientists and health professionals to know exactly which pathogen is causing a patient's symptoms so that they can select the appropriate response to treat the patient and limit the spread of disease to other people. Some of the methods scientists use to identify pathogens are summarised in Table 1.

Table 1 Methods of identifying pathogens

Method	Description
Physical	Visualising pathogens using microscopes to determine their structure (Figure 1)
Phenotypic	Selective media – an agar plate designed to allow certain pathogens to grow and multiply to test for their presence in a sample (Figure 2). For example, an agar of buffered charcoal yeast extract is highly selective for *Legionella pneumophila*, the bacteria that causes Legionnaires' disease. If this bacteria is present in a sample and combined with buffered charcoal yeast it will grow and multiply, allowing scientists to identify it and determine the appropriate course of treatment.
	Biochemical test panels – a series of tests designed to specify a sample's genus and species. For example, scientists can run a Gram stain to determine if bacteria are Gram-positive or negative, then test if they are aerobic or anaerobic, then run more tests until they can identify the species of bacteria present in a sample (Figure 3).
Immunological	**Serology** – the diagnosis of disease based on the presence of antibodies or antigens in a person's **serum**. One example used to detect the presence of pathogenic antigens in a sample is the **enzyme-linked immunosorbent assay (ELISA)**. There are four main types of ELISA tests – direct, indirect, sandwich, and competitive.
	The sandwich method of ELISA involves: (1) antibodies specific to a certain pathogen are attached to a plate; (2) the serum sample to be tested is then applied to the plate, resulting in any pathogen antigens present attaching to the antibodies; (3) a second detection antibody, linked to a colour-changing enzyme, is added to the plate, binding to any antibody-antigen complexes present; (4) a substrate is then added, reacting with the enzyme on the second antibody and changing colour/emitting a signal to reveal whether any pathogenic antigens were present in the sample (Figure 4).
Molecular	Hybridisation-based detection – labelled segments of genetic material that are complementary to a pathogen's genetic material are added to a sample. If a signal is generated, it means a pathogen is present.
	Whole-genome sequencing – provides detailed information about the pathogen.

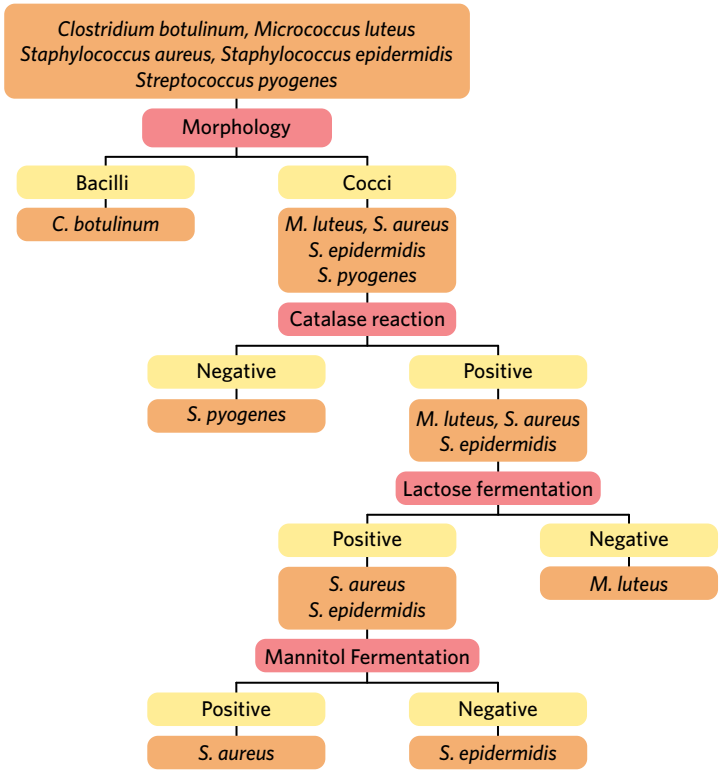


Figure 3 In this example of biochemical testing, scientists have a sample they believe consists of one of the five species of bacteria shown in the top box. Gradually, by conducting a series of different tests and reactions, and recording how their sample responds to each one, they can figure out the species of bacteria present.

epidemic a dramatically increased occurrence of a disease in a particular community at a particular time

pandemic an epidemic that has spread across multiple countries and/or continents

serology the study of blood serum, typically to determine the presence of antibodies and/or antigens

serum the fluid and solute component of blood that excludes blood cells, platelets, and clotting factors

enzyme-linked immunosorbent assay (ELISA) an experimental technique used to identify a pathogen by determining the presence of antigens or antibodies in a sample

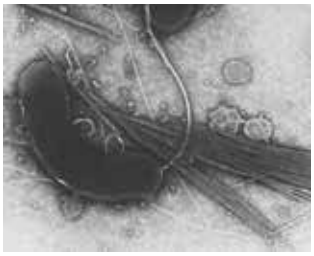


Figure 1 *Vibrio cholerae*, the pathogen that causes cholera, as seen under a microscope.

Image: AnaLysiSStudiO/Shutterstock.com

Figure 2 This agar plate is selective for *Escherichia coli*. Given that growth has occurred on the plate, it indicates to scientists that the sample used to swab the plate had *E. coli* present in it, as opposed to some other unknown bacteria.

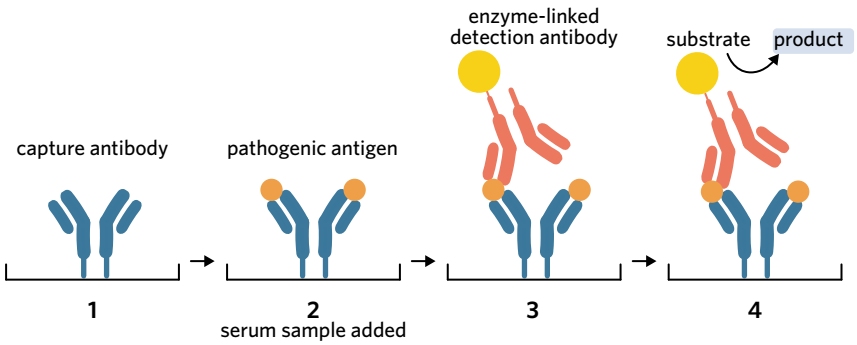


Figure 4 The sandwich method of ELISA is used to determine if a specific pathogenic antigen is present in a sample. The presence of pathogenic antigens can be identified by the colour change that occurs when the substrate is converted to a product by the enzyme-linked detection antibody.

Lesson link

If only a trace of your knowledge about PCR remains, head back to ***lesson 4C*** to amplify it.

Modes of disease transmission 4.1.8.2

OVERVIEW

Pathogens rely on five key methods of transmission to move from an infected host to a non-infected host: airborne transmission, droplet transmission, direct physical contact, indirect physical contact, and faecal-oral transmission.

THEORY DETAILS

In order for pathogens to continue their life cycle and ensure their survival, they need to move from one **host** to another in a process known as **transmission**. Pathogens can be transmitted between hosts in five key ways, outlined in Table 2 and Figure 5. Note that many pathogens utilise multiple modes of transmission – for example, they may be acquired via both airborne or droplet transmission. Likewise, many pathogens that spread via the faecal-oral route do so indirectly through contaminated food and water.

host an organism that harbours a pathogen

transmission the passing of a pathogen from an infected host to another individual or group

Table 2 Modes of pathogen transmission

Transmission route	Description	Examples
Airborne transmission	Pathogens spread via very small particles (traditionally <5 μm) that stay in the air for prolonged periods of time after a person sneezes, coughs, exhales, or talks. A person can inhale these particles and become sick even after the original host has left the vicinity.	Influenza virus – the causative agent of the flu SARS-CoV-2 – the causative agent of COVID-19 Rhinovirus – the causative agent of the common cold
Droplet transmission	**Respiratory droplets** containing pathogens can remain suspended in the air for a short period of time, before falling to the ground/onto a surface. If a person touches a surface containing droplets and then touches a mucosal surface (such as their eyes, mouth, or nose) the pathogen from the droplet may enter their system and infect them.	
Direct physical contact transmission	Pathogens can spread when a host physically touches another individual. This contact can occur either via skin-to-skin touch, sharing of bodily fluids, sexual contact, oral contact (kissing), from mother to baby *in utero* or post-birth (**vertical transmission**), or contact with a contaminated material during some medical procedures (**iatrogenic**).	*Tinea pedis* – the causative organism of athlete's foot Human immunodeficiency virus (HIV) – the causative agent of acquired immunodeficiency syndrome (AIDS) Epstein-Barr virus (EBV) – the causative agent of infectious mononucleosis (glandular fever)
Indirect physical contact transmission	Indirect transmission occurs when there is no direct host-to-host contact. Instead, pathogens are spread between hosts via **fomites** (e.g. food, water, tissues, needles) or a **vector** (e.g. mosquitoes).	*Plasmodium* – the causative agent of malaria, spread by mosquitoes
Faecal-oral transmission	Pathogens excreted in faeces can end up being consumed by another person indirectly via contamination of food or water by infected faeces. Additionally, faecal-oral transmission can also occur via airborne or droplet routes through the aerosilisation of pathogens when faeces is flushed.	*Vibrio cholerae* – the causative organism of cholera Rotavirus – the causative agent of diarrhoea, typically in young children

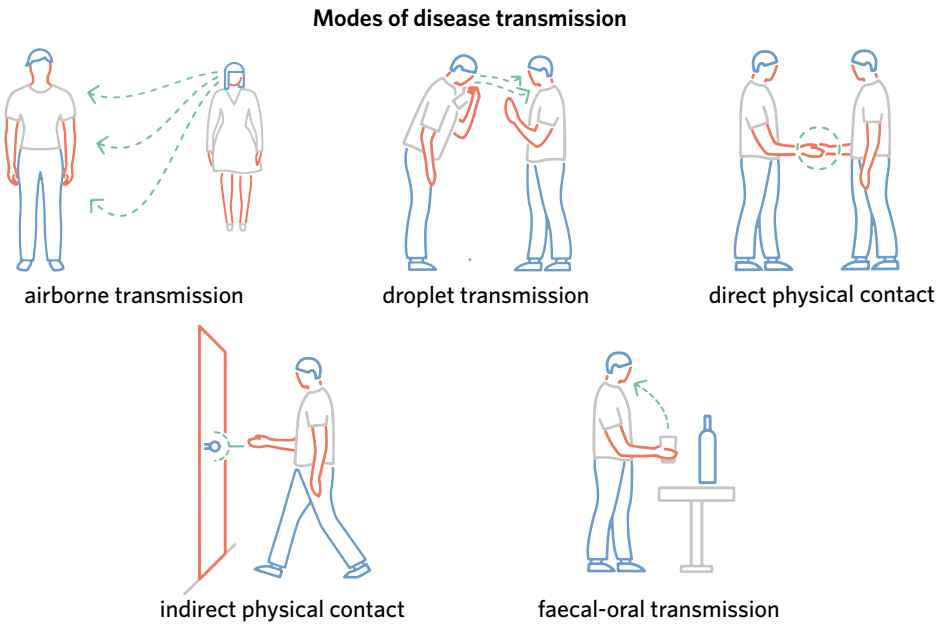


Image: Double Brain/Shutterstock.com

Figure 5 The various modes of disease transmission

Controlling disease transmission 4.1.8.3

OVERVIEW

The approach to managing a disease is complex and depends on the pathogen. Generally, strategies will include identification of the pathogen, prevention of disease, measures to control the spread of the pathogen, and treatment of those who are already sick.

THEORY DETAILS

There are many different ways we can stem the transmission of a pathogen. Exactly how this is achieved depends on a number of factors, including what type of pathogen it is and the mode/s of transmission it uses to spread between hosts. Table 3 outlines some key strategies for controlling disease transmission.

In order to effectively contain a disease, large amounts of coordination between local and national governments, as well as international organisations like the World Health Organisation (WHO) and the United Nations, is required. When this occurs, the transmission of disease can be stopped before it causes mass infections and death. For example, the SARS-CoV-1 virus that causes Severe Acute Respiratory Syndrome caused an outbreak in China in November 2002 that spread to 29 countries. The coordinated response and successful implementation of public health measures led to the WHO declaring the disease contained just a few months later in July 2003.

Table 3 Key strategies in controlling disease transmission

Strategy	Explanation
Prevention	• Improving hygiene and sanitation via handwashing • Sterilising hands and surfaces/tools using **antiseptics** and **disinfectants** • Ensuring access to clean water and food • Using personal protective equipment (PPE) such as gloves and masks when dealing with sick people (Figure 6a) • Vaccination, if a vaccine exists for the disease in question • Lockdown of areas/restrictions to reduce people's movement and the chance of spreading a disease
Screening	• Routine testing for the presence of disease in a population allows public health workers to quickly see who in a population is affected so they can target their response • Officials may observe medication sales at pharmacies and look for changes that might indicate that the prevalence of certain symptoms or illnesses has increased

cont'd

airborne transmission the spread of pathogens through air via small particles (traditionally <5 μm)

droplet transmission the spread of pathogens through air and contaminated surfaces via respiratory droplets

respiratory droplets droplets (traditionally >5 μm) produced by breathing, talking, vomiting, and coughing. They may contain saliva, mucus, and other substances from the respiratory tract, including cells/particles of pathogens

direct physical contact transmission the spread of pathogens through contact between a host and another individual

vertical transmission spread of pathogens from mother-to-child during gestation, during childbirth, or post-birth due to close physical contact and breastfeeding of a newborn

iatrogenic describes a disease caused by medical intervention

indirect physical contact transmission the spread of pathogens via contaminated objects or vectors

fomites an inanimate object that, when contaminated with a pathogen, can transmit that pathogen to a new host

vector an organism that is not affected by a disease but spreads it between hosts

faecal-oral transmission the spread of pathogens via oral consumption of contaminated faeces

antiseptic a substance that is applied to living tissue to kill or slow the growth of microorganisms

disinfectant a substance that is applied to non-living materials to kill or slow the growth of microorganisms

Table 3 Continued

Strategy	Explanation
Quarantine and isolation	• Once a person becomes ill or has the potential to become ill (e.g. is returning home from visiting an affected area overseas), they may be separated from healthy people to ensure they don't spread their disease to the community (Figure 6b)
Identification of the pathogen	• Using the methods outlined in Table 1, scientists will attempt to identify which pathogen is present in an individual so they can initiate the appropriate responses
Identify and control mode/s of transmission	• Once officials know which pathogen is present, they can take appropriate steps to mitigate its transmission • For example, if a respiratory pathogen is threatening to cause an outbreak, measures that reduce the risk of airborne and droplet transmission (e.g. wearing of surgical masks, social distancing) will be taken to control transmission
Treating infected individuals	• Specific curative treatment, including the use of medications such as **antibiotics** and **antivirals** to target the pathogen – Antibiotics are medicines that can be used to treat diseases caused by bacteria. They selectively affect bacterial cells by targeting specific biochemical pathways or components unique to bacteria, without damaging the patient's cells – Antivirals are medicines that can be used to treat diseases caused by viruses. Similar to antibiotics, antivirals are designed to specifically target viruses, interfering with their ability to attach to, replicate in, and exit from a host cell It is important to note that, given their specificity to bacteria, antibiotics will have no therapeutic effect in someone infected with a virus and therefore should not be prescribed to someone with a viral infection. Inappropriate use of antibiotics can lead to **antimicrobial resistance** in which bacteria are no longer affected by antibiotics (Figure 7).

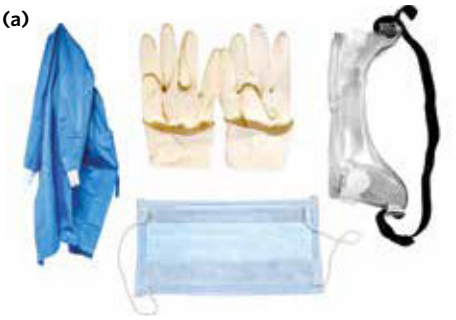


Image (a): H K Singh/Shutterstock.com

Figure 6 **(a)** presenting next flu season's hottest fashion accessories – PPE! **(b)** the crew of Apollo 11 in quarantine after returning from the moon

Theory in context

'SNOW MORE CHOLERA!

John Snow was an English physician who was living in London during the cholera outbreak of 1854 (Figure 8). At the time, a cholera pandemic was sweeping the globe, and people thought the disease was spread via airborne particles called 'miasmata.' John Snow, however, investigated the distribution of cholera in a street called Broad Street and determined that the illness was not due to people breathing 'bad air.' Rather, by speaking to people who had been ill and tracing their movements prior to becoming ill, Snow figured out that the source of the illness was contaminated water coming from the public water pump on the corner of Broad Street. The water from this pump was being contaminated by an old cesspit that had begun to leak faecal bacteria into the water.

Snow's evidence convinced the local parish to remove the handle from the pump, preventing people from using it. Not surprisingly, cholera rates rapidly decreased. This event is considered the founding event of modern epidemiology and is a great example of how studying a disease, identifying a pathogen, figuring out how the pathogen is transmitted between hosts, and finding ways to control transmission are vital components of disease management.

Figure 8 Find yourself a man who can cook, clean, and invent a whole new field of medicine.

Theory in action

Check out scientific investigations 8.1 and 8.2 to put this into action!

antibiotic medications used to kill bacteria or slow their growth

antiviral medications used to treat viral infections

antimicrobial resistance the ability of a microorganism to survive exposure to an antimicrobial agent

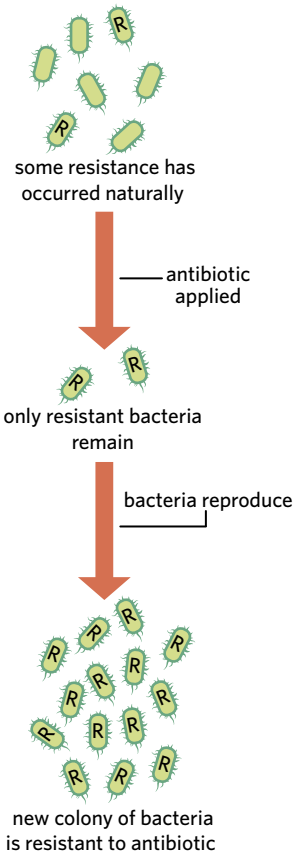


Figure 7 Resistance is developed in bacteria via natural selection.

Lesson link

If you can't resist the temptation to learn all about bacterial resistance, head to ***lesson 9F*** where it is explored in more detail.

Theory summary

Controlling the spread of pathogens consists of a number of stages, including identifying the pathogen, identifying the mode of transmission, and undertaking measures to control transmission. Using these management strategies we aim to prevent large outbreaks of disease from occurring.

Whilst the outfits of plague doctors may have looked unique, they actually did confer their wearers with some protection from Yersinia pestis*, the bacteria that causes plague.* Yersinia pestis *can be transmitted through direct physical contact, indirect physical contact via a vector (fleas), and through airborne transmission. By wearing long coats, gloves, and masks, plague doctors unknowingly reduced their likelihood of contracting* Yersinia pestis*. Remember, at this point in time they didn't know about the germ theory of disease, so any protection conferred by their attire was largely coincidental. This ensured plague doctors could continue their important work of bloodletting and applying leeches to plague patients (both of which, unsurprisingly, did absolutely nothing to help).*

A modern doctor in their bird/plague suit
Image: VIAVAL TOURS/Shutterstock.com

8C QUESTIONS

Theory review questions

Question 1

Which of the following is not a technique used to identify pathogens?

A visualising pathogens with a microscope
B enzyme-linked immunosorbent assay
C genome sequencing
D allergy testing

Question 2

Match the mode of disease transmission to its corresponding example. Terms may be used multiple times or not at all.

Mode of disease transmission

- airborne
- droplet
- direct physical contact
- indirect physical contact
- faecal-oral

Example

I _____ transmission of the Epstein-Barr virus between two individuals who are kissing
II _____ transmission of *Vibrio cholerae* via the leaking of infected nappies into a water supply
III _____ transmission of influenza virus via large particles that rapidly fall to the ground
IV _____ transmission of malaria via mosquitoes serving as a vector
V _____ transmission of rhinovirus via small particles that stay suspended in the air for a prolonged period of time

Question 3

Which of the following statements about controlling disease transmission is correct?

A To identify pathogens, doctors must quarantine sick people.
B Preventative measures of controlling transmission include vaccination.
C Treatment for a bacterial disease would include the use of antiviral medication.
D Vectors show symptoms of the disease and controlling them limits the spread of disease.

Question 4

Fill in the blanks in the following sentences.

_____ are antimicrobial agents that are applied to non-living surfaces, whereas _____ are a substance applied to living tissues to kill microorganisms. _____ are medications used to kill bacteria, whilst _____ are medications used to treat viral infections. Inappropriate use of antimicrobial agents can lead to the development of _____.

SAC skills questions

Data analysis

Use the following information to answer Questions 5–11.

Streptococcus agalactiae, also known as group B streptococcus, is a bacterium that is typically a harmless commensal organism, colonising the gastrointestinal and genitourinary tracts of up to 30% of the adult population. Group B streptococcal infections can occur, however, in babies, and is one of the leading causes of neonatal sepsis (a blood infection in an infant less than 90 days old).

The majority of cases of group B streptococcus infections in newborns are caused by vertical transmission during birth from a mother whose vagina is colonised with *Streptococcus agalactiae*. It is recommended that pregnant women are screened pre-birth to determine if their vagina is colonised with group B streptococcus. If they are found to be carriers, it has been established that they should receive antibiotics prior to giving birth vaginally to prevent vertical transmission.

A group of doctors in Spain sought to determine if the timing of these antibiotics affected the rate of *Streptococcus agalactiae* neonatal infections. To do so, they varied the timing of administration of antibiotics in a group of pregnant women who were *Streptococcus agalactiae* carriers. Their results are given in the table.

Time between administration of antibiotic and delivery (hours)	**Number of *Streptococcus agalactiae* carriers**	**Number of *Streptococcus agalactiae*-infected newborns (%)**
<1	24	11 (46)
1–2	21	6 (29)
>2–4	70	2 (2.9)
>4	86	1 (1.2)
Control group	253	120 (47)

Source: adapted from De Cueto et al. (1998)

Question 5

Neonatal sepsis is

A a blood infection in a newborn baby.

B an infection of a mother's genitourinary tract.

Question 6

Women who are found to be carriers of *Streptococcus agalactiae*

A are unable to give birth vaginally due to the risk of vertical transmission.

B can give birth vaginally, but it is advised that they receive antibiotics prior to delivery.

Question 7

Vertical transmission

A always results in neonatal sepsis.

B refers to transmission between mother and child during birth.

C refers to any transmission of a pathogen between a mother and her newborn child.

Question 8

How many newborns were infected with *Streptococcus agalactiae* in mothers who were carriers and received antibiotics 1–2 hours prior to delivery?

A 29

B 21

C 6

Question 9

Based on the data provided, which timing of antibiotic delivery gave the best results in terms of neonatal *Streptococcus agalactiae* infections?

A <1 hour prior to delivery

B 1–2 hours prior to delivery

C >2–4 hours prior to delivery

D >4 hours prior to delivery

Question 10

Which of the following would have been a suitable control group for this study?

A mothers receiving antibiotics 2–4 hours prior to delivery

B mothers receiving no antibiotics prior to delivery

C mothers receiving antibiotics during delivery

D mothers receiving antibiotics after delivery

Question 11

Which of the following statements regarding the administration time of antibiotics is correct?

A Without the delivery of antibiotics prior to birth, all babies born to *Streptococcus agalactiae* positive mothers will develop a *Streptococcus agalactiae* infection.

B Delivery of antibiotics less than 1 hour prior to delivery has little to no effect on *Streptococcus agalactiae* infection rates in newborns compared with no antibiotic exposure.

C Delivery of antibiotics between 2–4 hours prior to delivery was less effective at preventing *Streptococcus agalactiae* infection rates in mothers compared to administration of antibiotics more than 4 hours prior to delivery.

Exam-style questions

Within lesson

Use the following information to answer Questions 12 and 13.

Malaria is a disease caused by a eukaryotic parasite spread by mosquitoes. Female *Anopheles* mosquitoes transmit an infective form of the parasite into the bloodstream when they bite a vertebrate host such as a human.

Question 12 (1 MARK)

Using the information given, it can be reasonably concluded that

A antibiotics would be effective against malaria.

B mosquitoes are acting as the pathogen's vector.

C all people bitten by a female *Anopheles* mosquito will contract malaria.

D a person could be infected by coming into contact with a person with malaria.

Adapted from VCAA 2013 Section A Q16

Question 13 (1 MARK)

Based on the information provided, which of the following would not be an effective method of stopping the spread of malaria?

A culling of mosquitoes

B isolating infected people

C using nets to cover a person while they are asleep

D providing an infected population with access to antimalarial medication

Question 14 (6 MARKS)

The table compares how eight diseases spread and the number of people likely to be infected by one other infected person. For example, on average, each individual infected with measles is likely to infect a further 12 to 18 other people.

Disease	Measles	Whooping cough	Rubella	Polio	Smallpox	Mumps	Severe acute respiratory syndrome (SARS)	Ebola
How it spreads	airborne droplets	airborne droplets	airborne droplets	faecal-oral route	airborne droplets	airborne droplets	airborne droplets	bodily fluids
Number of people infected from one other person	12 to 18	12 to 17	6 to 7	5 to 7	5 to 7	4 to 7	2 to 4	1 to 4

Source: Thomson Reuters (2018), adapted from VCAA 2018 Section A Q32

a Using the information provided, identify an effective method for the prevention of the spread of polio during an outbreak. (1 MARK)

Adapted from VCAA 2018 Section A Q32

b Based on the information provided, which disease is the least contagious? (1 MARK)

c In 2018 an outbreak of Ebola in Kivu was referred to by scientists as an epidemic.

i Name and describe a modern method that scientists could have used to identify the pathogen in the Kivu outbreak. (2 MARKS)

Adapted from VCAA 2017 Sample Exam Section B Q10e

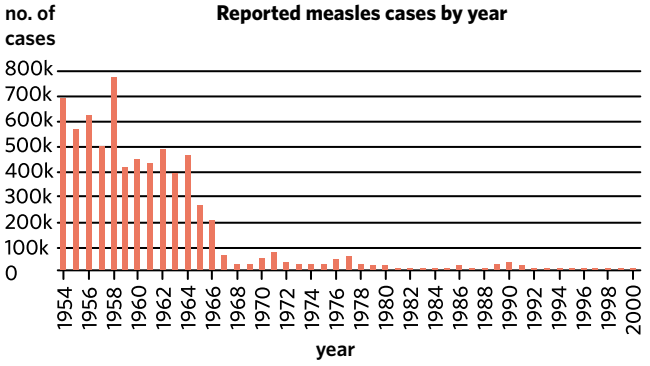
ii Explain what course of action Australian authorities may take for a person wanting to re-enter Australia after visiting Kivu during the epidemic, and state why this action would be taken. (2 MARKS)

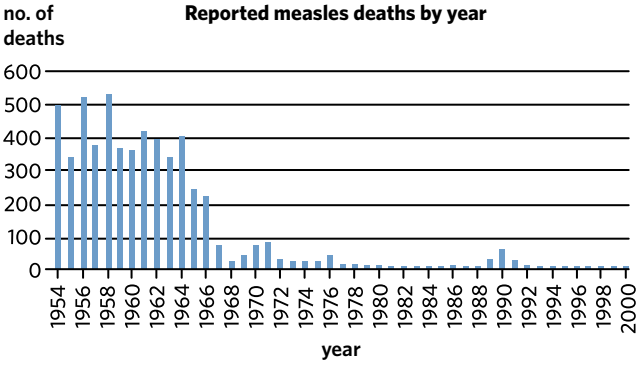
Adapted from VCAA 2012 Exam 1 Section B Q7c

Multiple lessons

Question 15 (8 MARKS)

Measles is a highly infectious and dangerous disease. Young children and individuals with impaired immunity are especially susceptible to measles. It is caused by the measles virus and is spread via airborne droplets. The following graphs show the number of people in the United States of America (USA) who were infected with measles during the period 1954–2000 and the number of people who died as a result of having measles during the same period.





Source: ProCon, adapted from VCAA 2018 Section B Q5

a Which year had the greatest number of reported measles deaths? (1 MARK)

Adapted from VCAA 2018 Section B Q5ai

b A person with measles visits a doctor and asks for treatment for their disease. Would antibiotics be an effective treatment? Justify your response. (2 MARKS)

Adapted from VCAA 2018 Section B Q8c

c In Australia, the government is aiming to achieve a vaccination rate against the measles virus of 95% in the Australian population.

i What kind of immunity is the government trying to achieve in this population with this high vaccination rate? (1 MARK)

ii How does this type of immunity protect the 5% of the population who have not been vaccinated? (2 MARKS)

Adapted from VCAA 2017 Sample Exam Section B Q5b

d Describe two ways in which the innate immune system of a person's body would protect against an infection by this virus. (2 MARKS)

Adapted from VCAA 2012 Exam 1 Section B Q7c

Key science skills and ethical understanding

Question 16 (10 MARKS)

Sharon wanted to investigate the efficacy of an antibiotic against the bacterium *Escherichia coli*. She prepared solutions containing five different concentrations of the antibiotic.

She wrote the following method:

1. Put on a pair of disposable gloves.
2. Collect five agar plates containing nutrient agar.
3. Label each agar plate with the five different concentrations of the antibiotic.
4. Collect a sample of *E. coli* in a broth culture.
5. Measure 0.5 mL of broth in a pipette and place it in the centre of the first agar plate.
6. Spread the bacteria evenly over the agar plate with the spreader provided.
7. Place a paper disk soaked in the antibiotic solution into the centre of the plate.
8. Close the lid of the agar plate and tape the lid to the bottom of the agar plate with sticky tape.
9. Repeat steps 5 to 8 with the other four concentrations of the antibiotic.
10. Place the agar plates on the side bench and leave overnight.
11. Wash your hands and dispose of the gloves.
12. After incubation, measure the zone of inhibition of bacterial growth using a ruler with 1 cm markings.

a Describe the relationship between bacterial growth and antibiotic concentration Sharon would expect to see in her results. (1 MARK)

b What sort of error could using the ruler with 1 cm markings create in Sharon's results? Justify your response, and suggest how this error could be reduced. (3 MARKS)

c Identify the dependent variable in the experiment. Justify your answer. (2 MARKS)

Adapted from VCAA 2017 Sample Exam Section B Q11b

d Sharon wanted to repeat the experiment to test the efficacy of an antifungal drug against *E. coli*. She prepared five different concentrations of the antifungal drug and followed the same steps that she used for the antibiotic.

Explain the results that Sharon would expect to obtain. (2 MARKS)

Adapted from VCAA 2017 Sample Exam Section B Q11d

e Sharon obtained results that did not support her hypothesis, yet she changed these results prior to publishing her findings so that her initial hypothesis appeared correct. Identify which bioethical concept is not being adhered to by Sharon's actions and explain why. (2 MARKS)

8D IMMUNOTHERAPY

From movies and TV shows, to your pantry and wardrobe, mice have taken over the world. What's the next venture on their mighty mouse agenda? Science. More specifically, curing cancer. How exactly are mice helping the fight against cancer? Has a medical school for mice opened somewhere that nobody told me about?

Two young medical students heading to their first day of university – future Drs. Michael Ice and Mary Ouse!

Image: George Sheldon/Shutterstock.com

Lesson 8D

In this lesson you will learn how monoclonal antibodies can be used to manage conditions such as cancer and autoimmune diseases.

◀ *Prerequisite knowledge* *Future applications* ▶

Lesson 7A
The body distinguishes between self and non-self. Pathogens are classified as non-self.

Lesson 7D
Monoclonal antibodies are created using selected plasma cells.

Lesson 9A
Mutations can lead to the development of cancer and autoimmune diseases.

Study design dot point

- the development of immunotherapy strategies, including the use of monoclonal antibodies for the treatment of autoimmune diseases and cancer

Key knowledge units

Immunotherapy	4.1.10.1
Monoclonal antibodies	4.1.10.2
Monoclonal antibodies and cancer	4.1.10.3
Monoclonal antibodies and autoimmune disease	4.1.10.4

Immunotherapy 4.1.10.1

OVERVIEW

Immunotherapy is a form of medical treatment that modulates the functioning of the immune system in order to treat disease.

THEORY DETAILS

Sometimes in life we need a little help to function at our best. If you have a Biology SAC first thing on a Friday morning, you might need a shot of coffee to help wake you up and get those neurons firing. The immune system is the same – sometimes it needs a bit of help to function at its best.

Immunotherapy is a category of medical treatments that change the way the immune system functions. There are two broad categories of immunotherapy:

- activation immunotherapies, which aim to induce or amplify an immune response
- suppression immunotherapies, which aim to prevent or reduce an immune response.

Immunotherapy is a very useful treatment when dealing with diseases related to the immune system. It is a relatively new approach to disease management, and as such a number of immunotherapy agents are still in experimental phases. Many different types of treatments can be classified as forms of immunotherapy. Some examples are summarised in Table 1.

immunotherapy medical interventions that treat disease by modulating the immune system, typically by either amplifying or reducing an immune response

chimeric an organism or cell containing genetic material from another organism or cell

monoclonal antibodies (mAbs) identical laboratory-made antibodies produced by plasma cell clones

Examiners' tip

While it is not necessary to memorise these treatment types for the purpose of VCE Biology, you should understand the following before continuing: (1) immunotherapy is a blanket term that refers to the altering of the immune system's function via medical intervention, and (2) some immunotherapies are designed to increase the action of the immune system, while others suppress or decrease it.

Table 1 Examples of immunotherapy

Treatment	Description
Dendritic cell therapy	Involves the priming of dendritic cells with tumour-associated antigens (TAAs) to facilitate the activation of lymphocytes, priming them to kill any cells expressing the tumour antigen. This priming can be achieved via a vaccination with TAAs, or by removing dendritic cells from the body and priming them with TAAs externally before infusing them back into the patient (Figure 1).
CAR-T therapy	Involves the modification of T cells to recognise and destroy cancer cells. T cells are extracted from the patient, and scientists add a gene coding for an antigen receptor into its DNA. This protein then gets made by the cell and inserted into its membrane, allowing it to recognise cancer cell antigens. These cells with **chimeric** antigen receptors (CAR) are then reintroduced into the patient and seek out and destroy cancer cells (Figure 2).
Antibody therapy	Involves the creation and use of antibodies to stimulate and enhance the functioning of the immune system, and is often used in the treatment of cancer. Antibodies used in antibody therapy are typically **monoclonal antibodies**.
Cytokine therapy	Involves the use of immune signalling molecules such as interferons and interleukins to modulate the effect of the immune system.

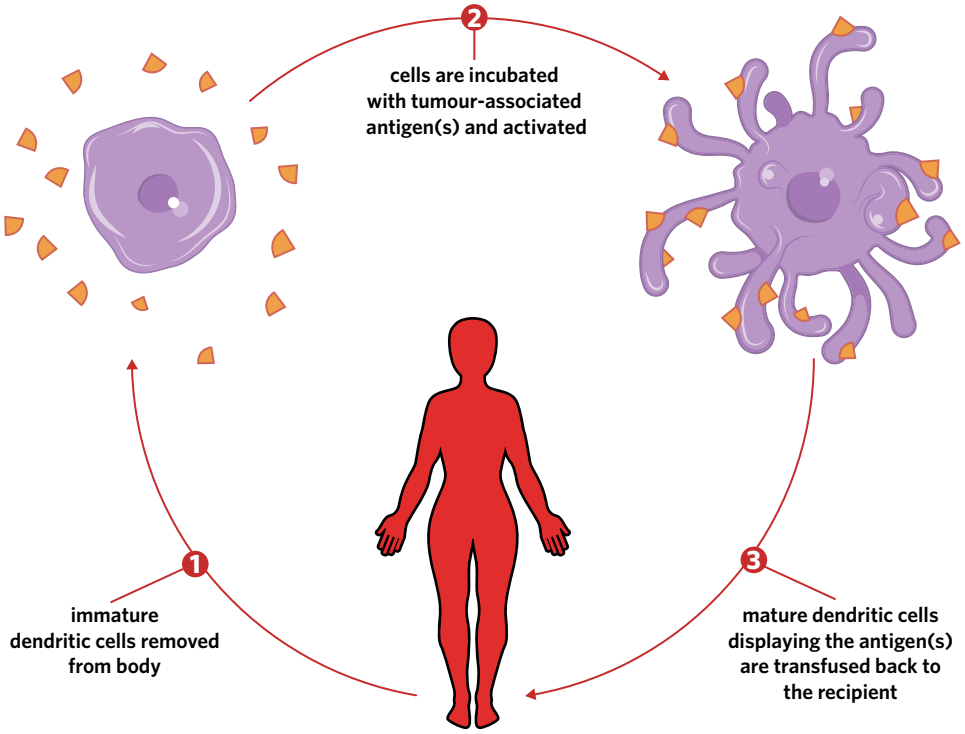


Figure 1 How dendritic cell therapy works. Note that the dendritic cells are first removed from the patient and combined with tumour-associated antigens in a lab before being transfused back into the patient in an effort to combat the tumour.

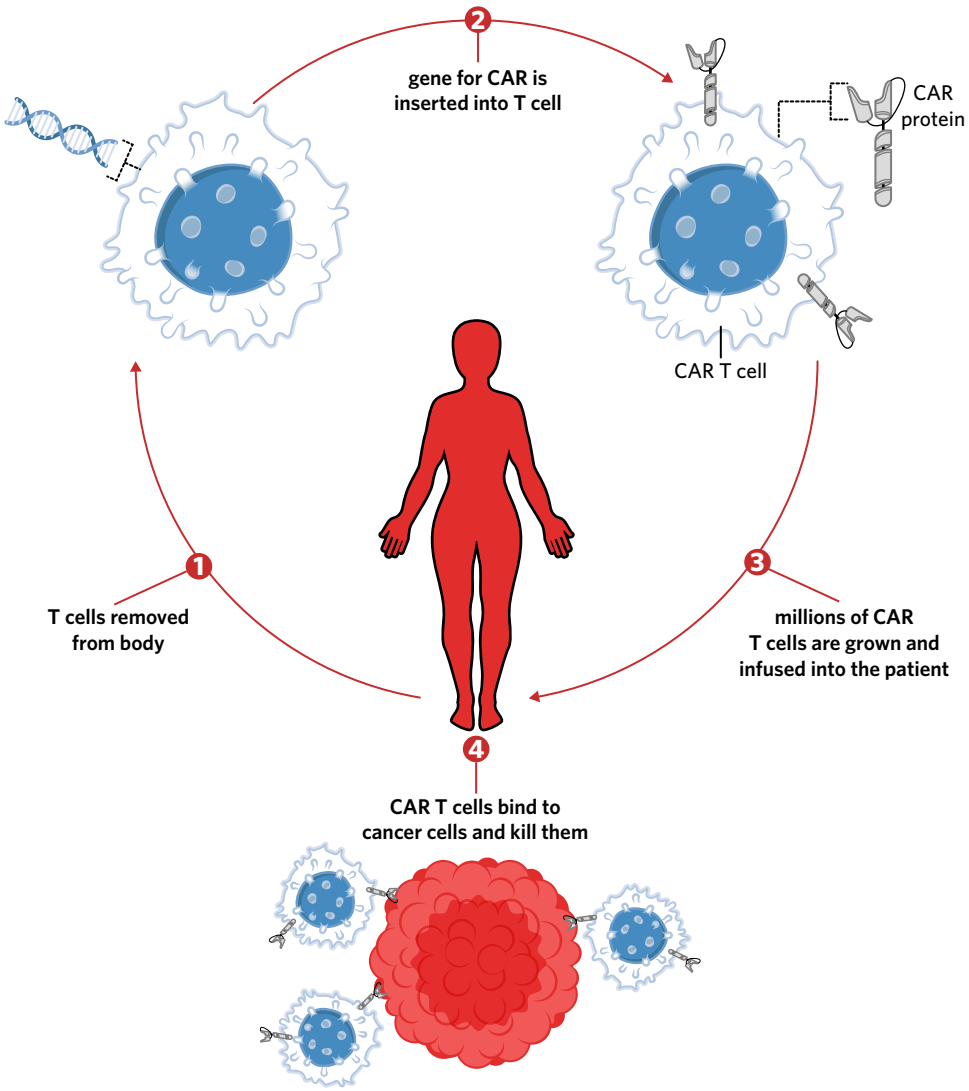


Figure 2 How CAR-T therapy works. Note the presence of CAR proteins on the surface of CAR T cells – these proteins go on to interact with cancer cells and induce T cell-mediated death.

Monoclonal antibodies are a very important component of antibody-based immunotherapy. Let's pause here and take a quick look at exactly what monoclonal antibodies are, as this will help when it comes time to learn about how they can be used to create immunotherapies to treat **cancer** and **autoimmune diseases** later in the lesson.

cancer a disease caused by the uncontrolled replication of cells with the ability to migrate to other parts of the body

autoimmune disease a disease in which an individual's immune system initiates an immune response against their own cells

Monoclonal antibodies 4.1.10.2

OVERVIEW

Monoclonal antibodies are laboratory-made proteins that can be used to treat a number of different diseases.

THEORY DETAILS

What are monoclonal antibodies?

Monoclonal antibodies are antibodies produced in a laboratory that bind to a specific antigen. Because of their specificity to one antigen, monoclonal antibodies can be used to target specific types or parts of cells for a variety of therapeutic purposes. For example, monoclonal antibodies can be used to treat cancer and autoimmune diseases due to their ability to trigger the killing of cancerous or self-recognising cells respectively. You'll learn more about the use of monoclonal antibodies as immunotherapy for these diseases later in this lesson.

Lesson link

Losing your sense of humour-al responses is no laughing matter! Head back to ***lesson 7D*** to brush up on your antibody knowledge.

How are monoclonal antibodies produced?

The process of making monoclonal antibodies is as follows (Figure 3):

1 Scientists identify and isolate an antigen that is present on a desired target cell. This cell will typically be one that is responsible for causing the disease that scientists want to treat. For example, it may be a cancer cell.

2 Scientists vaccinate an animal, usually mice, with an antigen. As you learned in lesson 8A, a vaccination stimulates an immune response against the antigen and results in the selection and proliferation of a **B lymphocyte** that matches the antigen.

3 Scientists extract these B lymphocytes from the spleen of the mice.

4 The extracted B lymphocytes are fused with rapidly-dividing cancerous human plasma cells known as **myeloma cells**. The products of this fusion are called **hybridomas**. The reason these myeloma cells are chosen to fuse with the B lymphocytes is because B lymphocytes do not grow well *in vitro*, whereas myeloma cells have the ability to grow indefinitely and produce large quantities of antibodies.

5 Hybridomas are screened so that only the cells with the appropriate antibody are selected. The hybridomas that produce the specific antibody are cloned, which results in the mass production of these antibodies.

6 Antibodies are then collected and purified before being administered to a patient.

B lymphocyte a type of lymphocyte that plays an important role in humoral immunity and differentiates into plasma cells and B memory cells

myeloma cells rapidly-dividing cancerous plasma cells which are fused with extracted B cells from mice to produce hybridomas

hybridoma the product of the fusion between a mouse's extracted plasma cell and a myeloma cell

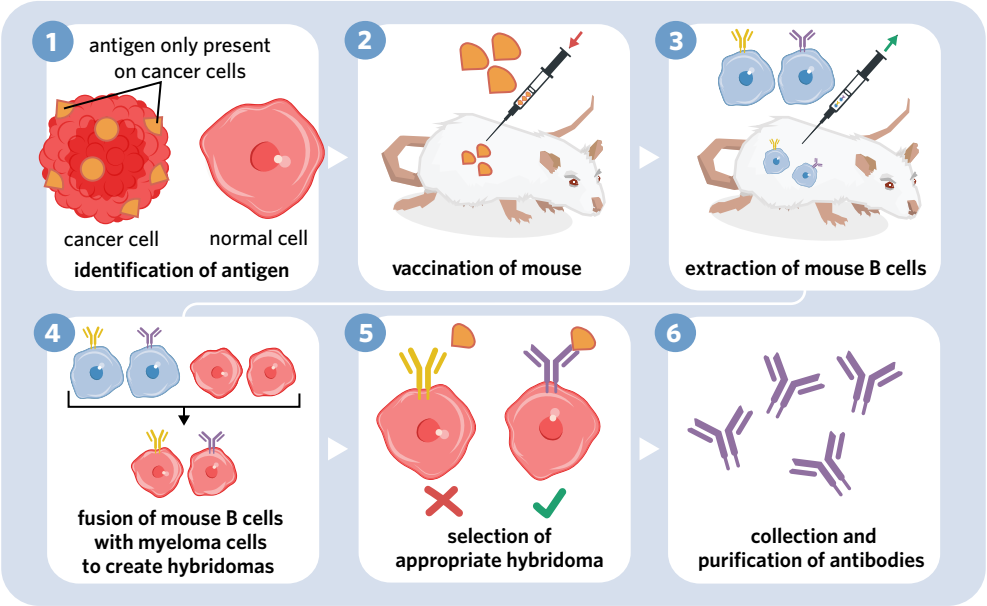


Figure 3 The process of producing monoclonal antibodies

Monoclonal antibodies and cancer 4.1.10.3

OVERVIEW

Monoclonal antibodies can be used as an activation immunotherapy to help the immune system recognise and kill cancer cells.

THEORY DETAILS

What is cancer?

Cancer is an incredibly complex group of diseases caused by the uncontrolled and unregulated replication of cells that then invade other sites of the body. The most important process in the development of cancer is the accumulation of mutations in a cancer cell's DNA that allow it to bypass normal regulatory checkpoints of the cell cycle and provide it with survival advantages.

The immune system is normally capable of recognising cells that have developed mutations and destroys them before they have a chance to replicate. Cancerous cells, on the other hand, are sometimes able to evade the immune system or develop mutations that allow them to suppress the immune response against them. The development of cancer, then, is in part due to the failure of the immune system to do its job of destroying abnormal cells. This is where immunotherapy comes into play.

Activation immunotherapy

Activation immunotherapies can be used to help the immune system recognise and destroy cancerous cells. Monoclonal antibodies are a key component of this and can be used in a number of different ways. There are two types of monoclonal antibodies used in immunotherapy:

- **naked monoclonal antibodies**
- **conjugated monoclonal antibodies**.

naked monoclonal antibodies monoclonal antibodies that do not have any other molecules attached to them

conjugated monoclonal antibodies monoclonal antibodies with other molecules (e.g. chemotherapy drugs or radioisotopes) attached to them

Naked monoclonal antibodies are simply antibodies that, in contrast with conjugated monoclonal antibodies (see below), do not have any drugs or added materials attached to them. They have three main mechanisms of action against cancer cells:

Antibody-dependent cell-mediated cytotoxicity (ADCC)

Monoclonal antibodies bind to cancer cells and interact with cells of the immune system, particularly natural killer cells (NK cells), causing them to recognise the antibody-coated cancer cell as foreign and kill it (Figure 4).

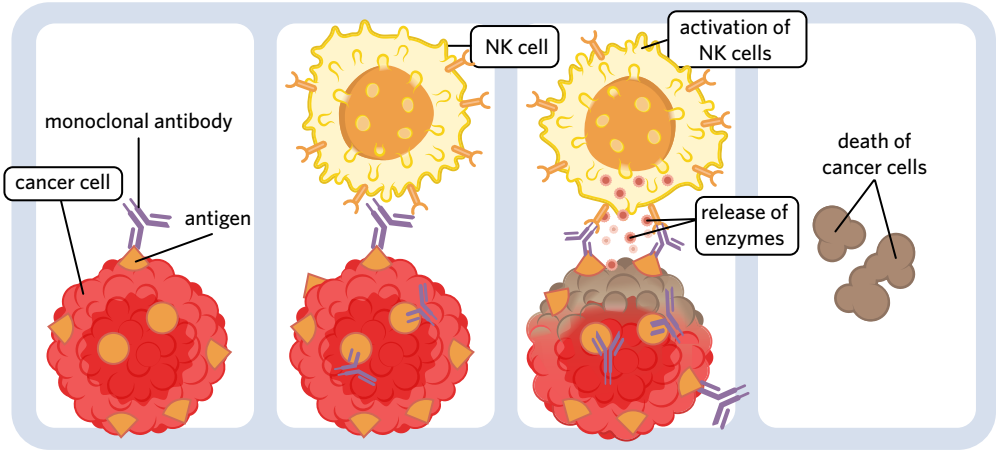


Figure 4 Monoclonal antibodies attaching to a cancer cell, causing a NK cell to recognise it as foreign and kill it.

Complement activation

Monoclonal antibodies bind to cancer cells and interact with **complement proteins**. Complement proteins can then go on to destroy the cancerous cell either by forming a **membrane attack complex (MAC)** or by enhancing the function of other immune cells (Figure 5).

complement proteins a number of different types of proteins found in the blood that opsonise, cause lysis, and attract phagocytes to invading pathogens

membrane attack complex (MAC) a pore formed by complement proteins in the cell membrane of a pathogen, disrupting the membrane and leading to the pathogen's destruction

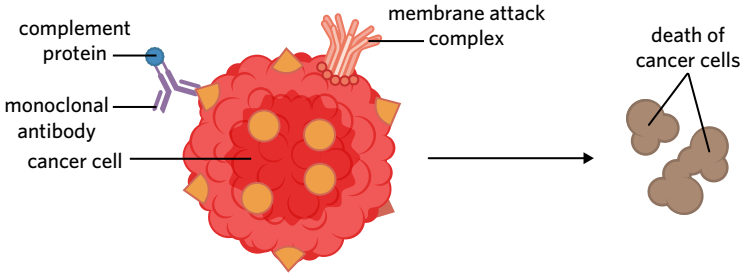


Figure 5 A monoclonal antibody interacting with a complement protein, initiating the formation of a MAC.

Checkpoint inhibition

Immune checkpoints are regulators in the immune system that, when activated, suppress the immune system. Whilst suppressing the immune system at times is a normal part of bodily function, some cancer cells secrete molecules that stimulate immune checkpoints, reducing the immune system's ability to recognise and destroy them. Monoclonal antibodies can be used to block immune checkpoints, meaning the immune system is able to function at a greater capacity and destroy cancer cells more easily.

Other uses of monoclonal antibodies

Monoclonal antibodies can be used to treat cancer in a variety of ways that don't alter the functioning of the immune system.

It is still useful to know about these mechanisms, however, as they help you to understand a more general picture of how monoclonal antibodies can be used against cancer.

For example, conjugated monoclonal antibodies are monoclonal antibodies that have other molecules attached to them. These molecules can be toxic to cancer cells, and include chemotherapy drugs or radioactive isotopes. Due to their specificity for cancer cell antigens, conjugated monoclonal antibodies can be used to specifically deliver these molecules to cancer cells, killing them (Figure 6).

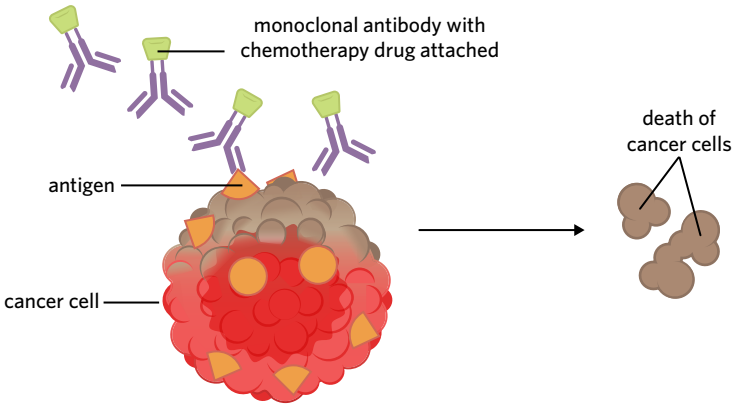


Figure 6 Conjugated monoclonal antibodies delivering a chemotherapy drug directly to a cancer cell.

Other non-immunotherapy ways monoclonal antibodies can be used to treat cancer include:

- blocking cell growth by blocking the connection between a cancer cell and proteins that promote cell growth
- triggering cell membrane destruction or **apoptosis**.

apoptosis the controlled death of cells in the body. Also known as programmed cell death

Immunotherapy and traditional cancer treatment

Traditional forms of cancer therapy such as chemotherapy and radiotherapy work by directly targeting and killing cells that are rapidly dividing, rather than stimulating the immune system. Cancer cells divide quickly, and are therefore killed by these therapies. A large problem, however, is that many other cells of the body – such as hair follicles, and cells lining the mouth and gut – also divide quickly and are killed by these treatments. This is one of the reasons why people on chemotherapy can suffer from side effects such as hair loss, nausea, and vomiting.

Antibody-based immunotherapies, by comparison, tend to be more specific and targeted in their attack. Because monoclonal antibodies have variable regions that bind with cancer antigens specifically, there is a lower chance that other cells in the body will be affected by the treatment and experience side effects. Unlike traditional cancer treatments that directly kill cancer cells, monoclonal antibodies can be used as a type of activation immunotherapy that stimulates the immune system to recognise and destroy cancer cells.

Having said this, immunotherapy still can cause a wide array of side effects, and is currently only available as a treatment for very specific types of cancer. Additionally, it is still typically used in conjunction with traditional cancer treatments such as chemotherapy and radiotherapy.

Monoclonal antibodies and autoimmune diseases 4.1.10.4

OVERVIEW

Monoclonal antibodies can be used as suppression immunotherapy to reduce the immune system's attacks on self-cells that cause autoimmune disease.

THEORY DETAILS

What are autoimmune diseases?

In lesson 7A, you learned that cells of the body express major histocompatibility complex (MHC) proteins that mark them as 'self'. If a person's immune system is functioning normally, their lymphocytes should recognise these markers and not launch an attack against a cell expressing them. Sometimes, however, lymphocytes fail to recognise these self-markers and end up inducing an immune response against self-cells.

When this occurs it can result in an autoimmune disease.

There are over 80 types of autoimmune diseases known, and nearly every part of the body can be affected by them. Examples include rheumatoid arthritis, type 1 diabetes, and coeliac disease. The symptoms of autoimmune diseases are brought on by both B and T cells responding to self-tissues as if they were foreign. B cells release **autoantibodies** and T cells become **autoreactive**.

autoantibodies antibodies directed against an organism's own tissues

autoreactive a cell that recognises a self-tissue or self-antigen as non-self

immunosuppression a reduction in the ability of the immune system to generate an immune response

immune deficiency a state in which the immune system is no longer able to protect the body against infection or disease. Also known as **immunodeficiency**

Suppression immunotherapy

Suppression immunotherapies can be used to dampen the immune system and reduce its ability to attack self-cells, leading to **immunosuppression**. Monoclonal antibodies are a key component of suppression immunotherapy, and can be used to reduce the immune response in a few different ways:

- Cytokine inhibition – Cytokines are messenger molecules used by the immune system to coordinate its response. Monoclonal antibodies that bind to and inhibit cytokines can be used to reduce the immune response (Figure 7).
- B cell and T cell depletion and inhibition – Monoclonal antibodies that bind to autoreactive B and T cells can be used to either inhibit these cells or stimulate other immune cells to destroy them.

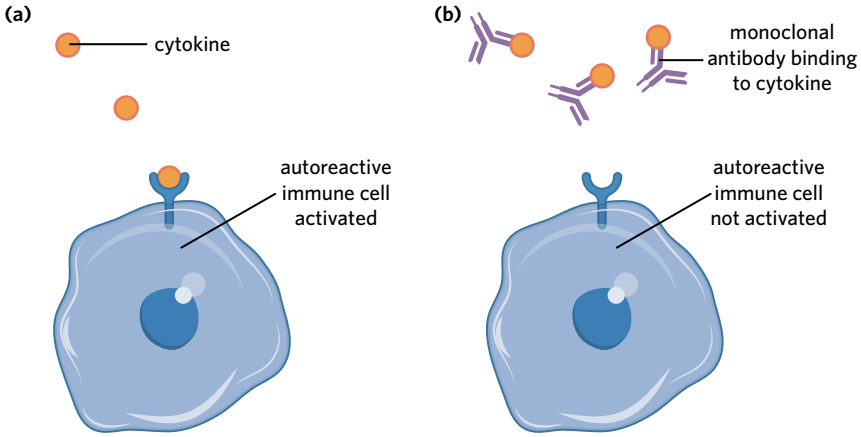


Figure 7 **(a)** Cytokines activating an immune cell; **(b)** monoclonal antibodies binding with cytokines before they reach the immune cell, preventing its activation

Immunotherapy and traditional autoimmune disease treatment

The majority of autoimmune diseases have no cure at this point in time. Instead, doctors try to reduce the symptoms experienced by patients. Treatments for autoimmune diseases have normally involved suppressing a patient's whole immune system via immunosuppressant medications such as non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. This type of broad immunosuppression has meant that these patients become **immunodeficient** and can be more susceptible to developing infections and cancer.

Whilst the use of immunotherapy to treat autoimmune conditions is a relatively new approach to disease management, it theoretically offers a lot of benefits. Immunosuppression via immunotherapy would be far more specific, suppressing only autoreactive cells and leaving the rest of the immune system to function normally. There are currently a few immunotherapy options available for those with autoimmune diseases, however most are still used in conjunction with more traditional treatments.

Figure 8 David Vetter was born with a condition that left him immunocompromised. He was known as the 'bubble boy' because he had to live in a sterile plastic bubble to prevent being exposed to pathogens.

Theory summary

Immunotherapy is a form of medical treatment that involves modulating the way the immune system functions. Monoclonal antibodies are antibodies produced by scientists that can be used as a form of immunotherapy to treat cancer (activation immunotherapy) and autoimmune diseases (suppression immunotherapies).

Unfortunately for mice, the application process for medical school is a little too challenging. Instead, mice help us in the fight against cancer by serving a crucial role in the creation of monoclonal antibodies. These antibodies can be used as immunotherapy to combat a variety of diseases, including cancer and autoimmune diseases. So next time a mouse runs across your desk, don't scream and run away – salute it for its contribution to medical science!

8D QUESTIONS

Theory review questions

Question 1

Immunotherapy is a type of medical treatment that

A changes the way the immune system works.

B always increases the ability of the immune system to destroy pathogens.

Question 2

Order the steps to correctly describe the process used in the production of monoclonal antibodies.

I fusion of mouse B cells with myeloma cells

II selection of appropriate hybridoma

III identification of target antigen

IV collection and purification of antibodies

V vaccination of mouse with target antigen

VI extraction of mouse B cells

Question 3

Which of the following describes a mechanism of activation immunotherapy?

A blocks activation signals to immune cells

B delivers chemotherapy drugs to cancer cells

C blocks growth factors needed by cancer cells to grow

D monoclonal antibodies attach to cancer cells causing NK cells to destroy them

Question 4

Fill in the blanks in the following sentences.

Diseases caused by the immune system not recognising and attacking self-cells are called _____. They are caused by B cells releasing _____ and T cells that are _____. Immunotherapy can be used to reduce the symptoms of these diseases, specifically a type of immunotherapy called _____.

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–9.

Organ transplantation is a complex surgical procedure in which an organ or body tissue is removed from one person (the donor) and placed into the body of another person (the recipient). The outcome of transplantation varies greatly, with one of the biggest problems faced by doctors and those who have received transplants being tissue rejection.

Tissue rejection primarily occurs as a result of cell-mediated immunity. The immune system recognises the non-self tissue/organ as foreign and attacks it via cytotoxic T cells. To prevent this from occurring, recipients will often be prescribed immunotherapy. For example, basiliximab is a medication consisting of monoclonal antibodies that is used to prevent organ rejection, especially in kidney transplants. It works by binding to the α chain of the IL-2 receptor of T cells, preventing the receptor from interacting with signalling molecules that activate T cells.

Organ transplant recipients may also be prescribed immunosuppressant medications, such as prednisone, that work by binding to glucocorticoid receptors that are found on almost every cell of the body. Glucocorticoids are a type of hormone that reduce immune system function, and medications such as prednisone mimic these effects.

Question 5

Organ transplantation involves

A the transfer of an organ from donor to recipient.

B the transfer or an organ from recipient to donor.

Question 6

The components of the immune system responsible for the rejection of organs are

A cytotoxic T cells.

B monoclonal antibodies.

Question 7

Based on the information provided, basiliximab is a type of

A activation immunotherapy.

B suppression immunotherapy.

Question 8

The monoclonal antibodies referred to in the text would have been created by first

A fusing a human B lymphocyte with a mouse myeloma cell to form a hybridoma.

B fusing a mouse B lymphocyte with a human myeloma cell to form a hybridoma.

C fusing a human B lymphocyte with a mouse hybridoma to form a myeloma cell.

D fusing a mouse B lymphocyte with a human hybridoma to form a myeloma cell.

Question 9

Which of the two forms of management for tissue rejection would theoretically result in fewer side effects?

A Basiliximab, because it is a monoclonal antibody that selectively inhibits T cells.

B Prednisone, because it is a monoclonal antibody that selectively inhibits T cells.

C Basiliximab, because it is a chemical that interacts with almost every cell in the body.

D Prednisone, because it is a chemical that interacts with almost every cell in the body.

Exam-style questions

Within lesson

Question 10 (1 MARK)

Monoclonal antibodies

A are lipid molecules.

B are produced by B memory cells.

C bind to the extracellular receptors of specific cells.

D all share the same universal active site that can bind to all cancerous cells.

Adapted from VCAA 2018 Section A Q24

Question 11 (1 MARK)

Monoclonal antibodies can be used to treat cancerous cells in the body. Which of the following is not a role of monoclonal antibodies?

A Triggers cell lysis through necrosis.

B Initiates an immune response by flagging the cancerous cells to immune cells.

C Delivers treatments such as radiation or chemotherapy drugs directly to cancerous cells.

D Prevents cell growth in the cancerous cells by blocking their connections to proteins that promote cell growth.

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q2c

Question 12 (6 MARKS)

Ipilimumab is a monoclonal antibody treatment that can be used in some cancer treatment regimens. It is a form of activation immunotherapy and works by blocking CTLA-4, a protein that inhibits the immune system.

a Explain the difference between activation and suppression immunotherapies. (1 MARK)

b Ipilimumab can be used to treat melanoma, a type of skin cancer. In addition to immunotherapy, patients will also typically be given chemotherapy.

i Explain why patients who receive chemotherapy will typically experience more side effects than those who only receive immunotherapy. (3 MARKS)

ii Based on the information provided, explain how ipilimumab could potentially be used as immunotherapy to treat cancerous melanoma cells. (2 MARKS)

Multiple lessons

Question 13 (10 MARKS)

Monoclonal antibodies: the invisible allies that changed the face of medicine

By Lara Marks

Published in *The Conversation* August 10th 2015

Monoclonal antibodies (mAbs) are tiny magic bullets that are quietly shaping the lives of millions of patients around the world. Produced in the lab, invisible to the naked eye, relatively few people are aware of these molecules' existence or where they came from. Yet monoclonal antibodies are contained in six out of ten of the world's bestselling drugs, helping to treat everything from cancer to heart disease to asthma.

In the years that have passed since 1975, mAb drugs have radically reshaped medicine and spawned a whole new industry. It is predicted that 70 mAb products will have reached the worldwide market by 2020, with combined sales of nearly $125bn.

Key to the success of mAb drugs are the dramatic changes they have brought to the treatment of cancer, helping in many cases to shift it away from being a terminal disease. mAbs can very specifically target cancer cells while avoiding healthy cells, and can also be used to harness the body's own immune system to fight cancer. In contrast, chemotherapy and radiotherapy can lead to debilitating effects on an individual's health, as there is not a high-cell specificity, meaning cancer cells are targeted with less direction.

mAbs have also radically altered the treatment of inflammatory and autoimmune disorders like rheumatoid arthritis and multiple sclerosis, moving away from merely relieving symptoms to targeting and disrupting their cause.

Aside from cancer and autoimmune disorders, mAbs are being used to treat over 50 other major diseases. Applications include treatment for heart disease, allergic conditions such as asthma, and prevention of organ rejection after transplants. mAbs are also under investigation for the treatment of central nervous disorders such as Alzheimer's disease, metabolic diseases like diabetes, and the prevention of migraines. More recently they were explored as a means to combat Ebola, the virus disease that ravaged West Africa in 2014.

Source: 'Monoclonal antibodies: the invisible allies that changed the face of medicine' By Lara Marks, Published in The Conversation August 10th 2015

a What is meant by the term 'autoimmune disorder'? (1 MARK)

b Do monoclonal antibodies cause less severe side effects than chemotherapy? Support your response with evidence from the article. (2 MARKS)

c Identify the type of immunity created by mAbs. (1 MARK)

d Identify and describe one mechanism by which mAbs can affect cancerous cells that does not require the use of other drugs. (2 MARKS)

e The article discusses the potential applications of mAbs, including 'treatment for heart disease, allergic conditions such as asthma, and prevention of organ rejection after transplants'.

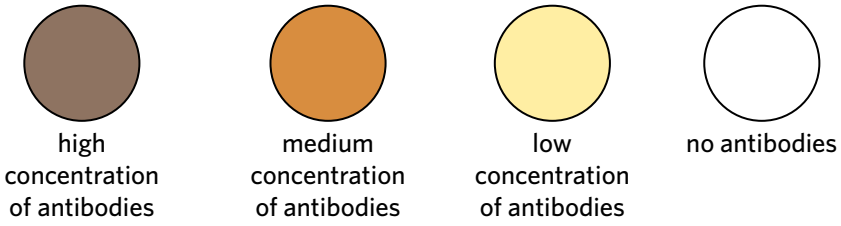
i State whether the mAbs used to treat cancerous cells would be identical to the mAbs used for these future applications. Justify your response. (2 MARKS)

ii Describe the potential role of mAbs in drug delivery for these applications and explain how they may improve the effect of the drugs. (2 MARKS)

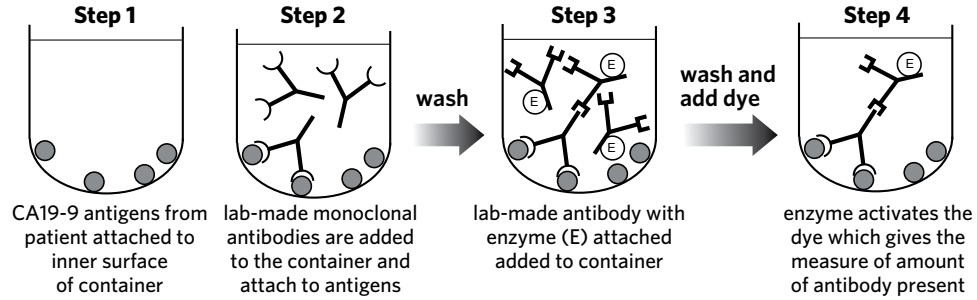
Key science skills and ethical understanding

Question 14 (8 MARKS)

Using monoclonal antibodies in an ELISA test, scientists can detect the presence of colorectal cancer by detecting the amount of the antigen CA19-9 in a patient's sample. If cancerous cells are present in the sample, mAbs will attach to CA19-9 antigens and cause a colour change. If cancerous cells are not present, the mAbs will be washed out and no colour change will be observed. The potential colour changes are shown:



One variation of the ELISA process is as follows:



Once the ELISA procedure is completed, the colour change can be observed.

The scientists performed the procedure with samples from five different individuals and displayed the results in the table.

Sample	Ab concentration
1	high
2	nil
3	low
4	medium
5	medium

a Identify and explain an uncontrolled variable that may have affected the observed results. (2 MARKS)

b The scientists used additional samples known as the positive and negative controls. A positive control involves a sample where scientists apply a treatment and know what results to expect, whereas a negative control does not have a treatment applied and is expected to produce no result. The results of these samples are not shown in the table. Identify what is in each control sample and state the predicted colour of each sample. (2 MARKS)

c Explain one way that monoclonal antibodies can be used to treat cancer in humans. (2 MARKS)

d One form of monoclonal antibody production involves injecting hybridomas into the gut of mice. These hybridomas develop into tumour cells that secrete fluid rich in monoclonal antibodies that can be harvested and used as immunotherapy. Using the bioethical concept of non-maleficence, discuss why the deliberate creation of tumours in mice is a bioethical issue. (2 MARKS)

Adapted from VCAA 2012 Exam 1 Section A Q24

Rotate page

Immunity

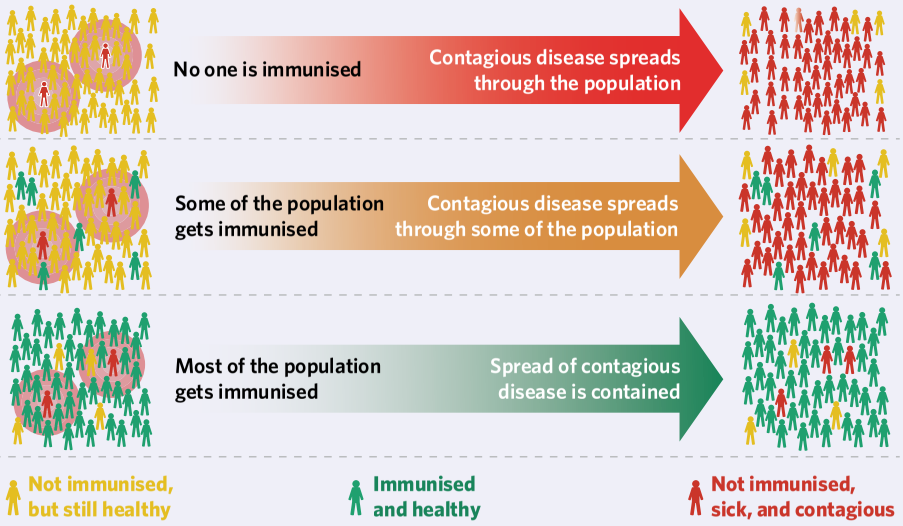
- **Immune system**
  - **Innate / non-specific**
  - **Adaptive immunity**
    - **Natural** — No medical intervention
      - **Active** — A person develops their own antibodies and memory cells; e.g. immune response generated against a pathogen encountered in the environment
      - **Passive** — Antibodies created by natural external source; e.g. breastfeeding, through the placenta
    - **Artificial** — Medical intervention
      - **Active** — A person develops their own antibodies and memory cells after getting vaccinated; e.g. vaccination
      - **Passive** — Antibodies created by artificial external source; e.g. antivenom

Vaccinations

Vaccines contain weakened pathogenic antigens that stimulate the production of immunological memory. In doing so, if an individual encounters the pathogen in the future, a large and rapid immune response will occur, preventing disease.

Herd immunity

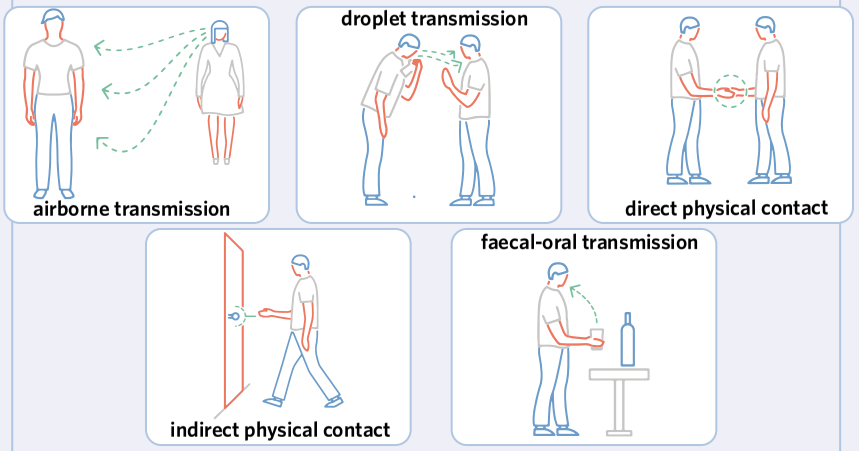
Herd immunity involves vaccinating a large proportion of a population to reduce the number of susceptible hosts, thereby protecting vulnerable members of the population.



Pathogen identification and spread

Pathogens can be identified using various methods, including physical, phenotypic, immunological, and molecular techniques. To help control their spread, strategies such as the use of personal protective equipment, routine screening, and medications like antibiotics and antivirals should be employed.

Modes of disease transmission

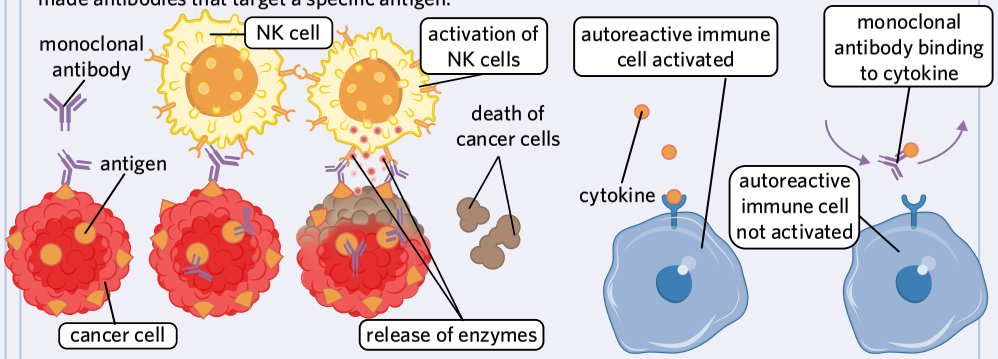


Unfortunately, new pathogens are constantly emerging. This can be due to the natural evolution of previous pathogens, globalisation and travel, increased exposure of humans to animals, increased human population and population density, and the inadequate vaccination of the population.

Image: Double Brain/Shutterstock.com

Immunotherapy

Immunotherapies refer to medical interventions that alter the immune system, and can act to either amplify the immune response (activation, e.g. for cancer) or reduce it (suppression, e.g. for autoimmune disease). Monoclonal antibodies can be used in immunotherapy, and are artificially made antibodies that target a specific antigen.



CHAPTER 8 SAC PRACTICE

SAC skills covered in this section:

✓ Case study analysis ✓ Data analysis ✓ Bioethical deep dive

MODERN MEDICINE (24 MARKS)

Discovery of penicillin

It's a bit scary to think about, but many modern drugs were discovered by accident or trial and error. The most famous example of this is the drug penicillin, which is an antibiotic that inhibits cell wall synthesis, and was discovered by Alexander Fleming in 1928. After returning from a holiday, Fleming noticed some mould growing on one of his staphylococci-infested Petri dishes that he had accidentally left uncovered on his bench. This mould, it turned out, prevented the growth of the bacterium *Staphylococcus* – meaning it had antibacterial properties! Fleming was able to use this to create the drug penicillin, one of the most important medicines ever produced.

1 Describe how penicillin inhibits bacterial growth. (1 MARK)

2 Explain whether penicillin can be used to treat viral infections. (1 MARK)

3 Describe two methods that could be used to prevent the spread of bacterial infections. (2 MARKS)

4 Describe one technique that could be used to visually identify bacteria. (1 MARK)

Rational drug design

More recently, scientists have come up with a more targeted and deliberate approach to designing new drugs. This process is called rational drug design and represents a massive step forward in modern medicine. In rational drug design, scientists identify a molecule crucial to the functioning of a disease or disorder and then design medications that use the complementary shape of the molecule to interfere with its functioning.

One of the most important examples of a rationally designed drug is Relenza, which was made by a team of Australian scientists in 1989. Relenza is an antiviral drug designed to specifically combat influenza by targeting one of its key enzymes.

On the surface of influenza, there is an enzyme called neuraminidase, which facilitates the release of viruses from an infected host cell. By acting as a competitive inhibitor of neuraminidase, Relenza is capable of inhibiting its action, thereby preventing newly replicated influenza viruses from being released into the extracellular environment. In doing so, Relenza slows the spread of influenza, allowing the body's immune system to catch up and overcome the infection.

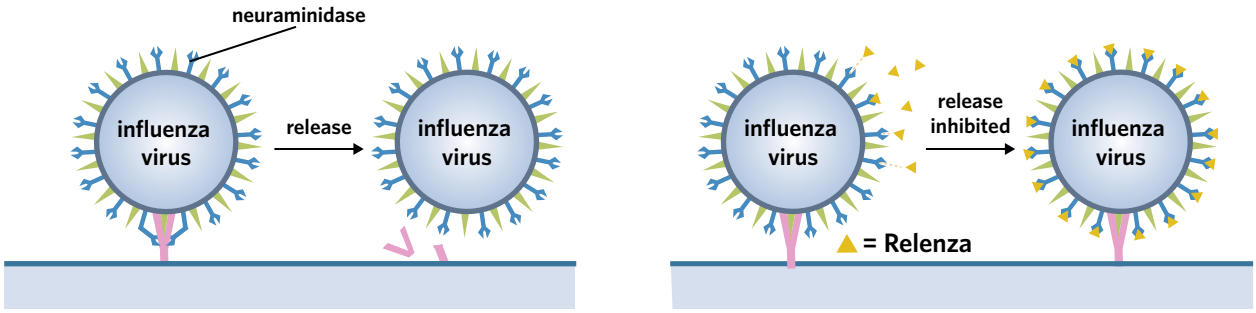


Image: Double Brain/Shutterstock.com

5 Due to the rapidly evolving nature of influenza, Relenza is not effective against all strains of the influenza virus. Suggest how resistance against Relenza may arise in strains of the influenza virus. (1 MARK)

6 Describe two reasons why a pathogen might rapidly spread within a local community. (2 MARKS)

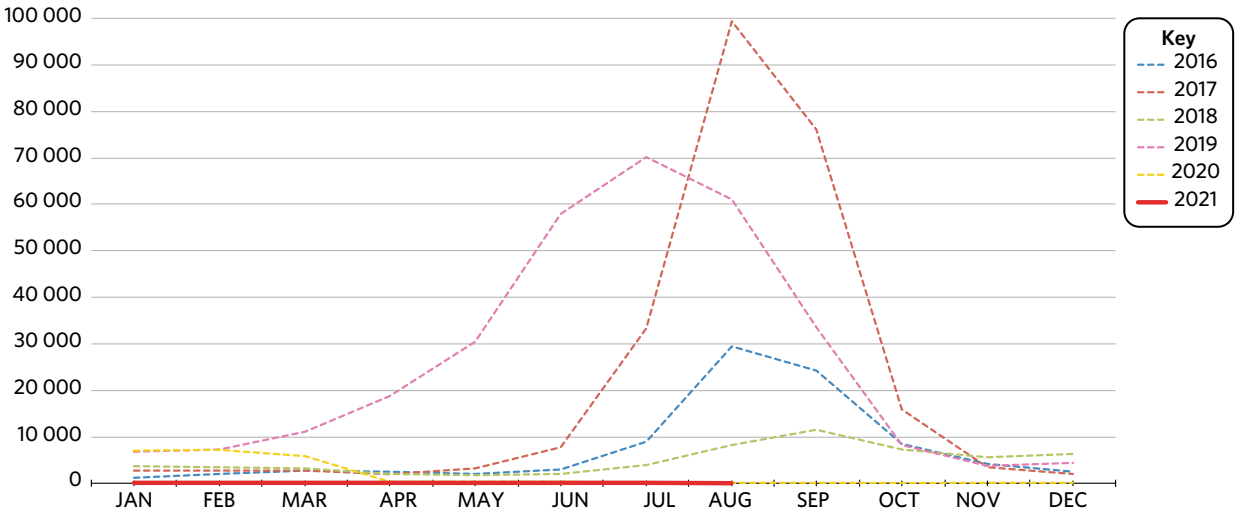
7 In preparation for the flu season, people receive the flu vaccine every year. Briefly describe how vaccines work. (2 MARKS)

8 In order to maintain a healthy population, governments aim to promote high vaccination levels. Explain why governments may wish to keep large proportions of their countries vaccinated. (2 MARKS)

Influenza statistics

The following graph illustrates the number of annual confirmed cases of influenza each year in Australia. Due to the reliance of the data on laboratory confirmed cases, it is likely that the number of influenza cases are underreported, as not all individuals with the flu will be tested.

Annual Australian laboratory confirmed flu numbers



Source: Australian Government, Department of Health, National Notifiable Diseases Surveillance System (2021)

9 Given the data shown, identify the month in 2019 that saw the highest total number of influenza cases. (1 MARK)

10 Suggest a possible reason for the significant increase in flu numbers observed in 2017. (1 MARK)

Monoclonal antibodies

Monoclonal antibodies are often designed using rational drug design – targeting specific molecules that belong to a particular pathogen or disease. In recent years, the use of monoclonal antibodies in treating infectious diseases has been extremely promising, with monoclonal antibodies being designed to target specific toxins released by bacteria as well as specific bacterial targets such as components of their cell wall. However, many hurdles still exist, such as cross-reactivity with other targets, which can cause the monoclonal antibody to fail or cause even greater damage to the body.

Nonetheless, monoclonal antibodies have been beneficial in the treatment of cancers and autoimmune diseases. The monoclonal antibodies that are used generally fall into two broad categories – activation and suppression immunotherapies.

11 Outline the process of developing a monoclonal antibody against a bacterial toxin. (2 MARKS)

12 State the type of immunity formed in an individual who receives monoclonal antibodies to treat a bacterial infection. Justify your response. (2 MARKS)

13 Describe how monoclonal antibodies could be used to combat cancer as an immunotherapeutic strategy. (2 MARKS)

14 Describe how monoclonal antibodies could be used to combat autoimmune diseases. (2 MARKS)

15 During the production of monoclonal antibodies, multiple clinical trials must be conducted prior to their release for community use. However, while waiting for approval, many individuals suffering from the diseases the monoclonal antibodies are designed to treat will pass away.

Based on the bioethical concept of non-maleficence, describe why monoclonal antibodies should not be approved prior to the completion of all clinical trials. (2 MARKS)

CHAPTER 8 EXAM PRACTICE

60 min

Section A (12 MARKS)

Question 1 (1 MARK)

Rubella is a highly contagious virus that commonly causes fevers, rashes, and enlarged lymph nodes. When infected with rubella, humans initially produce IgM antibodies and then IgG antibodies.

Tests for the presence of IgM and IgG antibodies are carried out on a newborn baby if the mother has been diagnosed with a rubella infection during pregnancy. Unlike IgG antibodies, IgM antibodies cannot cross the placenta. The results for the tests carried out on four newborn babies immediately after birth are summarised in the table.

	Antibodies for the rubella virus
Baby 1	none
Baby 2	IgG
Baby 3	IgG
Baby 4	IgM & IgG

Using the information provided, it would be correct to conclude that

A the mother of baby 1 has never produced IgG antibodies in response to the rubella virus.

B baby 3 was exposed to the rubella virus during fetal development.

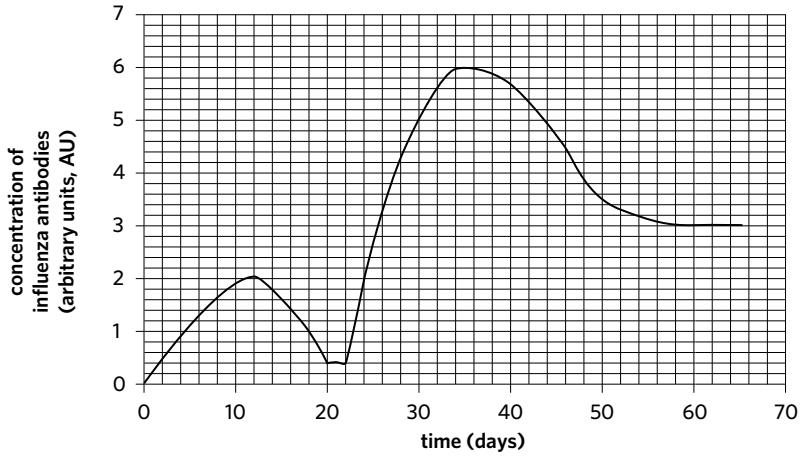
C baby 4 has been injected with the rubella vaccine.

D baby 2 has innate immunity to the rubella virus.

Adapted from VCAA 2012 Exam 1 Section A Q18

Question 2 (1 MARK)

A daily blood sample was obtained from an individual who received a single vaccination against a particular strain of the influenza virus. The individual had no prior exposure to this strain of influenza. The graph shows the concentration of antibodies present in the individual's blood for this strain of influenza over a period of 65 days.



Which one of the following conclusions can be made using this data?

A The patient received the influenza vaccination on day 12.

B The influenza virus produced the most antibodies on day 34 of the treatment.

C More of the influenza virus would be present within the blood on day 30 compared to day 12.

D B memory cells specific to this strain of the influenza virus were present in the patient's blood on day 22.

Adapted from VCAA 2017 Section A Q26

Question 3 (1 MARK)

People that have been infected with one or more different respiratory viruses develop antibodies in response to each kind of virus in their blood. The blood of four patients was tested to diagnose which viruses each patient had previously been infected with. The results are summarised in the table.

Note: ++ = agglutination and 0 = no agglutination

	Antibody to			
Blood from	Rhinovirus	Influenza A	Influenza B	RSV
Brenda	0	++	++	++
Nathan	++	0	0	++
Stacy	++	0	0	0
Jacqueline	0	++	++	0

Using the information in the table, it is reasonable to infer that

A Brenda and Jacqueline have each been infected with the same set of viruses.

B Stacy has been exposed to the fewest number of different viruses.

C Brenda has a stronger immune response to RSV than Nathan.

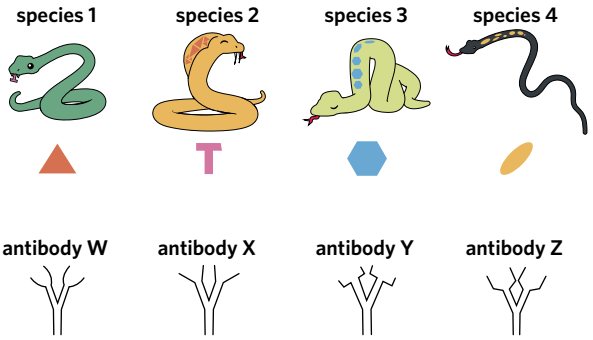
D Nathan has never been infected with the Rhinovirus.

Adapted from VCAA 2011 Exam 1 Section A Q24

Use the following information to answer Questions 4 and 5.

Following a snake bite, a park ranger was admitted to a hospital and urgently required a dose of antivenom. Fortunately, the ranger managed to capture and bring the snake with them to the hospital. To decide which antivenom to prescribe, doctors analysed that the given chart with the four most common venomous snake species in the area. Beneath each species is a diagram of their toxin. The four antibodies present in each antivenom are also shown. However, due to a design flaw, the antibodies have been mixed up.

After examining the captured snake, the doctors decided it belonged to species 1 and administered an antivenom containing one of the antibodies W–Z.



Question 4 (1 MARK)

Which antibody would be found in the administered antivenom?

A antibody W

B antibody X

C antibody Y

D antibody Z

Question 5 (1 MARK)

After being injected with the antivenom, the ranger's condition quickly improved. This is because the antivenom serum

A triggered the park ranger's immune system to mount an immune response against the venom.

B introduced antibodies that bound to and deactivated the snake venom.

C provided an active and induced (artificial) immunity.

D provided a passive and natural immunity.

Adapted from VCAA 2016 Section A Q23

Question 6 (1 MARK)

Monoclonal antibodies can be produced and used to treat different types of cancers. Which one of the following statements correctly describes monoclonal antibodies?

A Monoclonal antibodies are naturally produced in response to invading pathogens.

B The same monoclonal antibody can be used to treat all types of cancers.

C Monoclonal antibodies contain two identical antigen recognition sites.

D A monoclonal antibody will always initiate apoptosis in the target cell.

Adapted from VCAA 2018 Section A Q24

Question 7 (1 MARK)

Scientists are currently developing a vaccine to help prevent the spread of the Ebola virus. When developing a vaccine, scientists use a weakened form of the Ebola virus, which is then injected into the patient. They use a weakened form of the virus because

A initiation of an immune response relies on the virus being weak.

B there is a decreased chance of causing disease in the patient.

C active samples of the Ebola virus cannot survive in humans.

D it is difficult to isolate active samples of the Ebola virus.

Question 8 (1 MARK)

A doctor was consulting with a mother who had recently given birth. The doctor explained that once breastfeeding has stopped, she would no longer be able to offer her child protection against certain infections.

This is because the type of immunity achieved by breastfeeding is an example of

A active and natural immunity.

B passive and natural immunity.

C active and induced (artificial) immunity.

D passive and induced (artificial) immunity.

Adapted from VCAA 2016 Section A Q23

Question 9 (1 MARK)

Lupus is a disease that results in the increased production of self-targeting antibodies, which attach to antigens from otherwise healthy cells within a patient's body. The accumulation of these auto-antibodies leads to inflammation, joint pain, rashes, fatigue, and fever.

Which one of the following methods would be the most effective treatment for lupus?

A administration of monoclonal antibodies that target cytokines, suppressing the patient's immune system

B injecting signalling molecules that have been designed to initiate apoptosis in lupus-causing pathogens

C achieving lupus vaccination rates that are high enough to support herd immunity

D injection of hypersensitive mast cells into the bloodstream

Adapted from VCAA 2017 Northern Hemisphere Exam Section A Q15

Question 10 (1 MARK)

Researchers are trying to develop new antiviral therapies. These therapies could include a drug that

A stops the viral spindle fibres forming during mitosis.

B inhibits a bacterial-specific metabolic pathway.

C prevents virus attachment and entry.

D promotes flagellum development.

Adapted from VCAA 2013 Section A Q40

Question 11 (1 MARK)

Tetracyclines are a class of medication used against a number of infections in humans. They attach to cytoplasmic ribosomes inside bacteria and interfere with protein synthesis. From this information, it is correct to state that tetracyclines are

A a vaccine.

B an antiviral.

C an antibiotic.

D an antifungal.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q32

Question 12 (1 MARK)

A disease is more likely to be screened for if

A the disease significantly impacts quality of life.

B the screening test has a high rate of error.

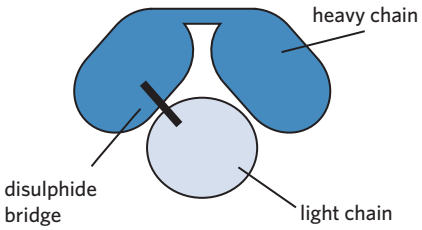
C there is no cure for the disease.

D the screening test is expensive.

Section B (28 MARKS)

Question 13 (8 MARKS)

The bacterium *Clostridium tetani* is capable of producing the toxin tetanospasmin, which is lethal even in extremely small amounts. In order to protect against *C. tetani*, a new vaccine against the polypeptide heavy chain of the tetanospasmin toxin has been developed. When injected by itself, the heavy chain of the tetanospasmin toxin is unable to cause disease. A simplified representation of the protein structure of the toxin is shown in the diagram.



a What is a vaccine? (2 MARKS)

b In an experimental trial, scientists injected sheep with the new vaccine. After 10 days, they withdrew blood from the sheep and mixed it with *C. tetani* that had been suspended in sterilised water. The scientists noted the almost immediate agglutination in the mixture after the sheep antibodies interacted with the tetanospasmin toxin.

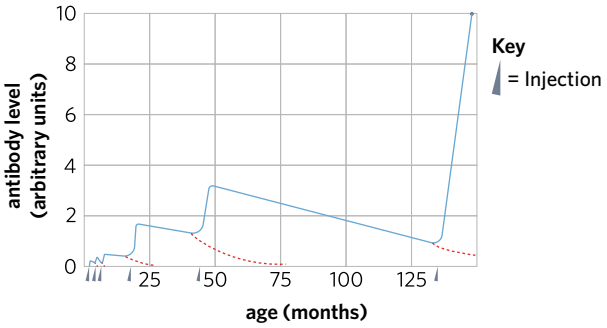
i Identify where the antibodies bind to the tetanospasmin toxin. (1 MARK)

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q11

ii Explain how the binding of antibodies provides the sheep in the trial with immunity to the tetanospasmin toxin. (1 MARK)

Adapted from VCAA 2014 Section B Q4cii

c The tetanus vaccination schedule for children involves multiple doses of the vaccine. The blue line on the graph shows the antibody levels of a child if the vaccination is given at the appropriate times, while the pink lines show what happens to the antibody levels if it is not administered according to the schedule.



i How many doses of the tetanus vaccine are required by the vaccination schedule? Justify your answer. (1 MARK)

ii Children are not considered to be immune to the *C. tetani* bacterium until the vaccination schedule is complete. Explain how the vaccination schedule enables a longer-lasting immunity to the toxin tetanospasmin than a single vaccination. (3 MARKS)

Adapted from VCAA 2015 Section B Q4a

Question 14 (11 MARKS)

Whooping cough is a highly contagious disease caused by an infection from the bacterium *Bordetella pertussis*. It is typically transmitted through coughing or sneezing. Whooping cough can be prevented by immunisation with the whooping cough vaccine. Individuals younger than two months old cannot receive the whooping cough vaccine.

a It is recommended that adults receive the whooping cough vaccine every 10 years, especially if they spend large amounts of time around children. Explain why this is necessary. (2 MARKS)

Adapted from VCAA 2013 Section B Q4c

b Explain how immunisation against whooping cough can help reduce the chances of developing the disease. (2 MARKS)

c Despite being unable to receive the whooping cough vaccine, individuals under the age of two months can still be protected from the whooping cough disease in a number of ways.

i Explain how pregnant mothers vaccinated against whooping cough would be able to provide a degree of protection to their foetus. (1 MARK)

ii Identify the type of immunity provided to the foetus through the mother. Justify your response. (2 MARKS)

Adapted from VCAA 2013 Section B Q4a

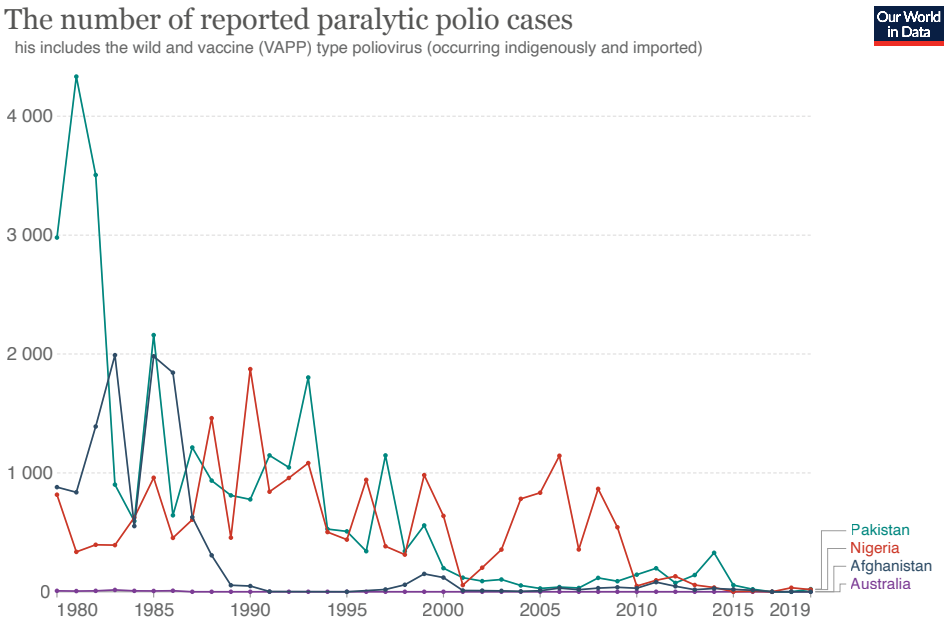
iii Describe how high rates of vaccination can protect unvaccinated infants from whooping cough. (2 MARKS)

Adapted from VCAA 2018 Section B Q5b

d Describe two possible methods of reducing transmission of whooping cough. (2 MARKS)

Question 15 (5 MARKS)

Polio used to be one of the most widespread and severe viral diseases. 1 in 200 individuals infected with the polio virus develop irreversible paralysis, which commonly results in death. The graph shows the number of reported paralytic polio cases in several countries since the 1980s.



Source: World Health Organisation (2017), adapted by Ochmann and Roser (2019)

a In 1983, which country reported the highest number of paralytic polio cases? (1 MARK)

b Many public health officials warn about the potential dangers of epidemics and pandemics in human populations.

i While polio has largely been eradicated, there have been several recent outbreaks around the world. Suggest two possible reasons for the occurrence of these recent polio outbreaks. (2 MARKS)

ii Suggest whether the graph indicates that there may have been a polio pandemic between the years of 1990 and 1995. (1 MARK)

c After European settlement of Australia, many new diseases were introduced into the Indigenous Australian populations and caused many First Nations peoples to lose their lives.

Explain why these diseases had such a significant effect on Indigenous Australians while leaving the Europeans relatively unscathed. (1 MARK)

Question 16 (4 MARKS)

Allergic reactions are potentially life-threatening reactions to typically non-harmful substances. These substances are called allergens, examples of which include pollen, dust, and eggs. When the body encounters an allergen, the allergen binds to IgE antibodies found on the surface of mast cells, causing them to degranulate and release histamine. This causes localised swelling, dilation of blood vessels, and itchiness.

Omalizumab is a monoclonal antibody designed specifically against IgE antibodies. It is often reserved as a last resort for individuals suffering from severe and persistent allergic asthma.

a At a molecular level, describe how omalizumab can be used to treat persistent allergic asthma. (1 MARK)

b Another form of asthma involves the use of a cytokine known as IL-5, which promotes the recruitment of eosinophils. Outline how monoclonal antibodies could be produced to reduce the recruitment of eosinophils. (3 MARKS)

UNIT 4

AOS2

How are species related over time?

In this area of study, students focus on changes to genetic material over time and the evidence for biological evolution. They consider how the field of evolutionary biology is based upon the accumulation of evidence over time and develop an understanding of how interpretations of evidence can change in light of new evidence, and/or as a result of technological advances, particularly in molecular biology. Students consider the biological consequences of changes in allele frequencies and how isolation and divergence are required elements for speciation. They consider the evidence for determining the relatedness between species and examine the evidence for major trends in hominin evolution, including the migration of modern human populations around the world.

Outcome 2

On completion of this unit, the student should be able to analyse the evidence for genetic changes in populations and changes in species over time, analyse the evidence for relatedness between species, and evaluate the evidence for human change over time.

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CHAPTER

9

How species evolve

Key knowledge

- causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow, and mutations as the source of new alleles
- biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity
- manipulation of gene pools through selective breeding programs
- consequences of bacterial resistance and viral antigenic drift and shift in terms of ongoing challenges for treatment strategies and vaccination against pathogens

9A THE GENE POOL

Looking around at other students in your class, you should notice that everyone looks unique. There's blonde hair, brown hair, black hair, red hair, brown eyes, blue eyes, green eyes – the variation present is almost unlimited. However, while you might look completely different to everyone else around you, it turns out that you still share 99.9% of your DNA with everyone else in your class. So how do you look so different from other students in your class given such genetic similarity? What is the mechanism responsible for creating variation between yourself and other students in your class?

Image: Areipa.lt/Shutterstock.com

Lesson 9A

In this lesson you will explore the significance of gene pools and allele frequencies. You will also learn how mutations serve as the source of new alleles.

◀ *Prerequisite knowledge* — *Future applications* ▶

Year 11
Mutations can affect the functioning of apoptosis, which in turn can lead to the formation and development of cancer.

Chapter 2
When mutations affect DNA sequences, the resulting mRNA sequence and primary protein structure may be changed, potentially leading to an abnormally functioning protein.

Chapter 9
Changes to gene pools and allele frequencies are induced by many different mechanisms such as environmental selection pressures, mutations, genetic drift, gene flow, and artificial selection pressures.

Chapter 10
Differences arising in DNA sequences and amino acid sequences via mutations can be used to determine the relatedness of different species.

Study design dot point

- causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow, and mutations as the source of new alleles

Key knowledge units

Gene pools and allele frequencies	4.2.1.1
Mutations	4.2.1.2

Gene pools and allele frequencies 4.2.1.1

OVERVIEW

The collection of all the genes and alleles within a specific population is known as the gene pool, and can be represented by allele frequencies.

THEORY DETAILS

Gene pools refer to the total aggregation of all the **genes** and **alleles** present within a particular **population** or species. **Allele frequencies** refer to the proportion of a particular allele appearing at a certain gene locus in a gene pool. For example, if there were two alleles of the gene coding for the colour of sheep (B and b), their frequencies could be calculated by totalling the number of a particular allele divided by the total number of alleles present in the population (Figure 1).

gene pool the complete set of alleles present within a particular population

gene a section of DNA that carries the code to make a protein

allele an alternate form of a gene

population a group of individuals of the same species living in the same location

allele frequency the proportion of certain alleles in a gene pool

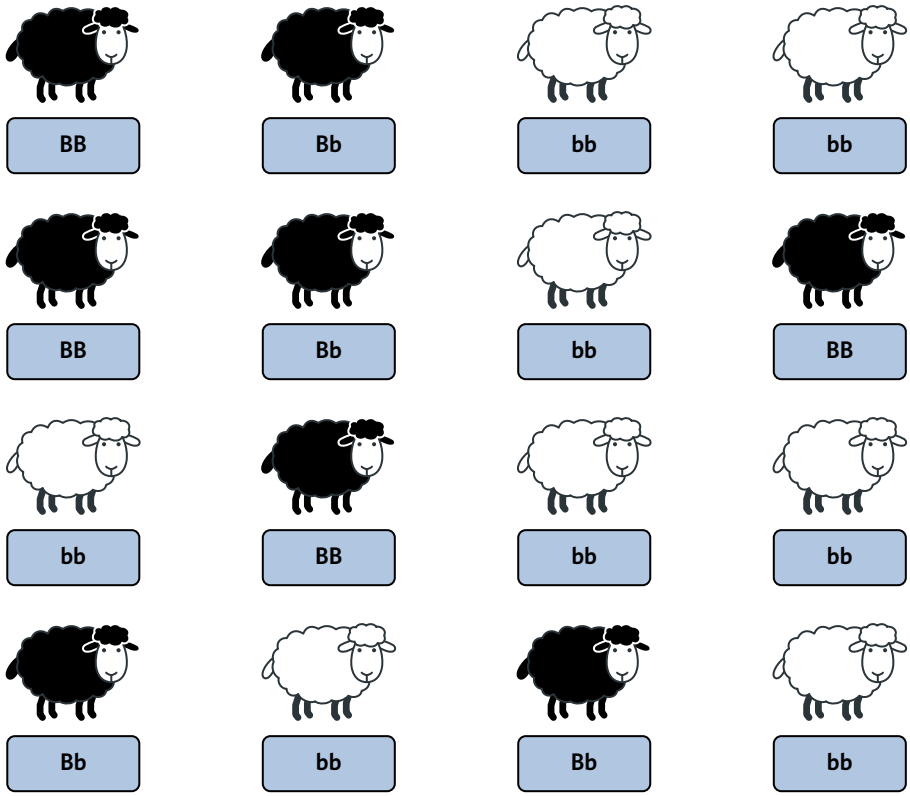


Figure 1 The frequency of alleles within a population of sheep

A larger and more diverse gene pool will contain a greater variety of genes and alleles, leading to a greater number of **genotypes** and **phenotypes**, and thereby resulting in increased **genetic diversity**. Throughout this chapter, we will explore the different factors which can influence gene pools and alter allele frequencies. Some of these factors include mutations, environmental selection pressures, genetic drift, gene flow, and artificial selection pressures.

genotype the genetic composition of an organism at a particular gene locus

phenotype the physical or biochemical characteristics of an organism that are the result of gene expression and the environment

genetic diversity the variation in genetic makeup or alleles within a population

Mutations 4.2.1.2

OVERVIEW

Mutations are responsible for introducing new alleles into a population via changes to DNA. These changes can involve the substitution, addition, or deletion of single nucleotide bases or larger blocks of DNA.

THEORY DETAILS

Mutations involve permanent changes to the DNA sequence of an individual and can occur either spontaneously or be induced by agents known as **mutagens** (e.g. UV radiation). When mutations occur in the DNA sequence of genes, they can have a significant downstream effect on the expression of that particular gene by altering the folding and functionality of the resultant protein.

Depending on the mutation's overall effect on the survivability of the individual affected, the mutation can be classified as advantageous, neutral, or **deleterious** (Figure 2). For example, if the mutation leads to the production of an abnormally functioning protein, then it would be classified as deleterious. However, if it led to the production of a protein which enhances the survivability of an individual, then it would be classified as advantageous.

mutation a permanent change to a DNA sequence

mutagen an agent that can cause mutations in DNA

deleterious used to describe alleles that have an overall negative effect on individual fitness when expressed

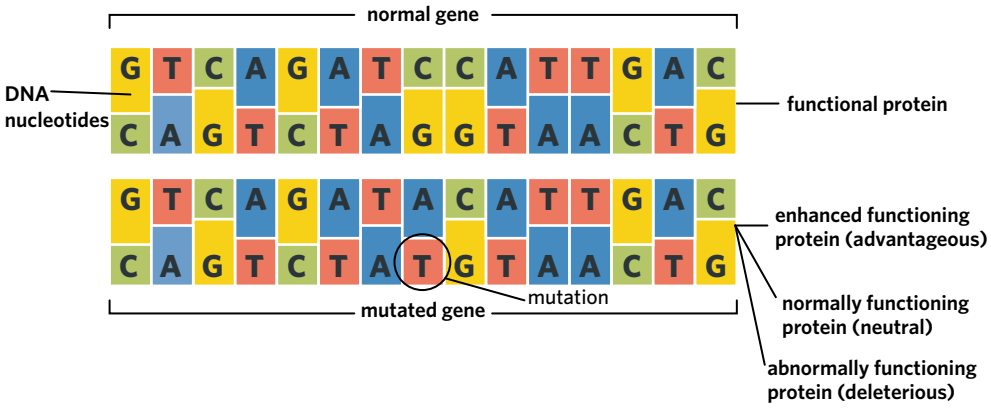


Figure 2 Mutations can be categorised as either advantageous, neutral, or deleterious.

The evolutionary significance of mutations is due to their ability to create and introduce new alleles into a population, thereby increasing genetic diversity. However, for the mutation to be **heritable**, it must occur in an individual's **germline cells**. If the mutation occurs in a **somatic cell**, then it is not heritable.

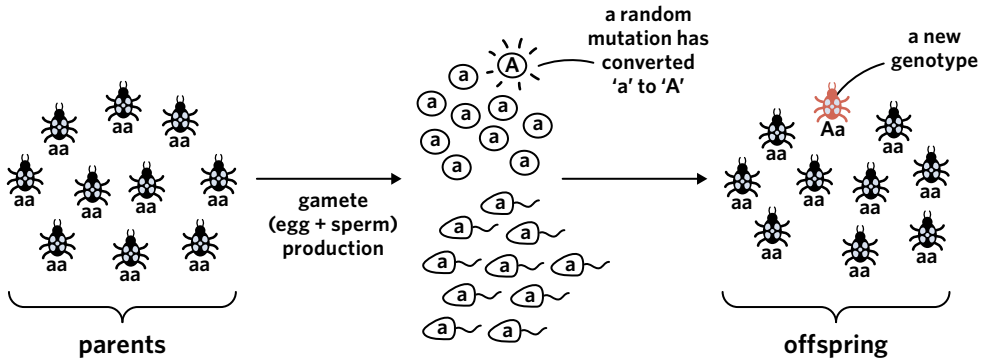


Figure 3 The effect of a single germline mutation on the gene pool of a beetle population.

Mutations can be categorised as either **point mutations** or **block mutations**, depending on whether they affect a single nucleotide base or a larger cluster of nucleotides.

Point mutations

Point mutations describe changes to a single nucleotide in a gene (Table 1, Figure 4). These modifications can include the substitution of a base, which can be further broken down into **silent**, **missense**, and **nonsense mutations** depending on their effect on the protein produced, or the addition or deletion of a single nucleotide, which triggers what is known as a **frameshift mutation**.

Table 1 Types of point mutations

Mutation	Description
Silent mutation	Substitution mutations that have no effect on the resulting amino acid sequence. Due to the **degenerate** nature of the genetic code, multiple different codons code for the same amino acid and, therefore, despite a change to the original DNA sequence, the same amino acid is incorporated into the protein.
Missense mutation	Substitution mutations which code for a different amino acid, altering the primary structure of the polypeptide. This in turn affects the folding of the polypeptide and could alter the functioning of the protein.
Nonsense mutation	Substitution mutations which prematurely end the translation of a gene's mRNA. Due to the substitution of a nucleotide that causes the affected codon to become a stop codon, the gene will not be completely translated, leading to a polypeptide that is too short to function as intended. These mutations are generally considered the most dangerous.
Frameshift mutation	Addition or deletion of one or two nucleotides, which alters the **reading frame** of all the following nucleotides. The reading frame is how DNA or mRNA is divided into triplets or codons respectively. Since the reading frame is shifted in frameshift mutations, all following codons and the amino acids they code for are affected, which can cause major disruptions to the structure and function of the protein.

heritability the transmission from parent to offspring (i.e. encoded in genes)

germline cell a cell involved in the generation of gametes in eukaryotes

somatic cell any cell in an organism that is not a germline cell

point mutation a mutation that alters a single nucleotide in a DNA sequence

block mutation a mutation that affects a large chunk of DNA, or an entire gene

silent mutation a mutation in which a nucleotide is substituted for another, changing the codon, but still coding for the same amino acid. Therefore, there is no effect on protein structure

missense mutation a mutation in which a nucleotide is substituted for another, changing the codon and coding for a different amino acid. Therefore, there can potentially be an effect on protein structure

nonsense mutation a mutation in which a nucleotide is substituted for another, changing the codon to a stop codon, prematurely ceasing translation of the gene's mRNA. Therefore, there is an effect on protein structure

frameshift mutation a mutation that involves the insertion or deletion of one or two nucleotides, altering every codon from that point forward

degenerate a property of the genetic code which means that a single amino acid can be coded for by more than one codon

reading frame the order in which nucleotide triplets or codons are divided into a consecutive, non-overlapping sequence

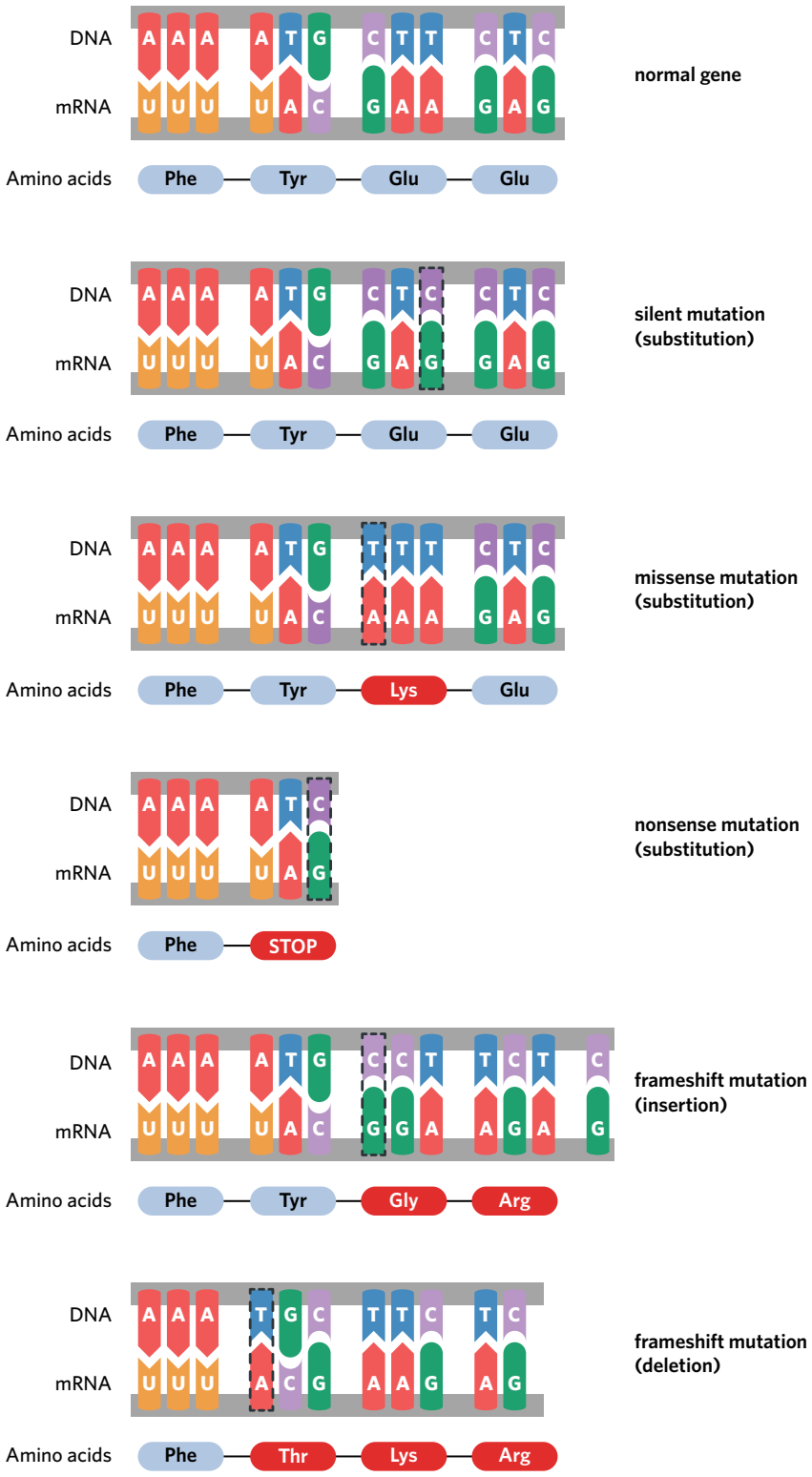


Figure 4 Types of point mutations

Theory in context

SICKLE CELL ANAEMIA

Sickle cell anaemia is a genetic disease which arises due to a missense mutation, leading to the formation of a new allele that causes a deformity in red blood cells. While normal red blood cells have a flattened disk-like shape to optimally transport oxygen around the body, sickle cells are shaped like crescents, with increased length and decreased width. Due to their reduced surface area to volume ratio, sickle cells are unable to carry oxygen as efficiently as normal red blood cells. Additionally, the sickle cell shape causes red blood cells to become trapped in capillaries, reducing blood flow.

cont'd

Theory in context

SICKLE CELL ANAEMIA – CONTINUED

However, while it may appear that the allele for sickle cell is entirely deleterious, in areas where malaria, (a disease caused by a parasite which infects red blood cells) is endemic, it turns out that having the allele for both normal red blood cells and sickled cells can be advantageous – a phenomenon known as a heterozygote advantage. This is because those with both alleles only display low levels of sickling, and the red blood cells that do become infected with malaria are more likely to sickle and become targeted by the immune system.

In contrast, those with both alleles for sickle cell (homozygous recessive) develop sickle cell anaemia, often resulting in premature death from damage to internal organs due to a lack of oxygen. Those with both alleles for normal red blood cells (homozygous dominant) are not conferred any resistance against malaria.

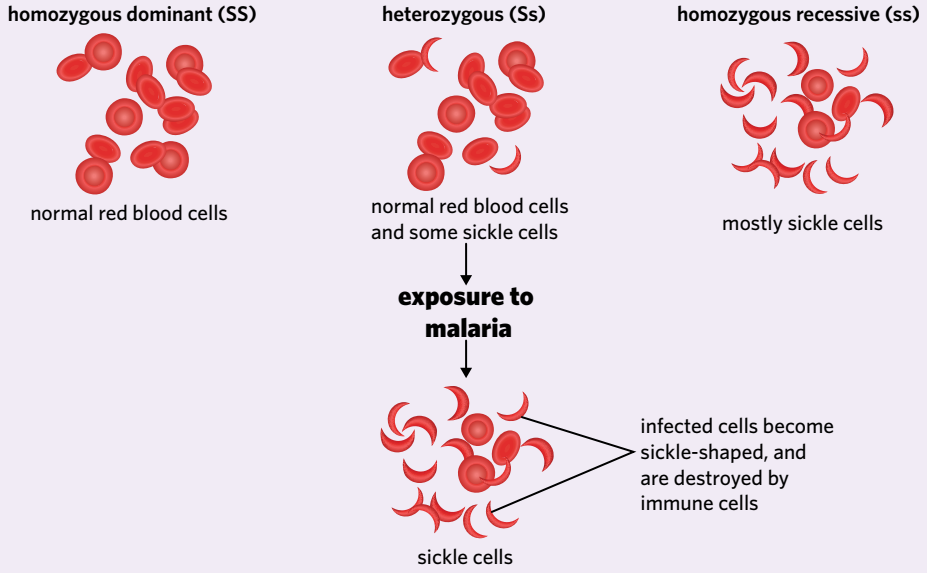


Figure 5 The genotypes and phenotypes for sickle cell anaemia

Block mutations

In contrast to point mutations, which are changes to a single base in the DNA, block mutations involve changes to larger sections of DNA, potentially causing significant changes to the DNA sequence of an organism. Block mutations involve the alteration of the structure of a chromosome by inserting, deleting, duplicating, or swapping a cluster of nucleotides, potentially involving multiple different genes (Figure 6). These mutations usually occur during the process of meiosis.

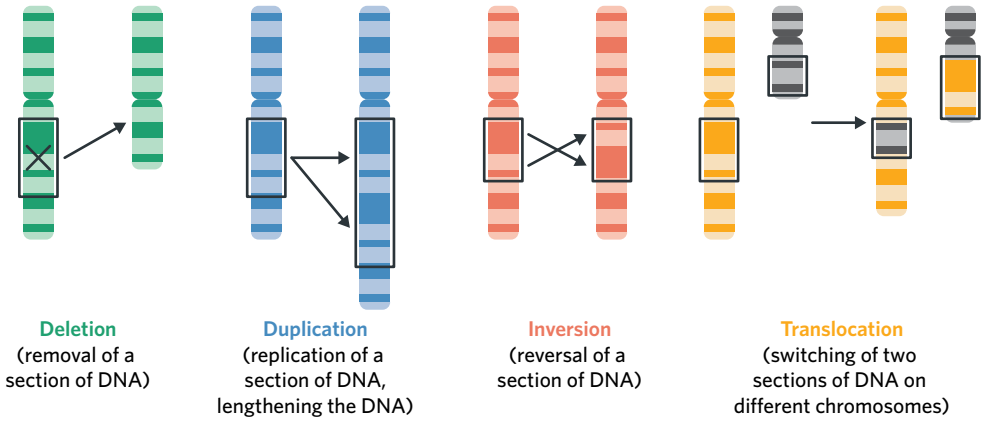


Figure 6 Types of block mutations, including deletion, duplication, inversion, and translocation.

Theory summary

Mutations involve permanent changes to the DNA sequence of an individual. Because mutations are capable of introducing new alleles into a population, they are a key source of genetic variation and are evolutionarily significant.

While there are many different mechanisms that can create variation between yourself and other students in your class, one reason could be mutations! Mutations are responsible for the creation of new alleles, which can introduce new characteristics and phenotypes into a population.

Image: Catalin Rusnac/Shutterstock.com

9A QUESTIONS

Theory review questions

Question 1

Fill in the blanks in the following sentences.

_____ involve the total aggregation of all the genes and alleles present within a particular population. They can be described through the use of _____, which involve calculating the proportion of each allele at each gene locus within the population. A population involves a group of individuals of the _____ living in the _____.

Question 2

Which one of the following statements about types of mutations is incorrect?

A Missense mutations result in the affected codon coding for a different amino acid.
B Silent mutations result in the affected codon coding for the same amino acid.
C Point mutations involve the substitution or addition of multiple nucleotides.
D Nonsense mutations result in the affected codon becoming a stop codon.

Question 3

Which one of the following statements about mutations is correct?

A Mutagens are protective against mutations, helping prevent them from occurring.
B By changing the DNA sequence, mutations always lead to deleterious effects.
C For mutations to be heritable, they must occur within germline cells.
D Mutations generally lead to a decrease in genetic diversity.

Question 4

Match the following mutations to their descriptions.

Mutation

- frameshift mutation
- nonsense mutation
- missense mutation
- silent mutation
- block mutation

Description

I _____ substitution of a single nucleotide which changes the affected codon, leading to the production of a different amino acid
II _____ substitution of a single nucleotide which does not lead to a change in the amino acid sequence
III _____ substitution of a single nucleotide leading to the production of a premature stop codon
IV _____ insertion or deletion of nucleotides affecting every codon from the point of mutation onwards
V _____ mutations that affect large amounts of DNA or entire genes

SAC skills questions

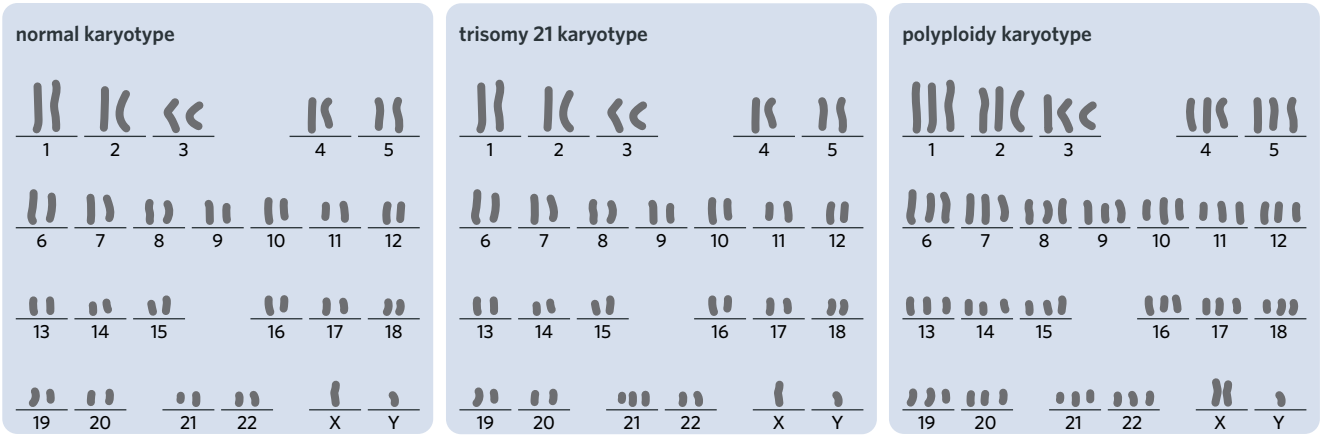
Case study analysis

Use the following information to answer Questions 5–9.

In addition to point mutations and block mutations, genomes can also change through the loss or gain of chromosomes or sets of chromosomes. These mutations are known as chromosomal abnormalities and can be further categorised as either aneuploidy or polyploidy.

Aneuploidy is a condition that describes the absence of a chromosome or the presence of an additional chromosome. These mutations can be seen in an individual's karyotype and usually stem from complications during meiosis. An example of aneuploidy includes Down syndrome, which involves the presence of an additional chromosome 21, leading to what is known as trisomy 21. Individuals diagnosed with Down syndrome often have decreased muscle tone, a short neck, a flattened facial profile, as well as cognitive impairment.

Polyploidy is a condition where an individual has an abnormal number of sets of chromosomes. For example, humans, like most eukaryotes, are diploid ($2n$) and have two sets of chromosomes. A person with polyploidy, however, may have three sets of chromosomes ($3n$) instead. While polyploidy in humans is fatal, many other organisms, such as plants, can often thrive with additional sets of chromosomes.



Question 5

Polyploidy involves

A an abnormal number of sets of chromosomes.

B the absence of a chromosome or the presence of additional chromosomes.

Question 6

In individuals diagnosed with Down syndrome, their cells have

A multiple extra sets of chromosomes.

B an additional singular chromosome.

C one extra set of chromosomes.

Question 7

An increase in chromosome number caused by aneuploidy would

A leave the allele frequency of the alleles located on the affected chromosome unchanged.

B decrease the allele frequency of the alleles located on the affected chromosome.

C increase the allele frequency of the alleles located on the affected chromosome.

Question 8

Polyploidy

A only involves additional sets of chromosomes.

B always leads to the creation of new alleles.

C is fatal in humans.

Question 9

Edward's syndrome involves an additional chromosome 18, often leading to death within 12 months from birth. Edward's syndrome is an example of

A a point mutation.

B aneuploidy.

C polyploidy.

Exam-style questions

Within lesson

Question 10 (10 MARKS)

Marfan syndrome is a genetic disorder affecting the formation of connective tissue within the body which is primarily responsible for holding cells, tissues, and organs of the body together. Many different bodily systems are affected, such as the cardiovascular, skeletal, and ocular systems. Due to a mutation in the gene coding for a protein known as fibrillin-1, an increased production of another protein known as transforming growth factor beta (TGF-β) occurs, disrupting the production of connective tissue. To date, there have been over 3 000 mutations identified to have an effect on the production of fibrillin-1.

The following codon table can be used to determine amino acids coded for by a nucleotide sequence.

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU phe UUC phe UUA leu UUG leu	UCU ser UCC ser UCA ser UCG ser	UAU tyr UAC tyr UAA STOP UAG STOP	UGU cys UGC cys UGA STOP UGG trp	U C A G
C	CUU leu CUC leu CUA leu CUG leu	CCU pro CCC pro CCA pro CCG pro	CAU his CAC his CAA gln CAG gln	CGU arg CGC arg CGA arg CGG arg	U C A G
A	AUU ile AUC ile AUA ile AUG met	ACU thr ACC thr ACA thr ACG thr	AAU asn AAC asn AAA lys AAG lys	AGU ser AGC ser AGA arg AGG arg	U C A G
G	GUU val GUC val GUA val GUG val	GCU ala GCC ala GCA ala GCG ala	GAU asp GAC asp GAA glu GAG glu	GGU gly GGC gly GGA gly GGG gly	U C A G

a One point mutation affecting the production of fibrillin-1 involves a mutation altering a codon coding for tryptophan (trp), resulting in a stop codon instead.

i Describe the type of mutation that has occurred. (1 MARK)

ii Identify a possible change in the DNA sequence of the template strand. (1 MARK)

iii Explain the effect of this mutation on the formation of fibrillin-1. (2 MARKS)

b Another mutation affecting the production of fibrillin-1 involves a mutation leading to the substitution of cysteine (cys) for glycine (gly) at a particular amino acid residue in the polypeptide product.

i Describe the type of mutation that has occurred. (1 MARK)

ii Identify a possible change in the DNA sequence of the template strand. (1 MARK)

iii Describe the effect of this mutation on the functioning of the fibrillin-1 protein. (2 MARKS)

c Some mutations do not affect the production of fibrillin-1. Suggest a reason for why these mutations do not affect the production of fibrillin-1. (2 MARKS)

Multiple lessons

Question 11 (1 MARK)

A repressor molecule is unable to bind to the operator region of the *trp* operon due to a mutation. The mutation could have occurred in the

A regulator gene.

B *trpE* structural gene.

C *trpD* structural gene.

D promoter region of the *trp* operon.

Question 12 (4 MARKS)

In populations of fruit flies, there are individuals that are resistant to the effects of insecticides. Insecticide-resistant fruit flies arose as a result of a mutation in a key metabolic gene. In normal insecticide-susceptible fruit flies, a specific section of this gene's mRNA has the corresponding anticodon CGA, whereas in the insecticide-resistant fruit flies, the anticodon sequence is AGA, which codes for a different amino acid.

a Considering the anticodon of the insecticide-resistant fruit flies, what is the corresponding sequence of nucleotides found on the template strand of their DNA? (1 MARK)

b Identify the type of point mutation that has occurred in the fruit flies' DNA and suggest which type of cells it occurred in. (2 MARKS)

c Describe the effect of the creation of the allele coding for insecticide-resistance on genetic diversity in the fruit fly population. (1 MARK)

Adapted from VCAA 2014 Section A Q22

Question 13 (6 MARKS)

Consider the template strand of the hypothetical gene shown. The exons are in bold type.

3′ **TAC GTA** CCG **AAA TAC GTT** CTT GAC **TAT ATC** 5′

The DNA triplet TAC indicates START for transcription, and the mRNA codon that it produces will code for the amino acid methionine, which remains in the resulting polypeptide. The DNA triplets ATC, ATT, and ACT code for a STOP instruction.

a Identify the sequence of the pre-mRNA strand produced from 5′ to 3′. (1 MARK)

b How many amino acids would be present in the polypeptide expressed by this gene? Justify your response. (2 MARKS)

c An allele for this gene codes for a polypeptide with only four amino acids.

i One reason for the formation of a polypeptide with only four amino acids could be due to a point mutation occurring in one of the exons. By referring to the original sequence, identify the nucleotide change that must have occurred to bring about this shorter polypeptide. Justify your response. (2 MARKS)

ii Another reason not involving a mutation in the exons of the gene exists. Suggest another possible reason for how a polypeptide with only four amino acids could be produced. (1 MARK)

Adapted from VCAA 2015 Section B Q8

Key science skills and ethical understanding

Question 14 (8 MARKS)

Scientists are exploring the effects of different strengths of UV radiation on mice. Cancer can be caused by mutations in tumour suppressor genes which are responsible for slowing the rate of cell division and repairing DNA errors to prevent tumours from forming. To measure the effect of radiation levels, scientists measured the number of mutations in a particular tumour suppressor gene of skin cells in each mouse at different levels of radiation strength.

a Identify the independent and dependent variables in this experiment. (2 MARKS)

b State a possible hypothesis for this experiment. (1 MARK)

c Name the general term for agents, such as UV radiation, which cause mutations. (1 MARK)

d Identify one possible uncontrolled variable in this experiment and explain its potential effect on the outcome of the experiment. (2 MARKS)

e When conducting experiments on animals, scientists must always balance the harms and benefits before proceeding. Describe how the bioethical concept of non-maleficence would have influenced the scientists' decision to proceed with the experiment. (2 MARKS)

Adapted from VCAA 2017 Northern Hemisphere Section B Q11

9B ENVIRONMENTAL SELECTION PRESSURES

*The rough-skinned newt (*Taricha granulosa*) is a formidable amphibian that produces a chemical in its skin called tetrodotoxin. Tetrodotoxin is a nerve agent which blocks the transmission of nerve signals, leading to asphyxiation, paralysis, and eventually death. In fact, tetrodotoxin is known to be almost 100 000 times more poisonous than cyanide, with the potential to kill you in less than 20 minutes. But why does such a small animal have such a powerful toxin?*

What does *Taricha granulosa* say when you tell it a fact it's already heard before? Newt!

Image: Peter K. Ziminski/Shutterstock.com

Lesson 9B

In this lesson you will learn how species evolve via natural selection as they adapt to environmental selection pressures.

◀ *Prerequisite knowledge*

Years 7–10
Changes caused by natural selection in a particular population result from specific environmental selection pressures.

Year 11
Adaptations for hot and cold environments can arise over time due to the extreme environmental selection pressures of an organism's environment.

Future applications ▶

Lesson 9D
Environmental selection pressures can drive some species towards speciation, which involves the formation of a new species, due to the creation of sufficient, heritable, genetic differences.

Lesson 9E
In contrast with environmental selection pressures, artificial selection pressures are imposed by humans and can be used to select for desirable phenotypes.

Chapter 11
Through the process of natural selection, environmental selection pressures have helped shape the evolution of human ancestor species into modern *Homo sapiens*.

Study design dot points

- causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow, and mutations as the source of new alleles
- biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity

Key knowledge units

Environmental selection pressures	4.2.1.3
The effect of selection pressures on genetic diversity	4.2.2.1

Environmental selection pressures 4.2.1.3

OVERVIEW

Environmental selection pressures select for the individuals best adapted to a specific environment, promoting their survival and the passing on of their alleles through a process known as natural selection.

THEORY DETAILS

Environmental selection pressures are factors within the environment that influence the survivability of a species within a given environment. Some common examples of environmental selection pressures include predation, disease, **competition**, and climate change. Through these factors, the process of **natural selection** can occur, which involves the selection of the phenotype most suited to overcome the environmental selection pressure.

Organisms more suited to a particular environment are considered to have higher genetic **fitness**, due to the presence of their **advantageous phenotype**, which arises due to the presence of certain alleles. Over time, because the fitter organisms with the advantageous phenotype have a **selective advantage** and are more likely to survive, they are more likely to pass on their alleles to the next generation, increasing the **allele frequency** of the alleles that code for the advantageous phenotype.

The mechanism of natural selection relies on the **heritability** of the advantageous allele and the presence of variation within the existing **population** to ensure that the alleles that confer an advantage are present within the environment. However, it is also important to remember that variation allows for **disadvantageous alleles** to arise, which can be selected against and subsequently removed from the population. Ultimately, there are four basic conditions that facilitate natural selection (Table 1).

Table 1 The four conditions of natural selection

Condition	Description
Variation	Individuals in a population vary genetically, which leads to phenotypic differences.
Selection pressure	An environmental selection pressure impacts the survivability of organisms within a population and their ability to reproduce.
Selective advantage	Individuals with phenotypes that are fitter or more advantageous under the environmental selection pressure are conferred a selective advantage, allowing them to survive and reproduce more successfully.
Heritability	The advantageous trait must be heritable, allowing it to be passed on from the parents to their offspring. Therefore, over time, the frequency of the advantageous allele will increase.

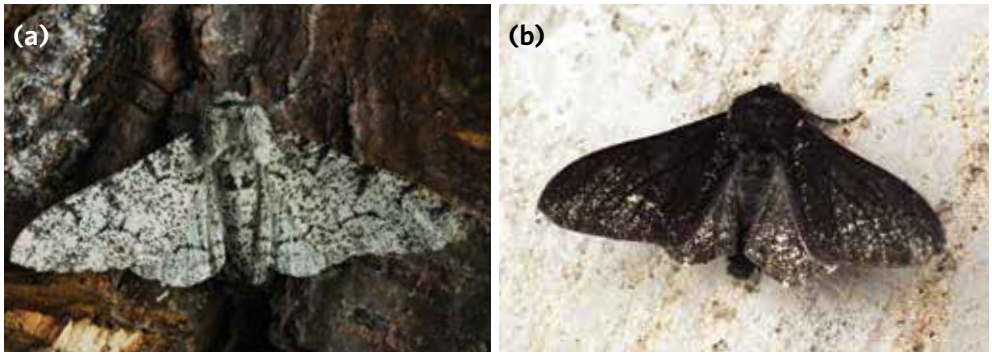


Figure 1 Phenotypic variation in peppered moths (*Biston beularia*) with **(a)** white body colour in a dark environment and **(b)** black body colour in a light environment

An overview of how environmental selection pressures can influence a species is summarised in Figure 2 using the peppered moth as an example.

environmental selection pressure a factor in the environment (e.g. limited resources, deforestation, changing temperature, predation) that impacts an organism's ability to survive and reproduce

competition interactions between organisms in which both are negatively impacted when vying for the same limited resource. Can exist within or between species

natural selection a mechanism through which organisms that are better adapted to their environment have an increased chance of surviving and passing on their alleles

fitness a measure of how well an organism survives and reproduces in its environment

advantageous phenotype a biochemical, physical, or behavioural trait that increases an organism's fitness in its local environment

selective advantage an organism conferred a beneficial allele, which increases its chances of survival against a specific environmental selection pressure

allele frequency the proportion of certain alleles in a gene pool

heritability the transmission from parent to offspring (i.e. encoded in genes)

population a group of organisms of the same species living in the same area

disadvantageous allele an allele that encodes for a biochemical, physical, or behavioural trait that lowers an individual's fitness in its local environment

Theory in action

Check out scientific investigations 9.1 and 9.2 to put this into action!

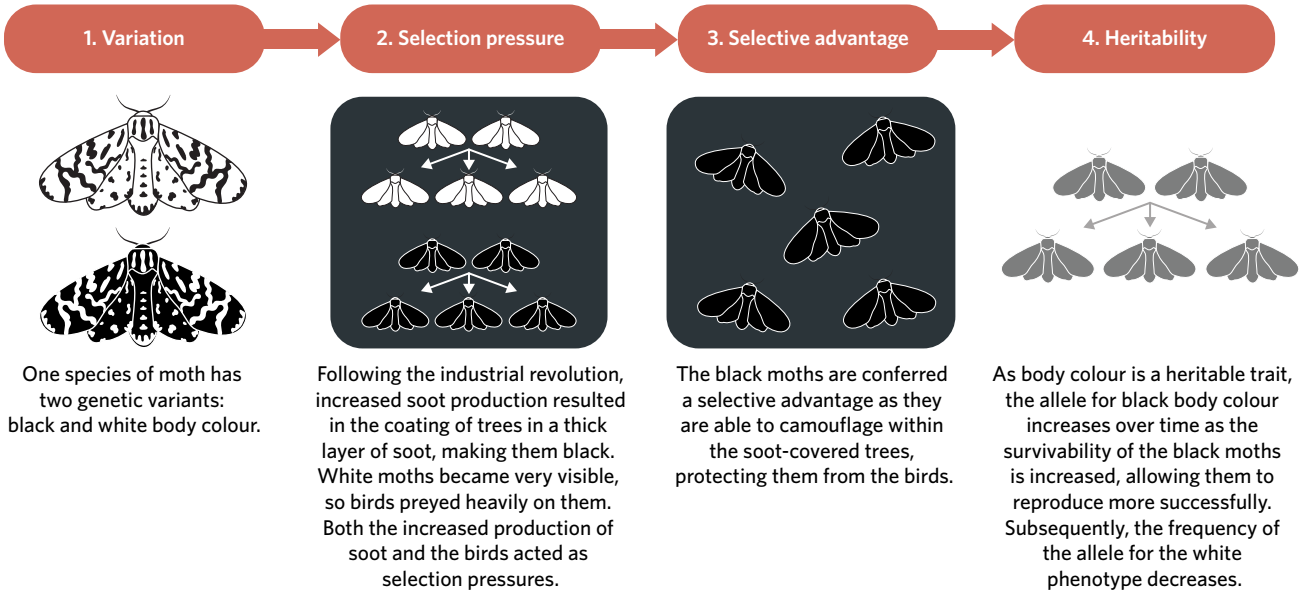


Figure 2 The effect of natural selection on the peppered moth

Theory in action

To simulate the effect of natural selection on a population of peppered moths, check out the following interactive simulation, where you will act as a bird preying upon white or black peppered moths in either a light or dark forest. askabiologist.asu.edu/peppered-moths-game/play.html

Examiners' tip

Students can sometimes struggle to answer short answer questions on natural selection. However, in most of these questions, you simply need to apply the following four conditions of natural selection to the scenario:

1. Variation – identify the variation that exists within the existing population.
2. Selection pressure – identify the environmental selection pressures within the particular environment that are acting on the population.
3. Selective advantage – explain the effects of the environmental selection pressure in terms of survivability and how it confers a selective advantage for the organisms with a fitter or more advantageous phenotype.
4. Heritability – state that organisms with the fitter or more advantageous phenotype will reproduce and pass on the advantageous alleles to the next generation, increasing their frequencies.

Theory in context

To show you how to approach short answer questions on natural selection, consider the following application of the four requirements of natural selection. In this scenario, we demonstrate how red-bellied black snakes (*Pseudechis porphyriacus*) were influenced by natural selection.

Cane toads (*Rhinella marina*) are an invasive pest in Australia. They are poisonous and will kill most native animals that try to eat them. However, research by Ben Phillips and Richard Shine from the University of Melbourne suggests that the red-bellied black snake has evolved to tolerate cane toads (Phillips & Shine, 2006). Compared to red-bellied black snakes that live in uninfested areas, red-bellied black snakes that live in Queensland alongside cane toads are less impacted by the poison. The four requirements of natural selection in this scenario are:

1. Variation – variation existed between individuals in the original population of red-bellied black snakes in Queensland.
2. Selection pressure – an environmental selection pressure influencing the survivability of red-bellied black snakes included the presence of cane toads which, if eaten, led to the death of the red-bellied snake.
3. Selective advantage – red-bellied black snakes with the allele conferring resistance to the poison of cane toads would have had a selective advantage, resulting in a higher chance of them surviving and reproducing.
4. Heritability – the allele conferring resistance to cane toad poison was heritable. The red-bellied black snakes with this allele had a selective advantage and therefore reproduced more, passing the advantageous allele to their offspring. Over time, this would increase the frequency of the advantageous allele in the population.

The effect of selection pressures on genetic diversity 4.2.2.1

OVERVIEW

Environmental selection pressures can reduce the genetic diversity of a gene pool as the fitter individuals with alleles that code for advantageous phenotypes are more likely to survive and reproduce.

THEORY DETAILS

Environmental selection pressures drive the adaptation of a population through the process of natural selection. It is important to note that:

- These environmental selection pressures determine which phenotypes are considered fitter and advantageous and, consequently, which traits are more likely to be passed on to the next generation.
- As advantageous traits become more common in a population, the allele frequencies of the population changes, with the frequency of the advantageous allele increasing. Eventually, the **evolution** of the species can occur.
- Due to the generational increase in the frequency of the advantageous allele, the **genetic diversity** of the population will also decrease as the phenotypes of the population are driven towards a specific allele.

evolution the change in the genetic makeup of a population over successive generations

genetic diversity the variation in genetic makeup or alleles within a population

For example, in plants, if light is limited, larger leaves may be selected over smaller leaves as large-leaved plants can absorb more light. Therefore, over time, the allele frequencies of the allele coding for larger leaves will increase, while the frequency of alleles coding for smaller leaves will decrease. Overall, this process leads to a decrease in genetic diversity within the population.

Images: PhotographerChiran85, Nemeziya/Shutterstock.com

Figure 3 Differences in leaf size can arise due to selection pressures such as light availability.

The survivability of a species relies upon a population having large genetic diversity. This is because a population with a greater variation in alleles has a higher chance of possessing a favourable allele that will help them survive if a new selection pressure arises.

Conversely, populations with low genetic diversity are often at risk of extinction due to their inability to adapt to changing environmental selection pressures. Moreover, small populations are more likely to have low genetic diversity when compared to large populations, making them more susceptible to pressures. Inbreeding is also more common in populations with low genetic diversity, which can lead to a high prevalence of disadvantageous alleles.

Considering the scenario of leaf size discussed above, due to the decrease in genetic diversity, the population may struggle to adapt to new environmental selection pressures. For example, a drought may occur, limiting water availability and potentially favouring smaller plants with smaller leaves due to their decreased water consumption. However, due to the decrease in genetic diversity, plants with the advantageous allele may be scarce, thereby threatening the survivability of the population.

Lesson link

Dramatic reductions in genetic diversity often occur through the process of genetic drift, which is explored in ***lesson 9C***. When this occurs, a species can often find it difficult to adapt to new environmental selection pressures.

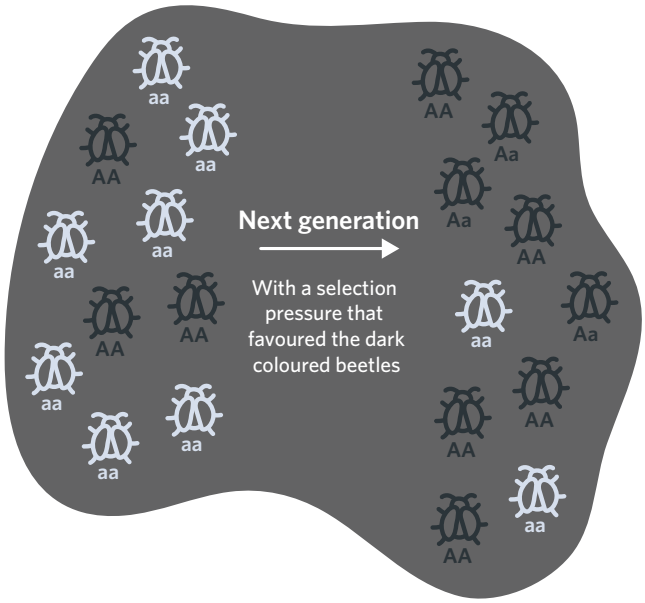


Figure 4 The change in allele frequencies of a beetle population affected by natural selection

Theory summary

Environmental selection pressures facilitate the mechanism of natural selection in which individuals best adapted to their environment are more reproductively successful and pass on their genes. These selection pressures determine which phenotype is fitter and more advantageous. As the fitter trait becomes more common in the population, the allele frequencies and genetic diversity within the population change.

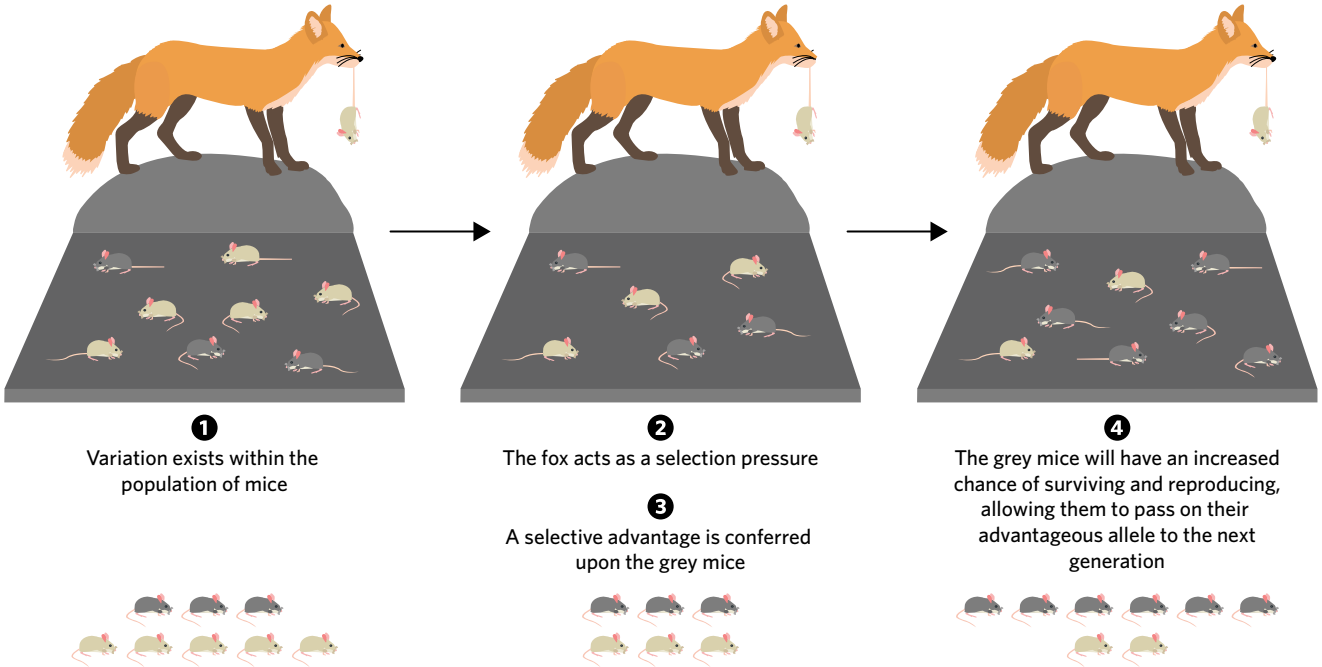


Figure 5 A summary of natural selection.

*The common garter snake (*Thamnophis sirtalis*) is the primary predator of the rough-skinned newt. While it isn't a very large snake, typically only around 55 cm in length, it has developed a powerful resistance to tetrodotoxin. Therefore, an evolutionary arms race has developed between the two organisms. Through the process of natural selection, the rough-skinned newts are increasing the frequency of alleles coding for a higher concentration of tetrodotoxin whilst common garter snakes, in turn, are increasing the frequency of alleles coding for increased resistance to tetrodotoxin.*

Image: Natalia Kuzmina/Shutterstock.com

9B QUESTIONS

Theory review questions

Question 1

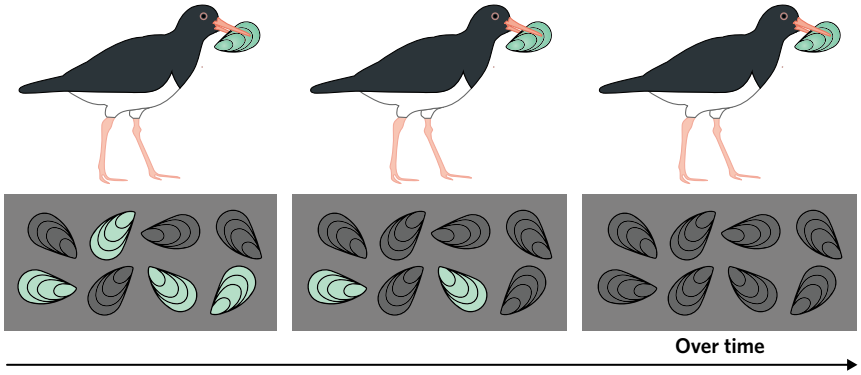
Environmental selection pressures

A decrease the survivability of all organisms within a population.

B confer a selective advantage to organisms with an advantageous allele.

Question 2

Fill in the blanks in the following sentences.



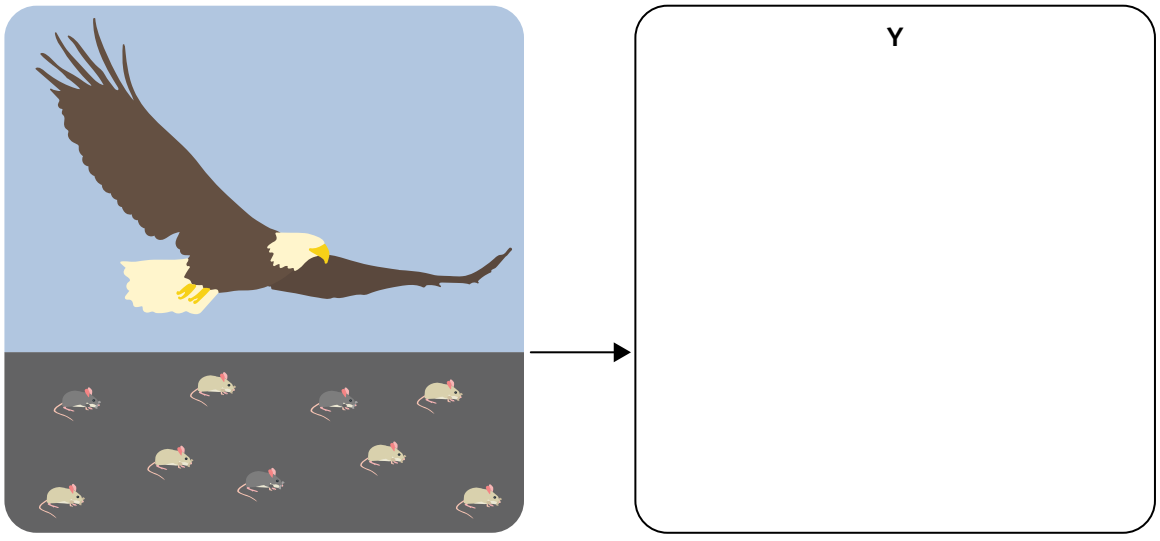
The image depicts the changes in a population of mussels over time. In the image, the selection pressure is the _____, the population that selection is acting against is _____, and the advantageous phenotype is _____.

Question 3

Match the condition of natural selection to its description.

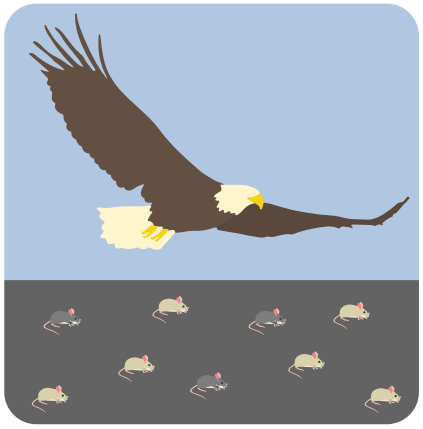
Condition of natural selection	Description
• variation	**I** _____ traits within the population can be passed on from parents to offspring
• heritability	**II** _____ external factors that affect an organism's ability to reproduce and survive
• selection pressure	**III** _____ individuals in a population are different genetically and phenotypically
• selective advantage	**IV** _____ individuals with specific phenotypes that increase their survivability

Question 4



Identify which image resembles Y according to the theory of natural selection. Note that grey mice are harder for birds to see.

A



B

C

D

Question 5

Which one of the following statements about natural selection is false?

A A selection pressure acts upon a population, increasing the difficulty of survival for certain individuals.

B For natural selection to occur, advantageous traits must be hereditary.

C Only small populations have low genetic diversity.

D Advantageous alleles increase genetic fitness.

SAC skills questions

Scientific methodology comparison

Use the following information to answer Questions 6–10.

The inheritance of acquired characteristics was a widely known theory about how traits were passed down generations. It was accepted for centuries and supported by many prominent individuals such as Hippocrates and Aristotle. However, it was not until the 19th century that Jean-Baptiste Lamarck linked the theory to biological evolution. He proposed that changes which arose during an organism's life from adapting to its external environment could be inherited in the next generation. For example, he believed that at one point in time, all giraffes had short necks and when they could no longer reach the tall branches for food, they simply adapted and stretched their necks. After stretching their necks, they could then pass this trait onto their offspring.

It was not until years later that Charles Darwin proposed another theory which would supersede Lamarck's theory on evolution, publishing *On the Origin of Species* in 1859. Darwin argued that instead of adapting to the environment, variation already existed within the original population. Therefore, he claimed that there would have already been shorter and longer-necked giraffes. In this population of giraffes, he proposed that those who could not reach the tall branches would have died from a lack of food and that those who were able to reach the tall branches would be able to survive and pass their advantageous alleles to their offspring, forming the basis of what we know today as natural selection. Eventually, the iconic phrase 'survival of the fittest' was coined, describing the mechanism of natural selection.

Question 6

Lamarck proposed that giraffes

A are capable of stretching their necks to reach taller trees.

B unable to reach tall trees would not be able to reproduce.

Question 7

In the example provided, the advantageous allele in giraffes would code for

A a long neck.

B a short neck.

Question 8

Over time, the genetic diversity of giraffes could decrease due to the

A presence of multiple different selection pressures.

B mechanism of natural selection.

Question 9

Both Lamarck and Darwin believed that

A organisms can develop heritable traits by adapting to their environment.

B the fitness of organisms could be changed throughout their life.

C organisms could pass traits down to the next generation.

D variation existed in the original population.

Question 10

Evidence that would support Darwin's theory of evolution and disprove Lamarck could include

A genetic sequencing.

B transformation.

C recombination.

D karyotyping.

Exam-style questions

Within lesson

Question 11 (1 MARK)

In 1954, copper waste in the Finniss River killed numerous fish. This caused various species in the area to die out. However, one species, the black-banded rainbowfish (*Melanotaenia nigrans*), increased in numbers. The black-banded rainbowfish have modified gills that enable the fish to filter and remove the copper before it enters their body.

With respect to the black-banded rainbowfish it is reasonable to conclude that

A there was more genetic variation in the black-banded rainbowfish population than in the populations of other fish species.

B the copper in the river caused a mutation in the black-banded rainbowfish that helped them survive.

C the ability of black-banded rainbowfish gills to remove copper already existed in 1954.

D the populations of other fish in the river were small even before the copper waste spill.

Adapted from VCAA 2009 Exam 2 Section A Q16

Question 12 (1 MARK)

Which one of the following statements does not align with Darwin's theory of natural selection?

A Selection pressures determine advantageous phenotypes.

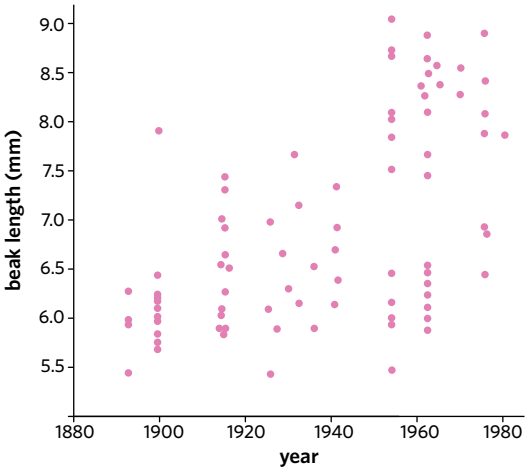
B All members of a species have an equal chance of survival.

C Individuals in a population have different chances of reproductive success.

D Offspring look more like their parents than they do unrelated individuals of the same species.

Question 13 (1 MARK)

The soapberry bug (*Jadera haematoloma*) uses its long beak to penetrate the fleshy fruit of plants to feed on the seeds at the centre. The bug feeds on the native soapberry tree. The bug also feeds on the fruit of the introduced golden rain tree. Investigators measured the average beak length of the soapberry bug over eighty years. The results are shown in the following graph.



From this information it would be reasonable to conclude that the

A soapberry bugs with longer beaks survive and reproduce more than soapberry bugs with shorter beaks.

B response of the soapberry tree to predation by soapberry bugs was to grow harder fruit.

C diameter of the golden rain tree seed acted as a selection pressure on beak length.

D population of soapberry bugs increased over the eighty years.

Adapted from VCAA 2011 Exam 2 Section A Q19

Question 14 (5 MARKS)

The blue mussel, *Mytilus edulis*, lives along the north-eastern coastline of the USA. A species of Asian shore crab, *Hemigrapsus sanguineus*, was accidentally introduced into the area about 15 years ago. As shown in the image, the Asian shore crab has only migrated to the southern two-thirds of the total area inhabited by the blue mussel.

The Asian shore crab preys on blue mussels. The thinner the mussel shell, the easier it is for the crab to crush and eat the mussel. In recent times, scientists have observed that the overall population of the southern blue mussel has a thicker shell than that of the northern blue mussel. This contrasts with 15 years earlier when there was no difference in the range of shell thickness in northern and southern blue mussel populations.

a Explain how the population of southern blue mussels has developed thicker shells. (3 MARKS)

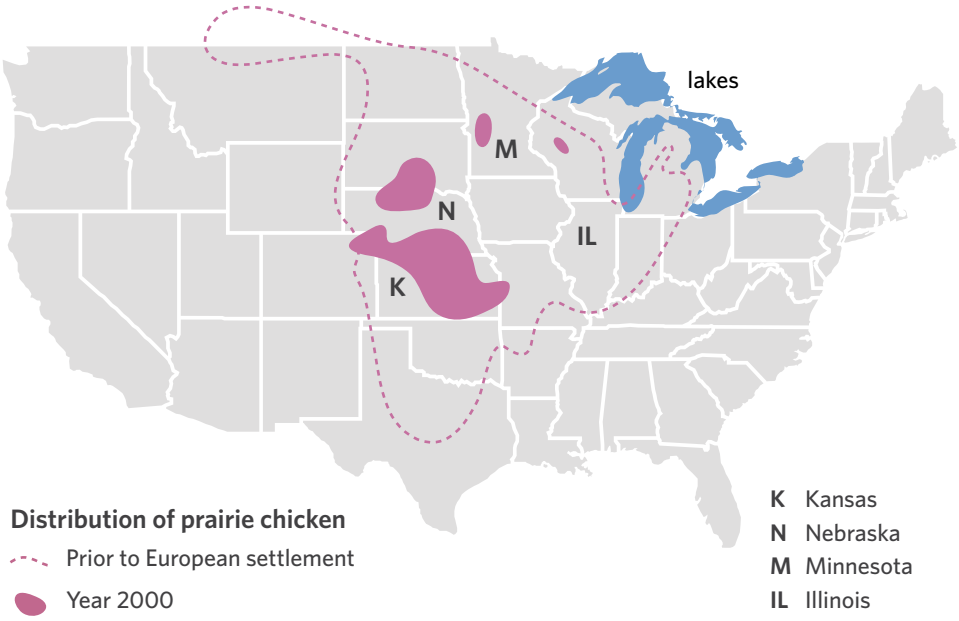
b Assume that the Asian shore crab migrates past the northern limit line into the northern blue mussel area. What would you expect to happen to the shell thickness of the northern blue mussels over time? Explain your reasoning. (2 MARKS)

Adapted from VCAA 2010 Exam 2 Section B Q4

Multiple lessons

Question 15 (8 MARKS)

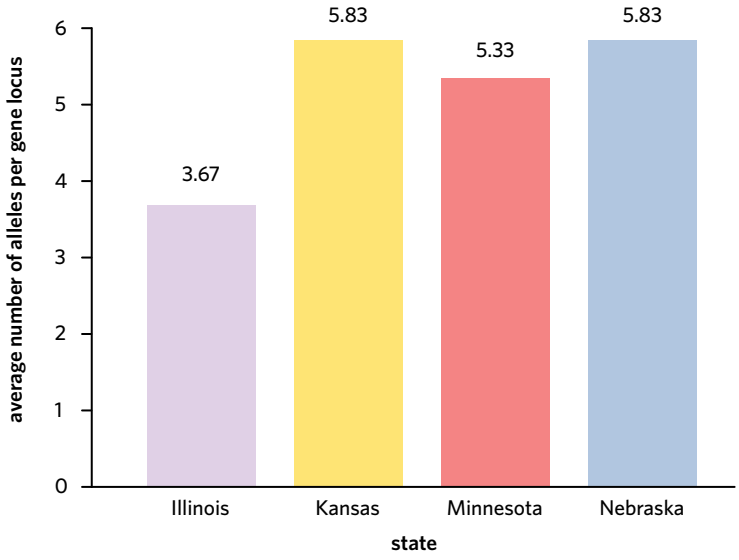
The prairie chicken (*Tympanuchus cupido pinnatus*) is a grassland bird native to North America. A prairie chicken spends its entire life within several kilometres of its birthplace. Prior to European settlement, prairie chickens numbered in the millions across the Midwest of the USA. As a result of the grasslands being replaced by plant food crops, the distribution of prairie chickens has diminished, as shown in the following diagram.



By 1994, Kansas, Nebraska, and Minnesota still supported large and widespread populations. However, in the state of Illinois, the number of prairie chickens fell to less than fifty individuals located in two separate geographical areas, as shown in the image.

Illinois – prairie chicken distribution

Representative samples of prairie chickens from the four states were selected for testing. Each prairie chicken had six gene loci tested. The average number of alleles at each gene locus for each prairie chicken group is shown in the graph.



a Define the term 'allele frequencies'. (1 MARK)

b Consider the graph and maps of prairie chicken distribution.

i What do these results mean for the future of the Illinois birds? (2 MARKS)

ii Suggest a reason for the differences in the average number of alleles per gene locus between the Illinois and Minnesotan populations. (1 MARK)

c Measures were taken in the 1990s to prevent the prairie chicken from dying out completely. Describe one measure that could be used to increase the average number of alleles per gene locus for Illinois prairie chickens. (1 MARK)

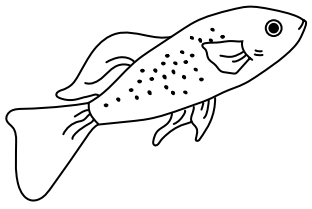
d How might natural selection have prevented prairie chickens from becoming extinct when humans replaced grasslands with food crops? (3 MARKS)

Adapted from VCAA 2011 Exam 2 Section B Q6

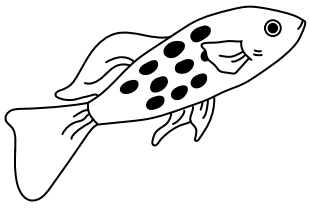
Key science skills and ethical understanding

Question 16 (12 MARKS)

Guppies, *Poecilia reticulata*, are small, active freshwater fish. Scientists have studied populations of guppies living in streams. In some of these streams, referred to as dangerous streams, guppies are found with predatory fish. These predatory fish eat guppies. In the dangerous streams, male guppies have small spots whose colours blend with the sand on the stream bed. In other streams, referred to as safe streams, the predatory fish are absent. In safe streams the male guppies have brightly coloured spots. The scientists suggested that the colour of the spots shown by the males in dangerous streams may be the result of natural selection.



male guppy from a dangerous stream (spots blend with sand)

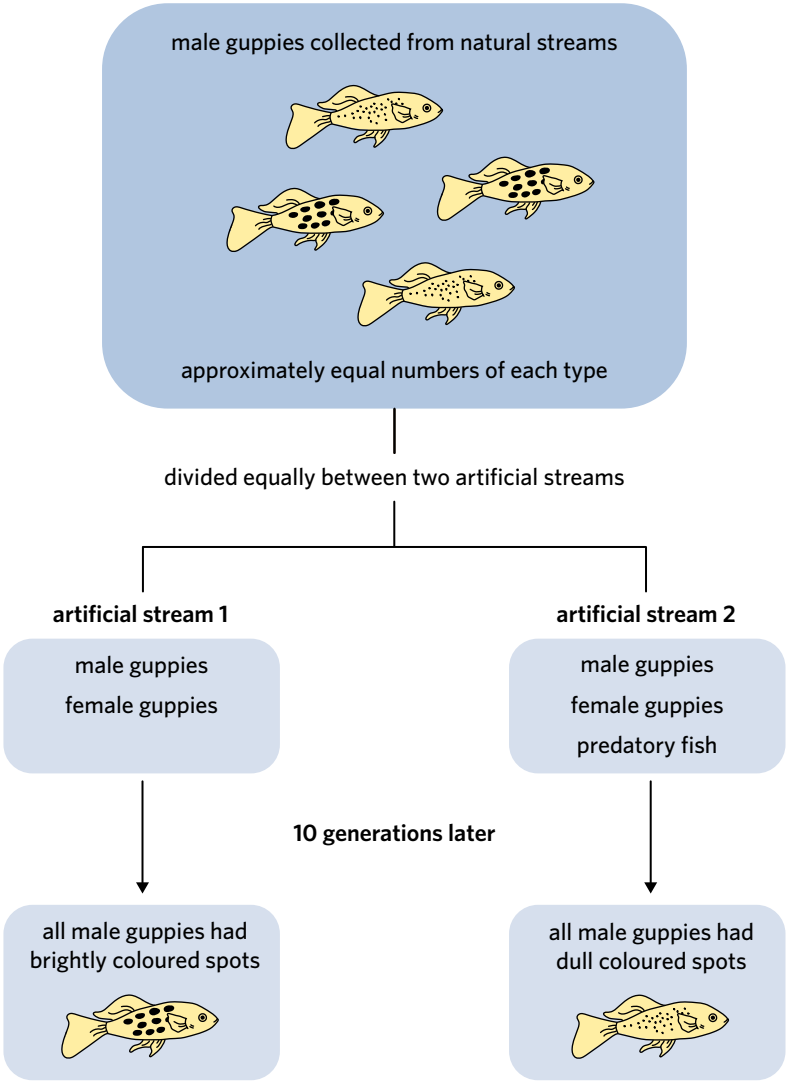


male guppy from a safe stream (spots brightly coloured)

a Name the selection pressure acting on the male guppies in the dangerous streams. (1 MARK)

b Suggest one reason why the male guppies in the safe streams have brightly coloured spots. (1 MARK)

c Scientists wanted to test the hypothesis that the colour of the spots of male guppies in dangerous streams is due to natural selection. Two identical artificial streams were built in a laboratory. A large number of male guppies were collected and divided into two equal groups with female guppies. The following diagram summarises the experiment in the laboratory.



i Describe the events that would have occurred to result in all male guppies having dull coloured spots in artificial stream 2. (3 MARKS)

ii Identify the independent and dependent variables in the experiment. (2 MARKS)

iii When designing the artificial streams in the laboratory, the scientists made streams with similar conditions to those occurring in the wild. Name two factors they would need to take into account when designing their artificial streams. (2 MARKS)

d At a later date, a similar experiment was carried out in the wild. Two hundred male guppies were moved from dangerous streams to safe streams two kilometres away. Within a year, most male descendants of those guppies had brightly coloured spots when compared with guppies in the dangerous streams.

i Why did the scientists carry out the experiment in the wild? (1 MARK)

ii Describe how the scientists could have used the bioethical concept of beneficence to justify the movement of guppies. (2 MARKS)

Adapted from VCAA 1997 CAT3 Article 5

9C GENETIC DRIFT AND GENE FLOW

In the 1800s, Ireland was struggling to feed its booming population. To solve this problem, they decided to grow enough potatoes to supply everyone with food for breakfast, lunch, and dinner. While this proved to be an effective solution for a couple of decades, in the 1840s, potato crops became infected with a fungus known as Phytophthora infestans, *which turned the potatoes into rotten slime. During this period, it is estimated that around one million individuals died from starvation, with another two million estimated to have emigrated from Ireland to other parts of the world. But why was the potato so vulnerable to* Phytophthora infestans*?*

Image: nednapa/Shutterstock.com

Lesson 9C

In this lesson you will learn about genetic drift and gene flow and their effects on allele frequencies.

◀ *Prerequisite knowledge*

Year 11
The survival and stability of species and ecosystems rely on a high degree of genetic diversity which allows species to adapt to new environments.

Lesson 9B
Populations with decreased genetic diversity resulting from either genetic drift or the emigration of individuals are less able to adapt to the introduction of new environmental selection pressures.

Future applications ▶

Lesson 9D
Allopatric speciation, which is the process of forming a new species through the presence of a geographical barrier, requires the absence of gene flow.

Chapter 11
The presence of gene flow between different species of hominins raises questions about the definition of species and the classification of humans.

Study design dot points

- causes of changing allele frequencies in a population's gene pool, including environmental selection pressures, genetic drift and gene flow, and mutations as the source of new alleles
- biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity

Key knowledge units

Genetic drift	4.2.1.4
The effect of genetic drift on genetic diversity	4.2.2.2
Gene flow	4.2.1.5
The effect of gene flow on genetic diversity	4.2.2.3

Genetic drift 4.2.1.4

OVERVIEW

Genetic drift involves changes to a population's allele frequencies due to sudden and random occurrences.

THEORY DETAILS

In response to random events, allele frequencies can change drastically and affect a population's overall **genetic diversity**. This phenomenon is known as **genetic drift** and occurs through either the **bottleneck effect** or the **founder effect**.

genetic diversity the variation in genetic makeup or alleles within a population

genetic drift a random event that dramatically alters a population's gene pool

bottleneck effect the reduction in genetic diversity that occurs when a large proportion of a population is removed due to a chance event

Bottleneck effect

The bottleneck effect occurs when a large portion of a population is wiped out by a random event such as a natural disaster. These events can dramatically decrease the **population** size, significantly impacting **allele frequencies**. Due to the severe reduction in population size, many individuals carrying unique alleles can be lost. Therefore, the new population has lower genetic diversity than the pre-disaster population.

For example, Figure 1 demonstrates the impact of the bottleneck effect on a multicoloured population of beetles. Due to a bottleneck event, the original population size is dramatically decreased, leading to the loss of various alleles. Therefore, the alleles present in the new population will depend on the remaining alleles within the surviving population. This significantly decreases the genetic diversity of the beetle population, leaving only green and yellow beetles.

founder effect the reduction in genetic diversity that occurs when a population is derived from a small unrepresentative sample of the original population

population a group of individuals of the same species living in the same location

allele frequency the proportion of certain alleles in a gene pool

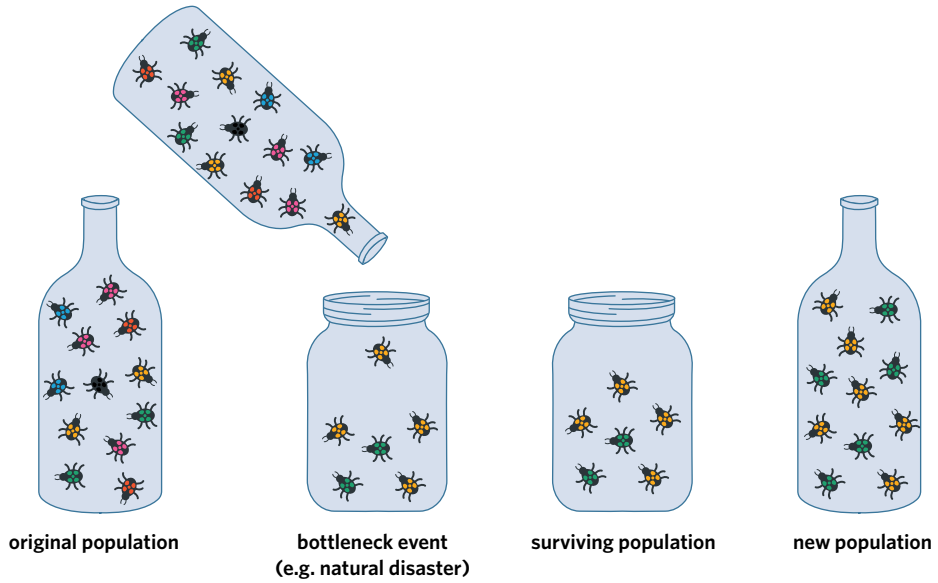


Figure 1 Bottleneck effect displayed in a beetle population

Founder effect

The founder effect occurs when a small **unrepresentative sample** of individuals separates from a larger population to colonise a new region and start a new population. Think of a population of beetles of many different colours. If ten green beetles left their original multicoloured population to form a new population, it would not mirror the initial **gene pool** and would therefore be considered an unrepresentative sample (Figure 2). This also means that the genetic diversity of the new population is significantly lower than the original and would be an example of the founder effect. However, if the original population of beetles were all green, establishing a new colony of green beetles would not be considered an example of the founder effect as the founding colony resembles the initial gene pool.

unrepresentative sample a small selection of individuals from a larger group that does not reflect the characteristics of the larger group

gene pool the complete set of alleles present within a particular population

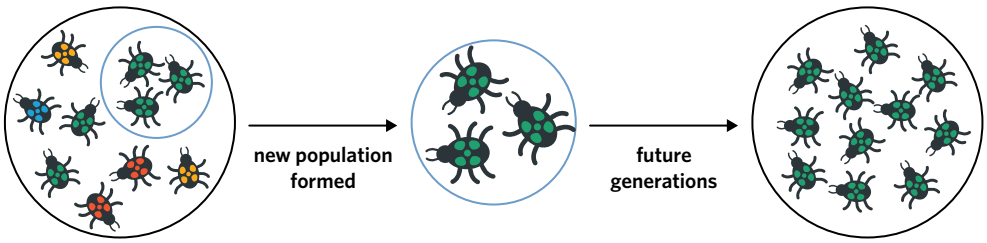


Figure 2 Founder effect displayed in a beetle population

The effect of genetic drift on genetic diversity 4.2.2.2

OVERVIEW

Both the bottleneck and founder effects decrease genetic diversity within a population.

THEORY DETAILS

Genetic drift reduces genetic diversity in a population through the random removal of alleles from the gene pool. The way through which each mechanism of genetic drift reduces genetic diversity is summarised in Table 1 and Figure 3.

Table 1 The consequences of the bottleneck and founder effects on genetic diversity

Mechanism of genetic drift	Consequence
Bottleneck effect	Reduces genetic diversity through the removal of alleles due to random events (e.g. natural disaster)
Founder effect	Reduces genetic diversity through the establishment of a new population with a small unrepresentative sample of the original population

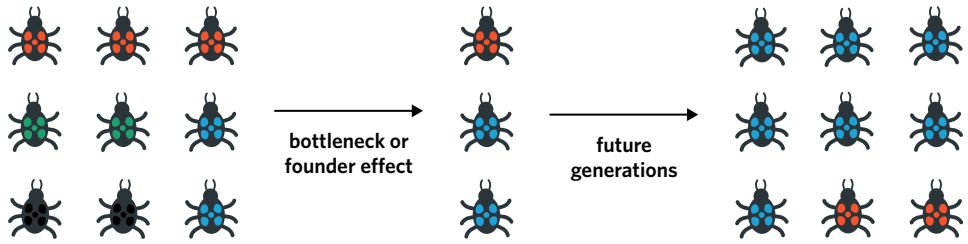


Figure 3 The effect of genetic drift on a beetle population. Both the bottleneck and founder effects reduce genetic diversity in future generations.

Reductions in genetic diversity have two major risks:

- **Inbreeding** – this keeps harmful alleles in the gene pool.
- Lower **adaptive potential** – populations become vulnerable to new selection pressures that could challenge and potentially wipe out the entire population due to the absence of advantageous alleles.

inbreeding sexual reproduction between two related individuals

adaptive potential the ability for a population to adjust to new environmental selection pressures

Additionally, smaller populations are more susceptible to the effects of genetic drift, as they typically already have lower genetic diversity when compared to larger populations. Subsequently, inbreeding and lower adaptive potential can aggravate the effects of genetic drift within smaller populations, causing greater negative effects. On the other hand, larger populations are more resistant to the effects of genetic drift.

For example, if a population is composed of ten beetles, and a given beetle died by chance before it sexually reproduces, all of its genes would be lost from the gene pool. This equates to losing 10% of a population's gene pool. However, if there was a population of 100 beetles and one died by chance, only 1% of the gene pool would be lost. This demonstrates that the impacts of random events are reduced in larger populations.

Theory in context

CHEETAHS

Cheetahs (*Acinonyx jubatus*) are a species severely affected by the bottleneck effect. It has been hypothesised that cheetahs have experienced two major bottleneck effects, including one from 100 000 years ago and another 10 000–12 000 years ago after the last ice age. During these events, large populations of cheetahs were lost, leading to the extinction of cheetahs from North America and Europe, leaving behind the Asian and African populations of cheetahs. Due to these bottleneck events, the genetic diversity of the remaining cheetahs was also severely reduced, leading to significant inbreeding.

From Chapter 7, you should remember that when receiving an organ transplant, individuals must undergo a series of tests to ensure compatibility to reduce the chances of rejection. However, because cheetahs are so inbred, they are nearly all genetically identical. Therefore, if a cheetah required an organ transplant, it could probably receive one from any other individual, including a complete stranger from the opposite side of Africa.

cont'd

Theory in context

CHEETAHS – CONTINUED

Unfortunately, due to this reduction in genetic diversity and widespread inbreeding, if a new environmental selection pressure was to arise, then the population of cheetahs is unlikely to be able to adapt. This is because the ability of a population to adapt relies on genetic diversity, with the presence of advantageous alleles which can confer a selective advantage against the environmental selection pressure. Without the presence of advantageous alleles, the population of cheetahs is likely to go extinct (Figure 4).

Image: Stu Porter/Shutterstock.com

Figure 4 Cheetahs are a species heavily affected by the bottleneck effect.

Gene flow 4.2.1.5

OVERVIEW

Gene flow involves the introduction or removal of alleles between populations through either migration or interbreeding.

THEORY DETAILS

The movement of alleles between individuals from two different populations through either migration or **interbreeding** is known as **gene flow**. Migration can occur when populations are physically close together, or due to external forces such as the clearing of a geographical barrier between populations. The migration into and out of a population is known as **immigration** and **emigration**, respectively.

When individuals enter a population via immigration, their alleles are added to the gene pool of that particular population. Conversely, when individuals exit a population via emigration, their alleles are removed from the gene pool (Figure 5). Additionally, when individuals temporarily enter a population and interbreed with local individuals before leaving again, they contribute to the gene pool of that particular population. This means that populations in different geographic locations can exchange alleles through either migration or interbreeding.

interbreeding when two individuals living in different populations mate and have offspring

gene flow the flow of alleles in and out of a population due to the migration or interbreeding of individuals between two populations

immigration the movement into a population

emigration the movement out of a population

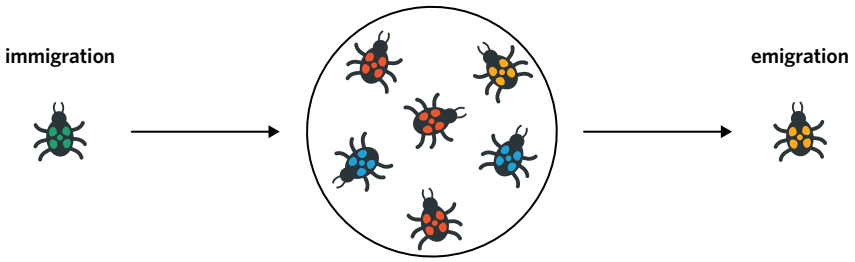


Figure 5 Gene flow in a beetle population

The effect of gene flow on genetic diversity 4.2.2.3

OVERVIEW

Gene flow can introduce or remove alleles from a population, thereby increasing or decreasing genetic variation.

THEORY DETAILS

Immigration and emigration can both affect allele frequencies (Figure 6). When new alleles are brought into a population through immigration, the genetic diversity in a population increases. This increase is more pronounced in smaller populations since they have a smaller gene pool to begin with. In larger populations, the immigration of new alleles into the population does not significantly affect the gene pool.

On the other hand, emigration removes alleles from a population's gene pool, decreasing genetic diversity. Once again, the effects of emigration are more pronounced in smaller populations compared to larger populations.

When individuals carrying unique alleles from different populations interbreed, the genetic diversity increases. These new alleles can often be permanently added to a population's gene pool, assuming that the allele is not selected against.

Figure 6 The effect of gene flow on a beetle population. The red beetle is emigrating from Population 1 which reduces its genetic diversity, while immigrating into Population 2 which increases its genetic diversity.

Theory summary

Genetic drift involves random events which dramatically alter a population's gene pool, while gene flow involves the movement of alleles into or out of a population. Both genetic drift and gene flow can influence allele frequencies. While genetic drift only reduces a population's gene pool, gene flow can either increase or decrease the genetic diversity (Table 2).

Table 2 The effect of genetic drift and gene flow on genetic diversity

Mechanism	Category	Effect on genetic diversity
Genetic drift	Bottleneck effect	Decrease
	Founder effect	Decrease
Gene flow	Immigration	Increase
	Emigration	Decrease
	Interbreeding	Increase

Potatoes are often grown through a technique known as vegetative propagation. Unfortunately, this technique produces genetically identical potatoes, which effectively eliminates the presence of genetic diversity. Subsequently, when Phytophthora infestans *was introduced it quickly spread throughout the potato population, which did not contain advantageous alleles to deal with the pathogen. The* P. infestans *infestation acted as a random event that drastically reduced the potato population size, serving as an agent of genetic drift. Due to the already reduced genetic diversity within the potatoes, they were unable to adapt to the new environmental selection pressure, resulting in the Great Irish Famine.*

Image: Thy/Shutterstock.com

9C QUESTIONS

Theory review questions

Question 1

Which one of the following statements is false?

A The bottleneck effect occurs as a result of a drastic reduction in population size.
B The founder effect involves a species colonising a new geographical area.
C Genetic drift occurs independently of an individual's genetic fitness.
D Emigration increases the genetic variation in a population.

Question 2

Categorise the following as either a mechanism which **increases** or **decreases** genetic diversity.

I bottleneck effect _____
II founder effect _____
III interbreeding _____
IV immigration _____
V emigration _____
VI inbreeding _____

Question 3

Match the following mechanisms to their scenario.

Scenario	Mechanism
• gene flow	**I** _____ volcanic eruption which kills the majority of a population
• founder effect	**II** _____ establishment of a new colony in Antarctica
• bottleneck effect	**III** _____ migration of Australians to America

Question 4

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- low adaptive potential
- inbreeding
- extinction
- smaller
- larger
- high
- low

Populations with a _____ genetic diversity often struggle with _____ and a _____. Therefore, they can often struggle to adapt to new environmental selection pressures, leading to _____. Additionally, the mechanisms of genetic drift affect _____ populations more than _____ populations.

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–9.

The Tasmanian devil is the largest surviving carnivorous marsupial in Australia. However, there have been several significant events throughout the history of Australia which have decimated Tasmanian devil populations. The first event was the extinction of Tasmanian devils from mainland Australia, which is thought to have occurred 3 000 years ago, most likely due to predation by dingos, climate change, and the destruction of food sources. The next event occurred throughout the 1800s, when there was a significant effort to eradicate the Tasmanian devil by Tasmanian farmers, who thought that the devil was responsible for killing livestock. Therefore, Tasmanian devil numbers were significantly reduced. However, they were lucky to escape extinction, unlike their close relative the Tasmanian tiger.

Now, the Tasmanian devil is in danger of extinction due to the deadly Devil Facial Tumour Disease (DFTD), a type of cancer. DFTD is an unusual type of cancer because it can be passed from one individual to another when deep wounds occur as they fight over food or as they mate. When tumour cells in the mouth or cheek of an infected Tasmanian devil break off and enter the wound of another Tasmanian devil, it can rapidly multiply and form a new tumour that can kill the devil.

Fortunately, Tasmanian devils genetically resistant to DFTD are beginning to arise, with researchers hoping that by establishing protected breeding grounds and conservation programs, the Tasmanian devil population will increase. In 2020, researchers also reintroduced a group of 26 Tasmanian devils back onto mainland Australia.

Question 5

The first major event affecting Tasmanian devil populations involved the

A destruction of food sources on mainland Australia.

B hunting of Tasmanian devils in Tasmania.

Question 6

The effects of the Devil Facial Tumour Disease can be described as an example of

A the bottleneck effect.

B the founder effect.

C gene flow.

Question 7

The reintroduction of Tasmanian devils back onto mainland Australia can be described as an example of

A the bottleneck effect.

B the founder effect.

C gene flow.

Question 8

Interbreeding between mainland and Tasmanian populations of Tasmanian devils can be described as an example of

A the bottleneck effect.

B the founder effect.

C gene flow.

Question 9

The allele conferring resistance against DFTD could have arisen due to

A mutations.

B genetic drift.

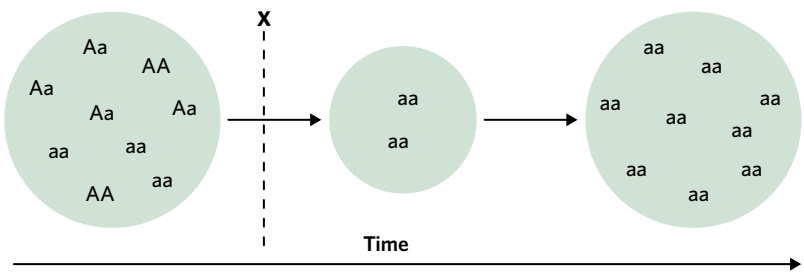
C natural selection.

Exam-style questions

Within lesson

Question 10 (1 MARK)

Consider the diagram that models changes in allele frequencies for one trait in a population over two generations. The original population is shown on the left.



If event X is a natural disaster, then the diagram models

A the bottleneck effect.

B the founder effect.

C random mating.

D gene flow.

Adapted from VCAA 2014 Section A Q33

Question 11 (1 MARK)

Northern elephant seals, *Mirounga angustirostris*, were nearly hunted to extinction in the 1890s, with only about 20 individuals left at the end of the century. The population has now grown to more than 120 000. In the 1890s, southern elephant seals, *Mirounga leonina*, were not as severely hunted and currently there are estimated to be 600 000 southern elephant seals.

Which one of the following statements is false?

A Northern elephant seals are more likely to suffer from a bottleneck effect than southern elephant seals.

B Southern elephant seals would have a greater genetic diversity compared to northern elephant seals.

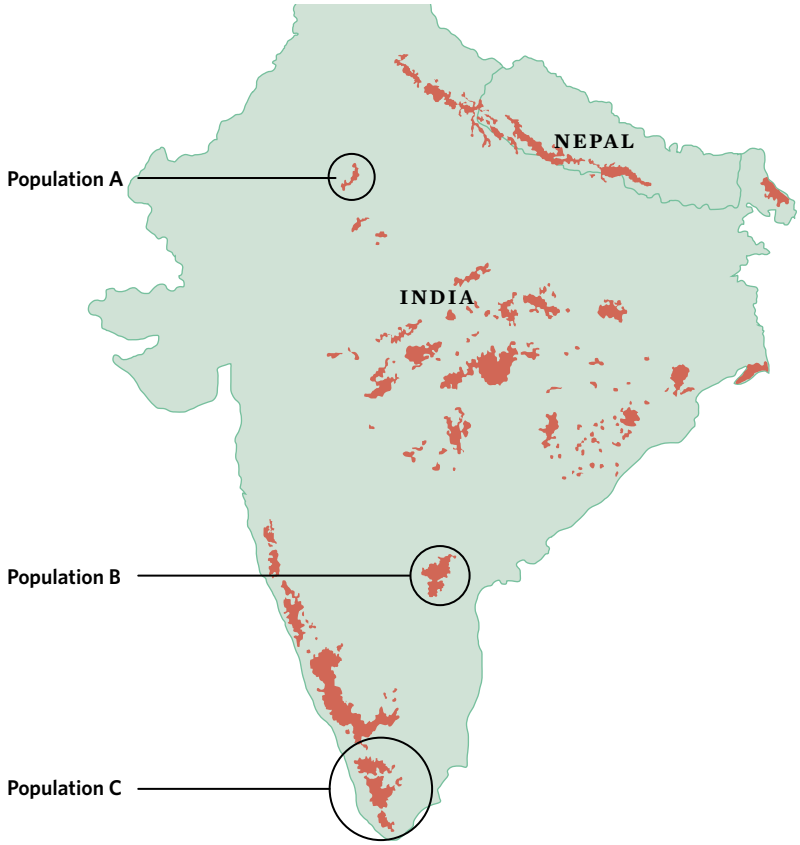
C Northern elephant seals would have experienced greater genetic drift than southern elephant seals.

D Northern elephant seals evolved into southern elephant seals as a result of the bottleneck effect.

Adapted from VCAA 2015 Section A Q40

Question 12 (3 MARKS)

Currently, tigers are classified as an endangered species, with only 2 000–3 000 individuals left in the wild. They can be primarily found in China, India, Indonesia, Malaysia, Nepal, Russia, and Thailand, inhabiting a wide variety of different habitats, including rainforests, grasslands, savannas, and mangrove swamps. The following image depicts the distribution of tigers across India.



Source: Adapted from International Union for Conservation of Nature Red List (2021)

a Explain how the genetic diversity of a population is affected by gene flow. (2 MARKS)

b Suggest why the amount of gene flow is higher between populations B and C when compared to populations A and B. (1 MARK)

Multiple lessons

Question 13 (5 MARKS)

Huntington's disease is a rare genetic disorder that develops during adulthood and involves the progressive degeneration of nerve cells, resulting in cognitive, physical, and psychiatric impairments. Unfortunately, in certain populations, the prevalence of the allele for Huntington's disease is relatively high. For example, the prevalence of the allele for Huntington's disease in Afrikaners living in South Africa is particularly high. It has been hypothesised that this high prevalence in the allele for Huntington's disease originates from a small group of Dutch settlers who established a new colony in South Africa in the 1600s.

a Explain the high prevalence of the allele for Huntington's disease in Afrikaners. (2 MARKS)

b Identify two possible mechanisms of how new alleles could be introduced into the gene pool of the Dutch settlers. (2 MARKS)

c Given that Huntington's disease results in severe impairments, suggest a reason why it still persists in the gene pool. (1 MARK)

Question 14 (7 MARKS)

Blue whales (*Balaenoptera musculus*) are the largest animals currently living on the planet, measuring up to 30 m and weighing over 150 tonnes. Unfortunately, after the rise of commercial whaling in the early 1900s, populations of blue whales have significantly declined from 250 000 to 25 000.

Recently, researchers have raised concerns over the increase in boat traffic through the eastern South Pacific, which represents a crucial feeding ground for blue whales. It has been reported that over 1 000 boats travel through the eastern South Pacific, with 83% of those vessels belonging to the salmon industry operating in northern Chilean Patagonia. This increase in boat traffic has severely affected the ability of the blue whales to feed, as they must cautiously navigate around these boats to prevent colliding with them. Sadly, when blue whales collide with these boats, they are often killed or injured, further contributing to their declining numbers. The following image depicts the movement of a single blue whale among the boat traffic in Chilean Patagonia.

a A large number of blue whale deaths can be attributed to collisions with boats, reducing their population size. Explain the effect of a decline in population on the blue whale species' ability to adapt to new environmental selection pressures. (2 MARKS)

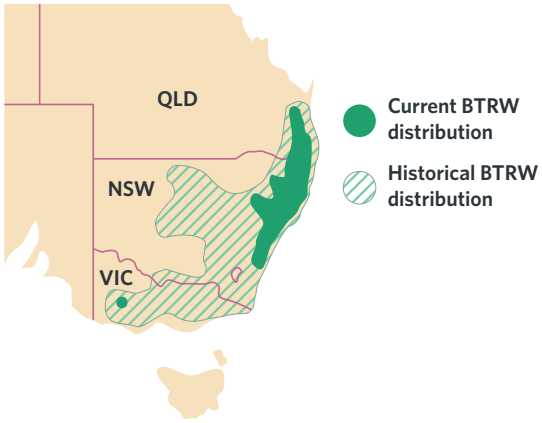
b Assuming that boat traffic continues to grow, suggest two possible future outcomes for the population of blue whales living in Chilean Patagonia. (2 MARKS)

c In order to increase populations of blue whales, four healthy individuals from the blue whale population (two male, two female) were translocated into another ocean habitat and bred intensively over several generations. Outline an issue with such a breeding program, referring to the genetic diversity and fitness of the future population. (3 MARKS)

Key science skills and ethical understanding

Question 15 (7 MARKS)

The brush-tailed rock-wallaby (*Petrogale penicillata*) was once an abundant species across southeastern Australia, however now faces extinction. Currently, the rock-wallaby is restricted to two populations: one larger population along the east coast spanning Queensland and New South Wales, and one smaller population in western Victoria. To explain the decrease in the rock-wallaby population, scientists have been investigating the distance between colonies of the species to determine the effect of gene flow on a population's viability.



Source: Adapted from Department of Environment & Climate Change (2008)

The scientists hypothesise that if the amount of gene flow between two populations is dependent upon the distance between rock-wallaby colonies, then the greater the distance, the lower the amount of gene flow that would occur.

a State the independent and dependent variables. (2 MARKS)

b Explain how gene flow can increase genetic diversity. (2 MARKS)

c Outline the importance of genetic diversity for the brush-tailed rock-wallaby. (2 MARKS)

d By referring to the map, suggest a reason why scientists could conclude that the rock-wallabies were not affected by the founder effect. (1 MARK)

9D SPECIATION

There are many unique animals that can be produced by breeding two different species together. For example, by breeding a lion with a tiger together, it is possible to create a liger, and by breeding a donkey with a horse together, it is possible to create a mule. But would these hybrid animals produced be considered a separate species?

Image: s7chvetik/Shutterstock.com

Lesson 9D

In this lesson you will learn how new species evolve through allopatric and sympatric speciation.

◀ *Prerequisite knowledge*

Lesson 9B

Environmental selection pressures select for particular phenotypes suited to surviving within a specific environment, allowing for genetic differences to accumulate between populations subjected to different environmental selection pressures.

Lesson 9C

The process of speciation relies on a lack of gene flow between populations.

Future applications ▶

Chapter 10

The formation of new species can be visualised through the discovery of transitional fossils. Additionally, the relationships between different species can be determined by comparing both morphological and genetic differences.

Chapter 11

The timeline and eventual formation of the modern human species is heavily debated, with new species of humans constantly being discovered and recategorised.

Study design dot point

- evidence of speciation as a consequence of isolation and genetic divergence, including Galápagos finches as an example of allopatric speciation and *Howea* palms on Lord Howe Island as an example of sympatric speciation

Key knowledge units

Speciation	4.2.6.1
Allopatric speciation	4.2.6.2
Sympatric speciation	4.2.6.3

Speciation 4.2.6.1

OVERVIEW

Speciation is the process by which populations genetically diverge until they become distinct species.

THEORY DETAILS

Throughout this chapter, you have learned about different evolutionary processes such as mutations, natural selection, genetic drift, and gene flow. All of these processes influence the frequency of alleles, leading to changes in the genetic composition and genetic diversity of a population. When genetic differences accumulate through these processes, speciation can occur, which involves the formation of a new **species**. For VCE Biology, speciation can be categorised as either **allopatric speciation** or **sympatric speciation**.

species a group of individuals who are able to breed with each other and produce viable and fertile offspring

allopatric speciation the geographic separation of a population from a parent population resulting in the formation of a new species

sympatric speciation the divergence of a species from an original species without the presence of a geographical barrier

Individuals are recognised as different species if they can no longer interbreed with one another to produce **viable** and **fertile** offspring. Other methods that indicate whether two individuals are of the same species include comparing their genetic composition through the analysis of their amino acid sequences and DNA sequences and the comparison of structural features.

It is important to remember that speciation is a slow and gradual process. Subspecies, which include individuals phenotypically different from their original population, can often arise. While these subspecies are phenotypically different from each other, they are still capable of interbreeding to produce viable and fertile offspring (Figure 1).

viable able to survive

fertile the ability to produce offspring

Lesson link

The techniques used to determine relatedness between different species are explored in ***lesson 10B***.

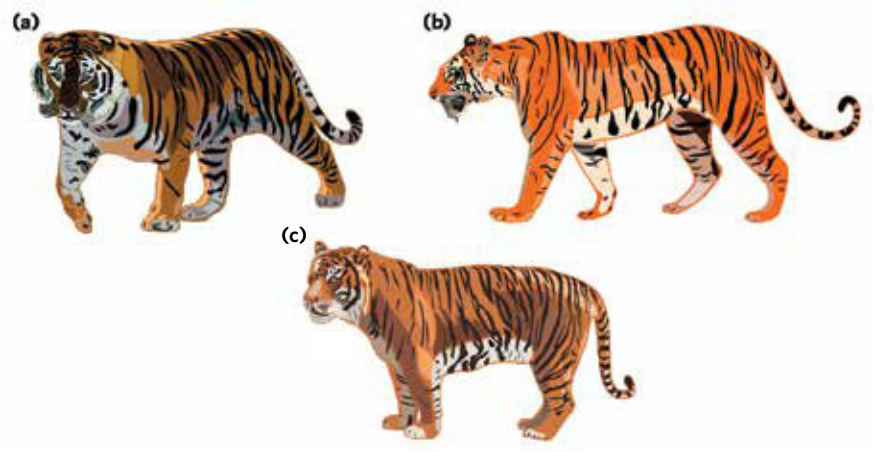


Image: ylq/Shutterstock.com

Figure 1 The **(a)** Siberian tiger (*Panthera tigris altaica*), **(b)** Sumatran tiger (*Panthera tigris sumatrae*), and **(c)** Bengal tiger (*Panthera tigris tigris*) are three examples of tiger subspecies. While they are phenotypically different, they are still capable of breeding with each other.

Isolating mechanisms

The mechanisms which prevent species from interbreeding to produce fertile and viable offspring can be categorised into pre- and post-reproductive isolating mechanisms (also known as pre-zygotic and post-zygotic isolating mechanisms). While these isolating mechanisms are unlikely to be assessed by the VCAA, they will help your understanding of what prevents viable and fertile offspring from forming (Table 1).

Table 1 Pre-reproductive and post-reproductive isolating mechanisms

Isolating mechanism	Examples
Pre-reproductive	Geographical – individuals may not be able to interact with each other due to separation by barriers (e.g. body of water). Ecological – individuals may inhabit different ecological niches or habitats so they do not interact with each other. Temporal – the time of the day or year when individuals are ready to breed may differ. Behavioural – the type of mating behaviours, such as mating call, of individuals may vary. Structural – the physical characteristics of individuals may drastically vary, physically preventing breeding.
Post-reproductive	Gamete mortality – the sperm may be unable to penetrate the ovum for fertilisation. Zygote mortality – fertilisation may occur and a zygote may be formed, however, it will not survive. Hybrid sterility – a viable offspring may be formed and may survive until adulthood, but will not be fertile.

Allopatric speciation 4.2.6.2

OVERVIEW

Allopatric speciation involves the formation of a new species as a result of a geographical barrier.

THEORY DETAILS

Geographical barriers which can facilitate the process of allopatric speciation include the presence of a mountain range or the development of a river. These barriers isolate populations from each other, preventing gene flow between them and allowing genetic differences to accumulate. Genetic differences can accumulate due to the presence of different selection pressures, driving the geographically separated populations towards different phenotypes. Once sufficient differences accumulate, the formation of a new species occurs. The process of allopatric speciation is demonstrated in Figure 2.

geographic barrier a physical factor that prevents gene flow, and thereby stops two populations from breeding together

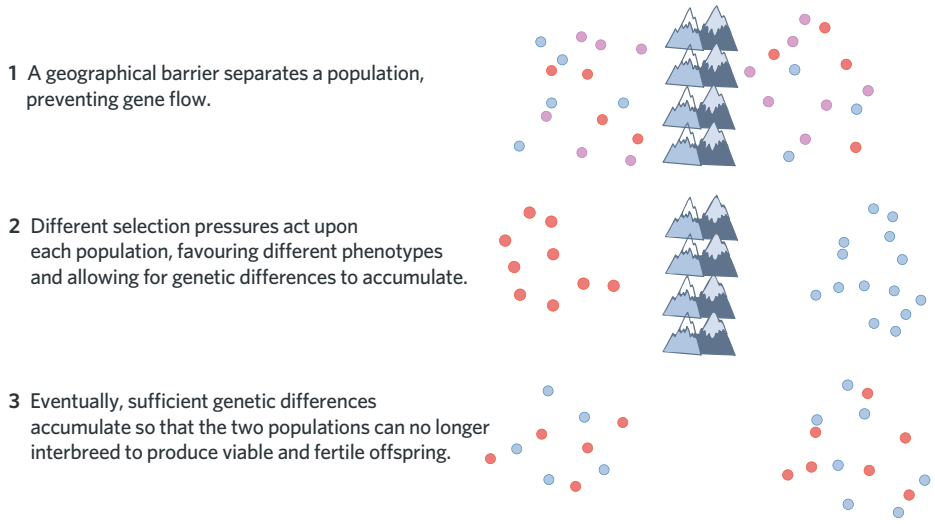


Figure 2 The process of allopatric speciation

Examiners' tip

Students can sometimes struggle to answer short answer questions on allopatric speciation. However, in most of these questions, you simply need to apply the following principles to the scenario provided:

1. Geographical barrier – state that a geographical barrier (e.g. a mountain) has isolated a population (or populations) of the same species from each other, thereby preventing gene flow.
2. Environment – state that the isolated populations are subjected to different selection pressures, allowing for the accumulation of genetic differences.
3. Speciation – explain that once sufficient genetic differences accumulate and the two populations can no longer interbreed to form viable and fertile offspring, a new species has been formed.

Lesson link

If the concept of gene flow has flowed out of your brain, head back to ***lesson 9C*** for a quick refresher.

Galápagos finches

The Galápagos Islands are a collection of 19 islands situated in the Pacific Ocean west of Ecuador. Each of the 19 islands represents a specific **ecological niche**, each with its own different selection pressures and species. Additionally, these islands are separated by the ocean, which serves as a geographical barrier, preventing gene flow between them.

ecological niche the specific environmental conditions and resources or selection pressures within a particular environment

One of the many different organisms that inhabit the Galápagos Islands includes the Galápagos finches, which are also known as Darwin's finches. Currently, there are 18 known species of Galápagos finches, belonging to various genera, with more species constantly being speculated and discovered. These finches have a vast array of beak shapes and sizes, each tailored towards a specific food source. Three of the 18 current species of Galápagos finches are described in Table 2.

Image: Ppito00/Shutterstock.com

Figure 3 The Galápagos Islands

Table 2 Three different species of Galapagos finches

Species	Beak shape	Food source	Image
Cactus finch (*Geospiza scandens*)	Thin and elongated	Cactus finches primarily source their food from cacti, with their thin and elongated beak allowing them to easily pick cactus seeds from cacti without coming into contact with the spikes protruding from the surface of cacti.	 Image: Kjersti Joergensen /Shutterstock.com
Large-ground finch (*Geospiza magnirostris*)	Thick and short	Large-ground finches primarily source their food from hard woody nuts, with their thick and short beak providing them with a significant amount of force to break open and extract the interior of woody nuts.	 Image: spatuletail/Shutterstock.com
Medium-ground finch (*Geospiza fortis*)	Moderate width and short	Medium-ground finches primarily source their food from soft seeds, with their moderate width and short beaks allowing them to only eat soft seeds due to the inability to break open harder seeds.	 Image: Steven Blandin/Shutterstock.com

It has been hypothesised that the formation of these different species of Galápagos finches has largely been a result of allopatric speciation. This is because each of the islands is separated by the ocean, preventing gene flow. Moreover, different islands have different food sources and each island presents its own selection pressures, selecting for different phenotypes (such as beak shape) and allowing for genetic differences to accumulate. Subsequently, once sufficient differences accumulated and viable and fertile offspring could no longer be produced through interbreeding, new species of finches were formed.

Sympatric speciation 4.2.6.3

OVERVIEW

Sympatric speciation involves the formation of a new species in populations located in the same geographical location.

THEORY DETAILS

In contrast to allopatric speciation, sympatric speciation does not rely on the presence of a geographical barrier preventing gene flow. Instead, sympatric speciation occurs within populations sharing the same geographical location, where different selection pressures act on different phenotypes within a population, causing individuals with certain phenotypes to diverge from others and form a new species.

Alternatively, sympatric speciation can also arise from genetic abnormalities that occur during gamete formation, producing **polyploid** variants, which involve differences in the number of sets of chromosomes compared to the original parent (Figure 4). For example, due to an error in meiosis, a diploid ($2n$) parent consisting of two sets of chromosomes may produce a diploid gamete ($2n$), instead of a haploid gamete (n). Therefore, after fertilisation where two diploid gametes ($2n$) fuse together, a tetraploid organism ($4n$) could be produced. If this tetraploid organism can produce viable and fertile offspring, then it would be considered a new species.

polyploidy when an organism contains additional sets of chromosomes in its genome

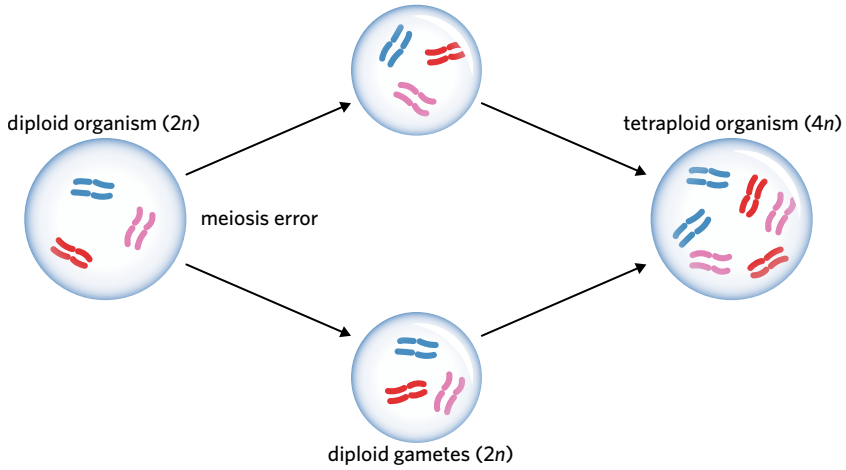


Figure 4 Errors in meiosis can lead to polyploid variants.

While errors in sets of chromosomes in humans and animals often lead to the death of the developing embryo, plants are generally tolerant to changes in their sets of chromosomes. Therefore, sympatric speciation arising from genetic abnormalities is nearly exclusively seen in plants.

Images (left to right): Inga Gedrovicha, Tony Baggett, Natalia Vasylieva /Shutterstock.com

Figure 5 Garden onions (*Allium cepa*) are diploid, keeled garlic (*Allium carinatum*) are triploid, and Chinese chives (*Allium tuberosum*) are tetraploid.

Howea palms

Lord Howe Island is a small Australian island located in the Tasman Sea, 580 km off the eastern coast of Australia. This island has become a place of interest in investigating sympatric speciation, with the *Howea* palms serving as one of the most conclusive examples of sympatric speciation. While there are many different species of palms that inhabit Lord Howe Island, two species of particular interest include *Howea forsteriana* and *Howea belmoreana*.

Image: Tomacrosee/Shutterstock.com

Figure 6 Lord Howe Island

By analysing the distribution of the two species of Howea palms, researchers hypothesised that differences in soil pH were the catalyst for speciation. While *Howea belmoreana* inhabits neutral and acidic soils (low pH), *Howea forsteriana* inhabits a region of alkaline soil (high pH) known as calcarenite. This led researchers to suggest that *Howea forsteriana* diverged from its sister species *Howea belmoreana* after the initial population colonised the alkaline soil, which acted as a selection pressure.

After inhabiting the alkaline soil, physiological differences began to develop. One of these differences included changes in flowering times, which served as a reproductive isolation mechanism. Therefore, after several generations and as differences continued to accumulate, a new species was finally formed once they could no longer interbreed to produce viable and fertile offspring. Additionally, given the relatively small size of Lord Howe Island, it is unlikely that the *Howea* palms were ever geographically isolated from one another, further supporting the idea that the speciation event occurred sympatrically.

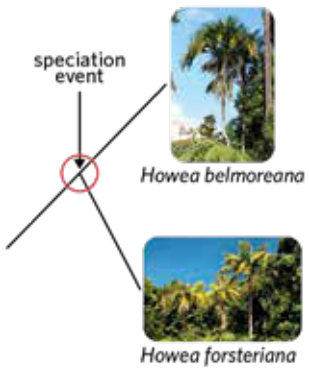


Figure 7 The divergence of the two *Howea* palm species

However, while the evidence supporting the speciation of *Howea* palms is significant, there are still areas requiring further investigation. For example, there are many different possible hypotheses as to the order of events. Instead of the differences in flowering time arising prior to speciation, some researchers have suggested that those differences may have arisen after the speciation event.

Examiners' tip

The process of speciation relies on the presence of mutations, which introduce new alleles and help create variation. In doing so, differences in physiology, behaviour, and morphology can arise. Additionally, the variation created helps facilitate the selection of advantageous phenotypes in response to changing environmental selection pressures through the process of natural selection.

Theory summary

While allopatric speciation involves the presence of a geographical barrier that inhibits gene flow, sympatric speciation can occur in populations existing in the same geographical area. When populations accumulate sufficient genetic differences and can no longer interbreed to produce viable and fertile offspring, a new species is formed.

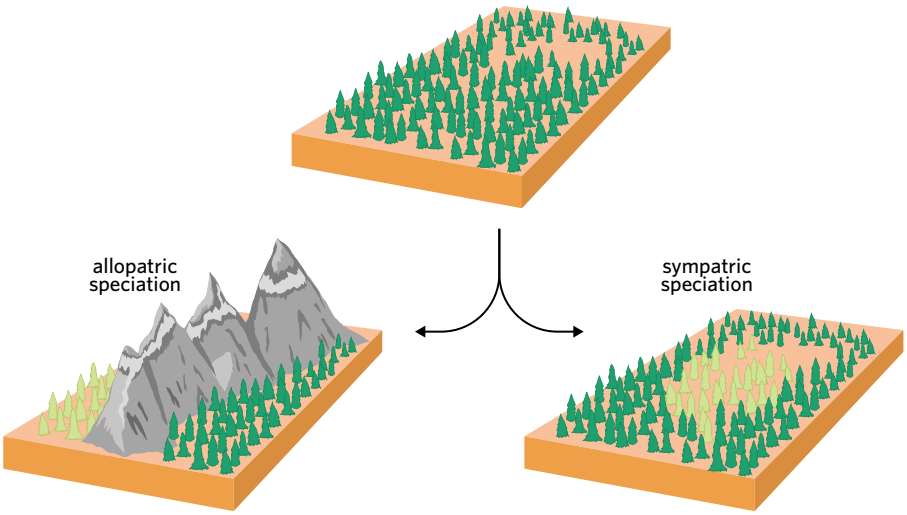


Figure 8 Comparison of allopatric and sympatric speciation

Unfortunately, because ligers and mules are hybrids which cannot produce fertile offspring, they are not considered distinct species. To be considered a distinct species, members of that species would be required to interbreed to produce viable and fertile offspring.

Image: Korawat photo shoot/Shutterstock.com

9D QUESTIONS

Theory review questions

Question 1

Fill in the blanks with the following terms.

- fertile
- viable
- genetic differences
- selection pressures

Speciation involves the formation of a new species. For a population to be considered a new species, it must no longer be able to interbreed to produce _____ and _____ offspring with its original population. The process of speciation largely depends on the presence of _____ which facilitate the accumulation of _____.

Question 2

Which one of the following statements is false?

A Speciation is a result of different selection pressures affecting a species.

B Sympatric speciation does not involve different selection pressures affecting a population.

C Mutations that are involved in speciation create variation between the original and newly formed species.

D The process of natural selection is part of allopatric speciation due to the presence of multiple selection pressures.

Question 3

Order the steps to correctly describe allopatric speciation.

I Genetic changes accumulate over time until the morphology of the populations is significantly different.

II Individuals can no longer interbreed to produce viable and fertile offspring.

III A geographic barrier divides a population, preventing gene flow.

IV Different selection pressures act upon the different populations.

Question 4

Categorise the following statements as **allopatric speciation**, **sympatric speciation**, or **both**.

I The formed species cannot interbreed with the original species to produce viable and fertile offspring. _____

II Gene flow is still possible between the two populations. _____

III Different selection pressures act upon the population(s). _____

IV Natural selection is embedded within the process. _____

V A geographic barrier splits a population. _____

VI Populations are physically isolated. _____

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–9.

Master genes control the expression of other genes by regulating the timing and expression of those genes during embryonic development. For example, early expression of a gene may have a different effect on development compared to late expression and certain genes may only be expressed in certain parts of the body. If mutations occur within master genes, their timing and expression of other genes can be altered, producing significant variation within a population.

In the Galápagos finches, there are two master genes of particular interest:

- *BMP4* produces the bone morphogenetic protein 4 (BMP4) signalling protein which controls beak width. High *BMP4* expression in the upper beak of developing finches results in a wider and deeper beak, whereas low *BMP4* expression results in a thinner and shallower beak.
- *CaM* produces the calmodulin (CaM) signalling protein which controls beak length. High *CaM* expression results in an elongated beak, whereas low *CaM* expression results in a short beak.

The huge variation in the beak shapes of the Galápagos finches has largely been attributed to mutations in these master genes. As a result, a huge variety of phenotypes have been created, allowing the finches to fill specific ecological niches and, over time, form new species.

Question 5

Significant variation can arise due to

A mutations in master genes.

B strong selection pressures.

C sympatric speciation.

D allopatric speciation.

Question 6

A wide and short beak could be achieved by

A an increased expression of *BMP4* and an increased expression of *CaM*.

B an increased expression of *BMP4* and a decreased expression of *CaM*.

C a decreased expression of *BMP4* and an increased expression of *CaM*.

D a decreased expression of *BMP4* and a decreased expression of *CaM*.

Question 7

Provided that the Galápagos Islands are geographically separated by the ocean, the accumulation of mutations within *BMP4* and *CaM* could result in

A sympatric speciation.

B allopatric speciation.

C bottleneck effect.

D genetic drift.

Question 8

If two Galápagos finches with significant differences in *BMP4* and *CaM* expression were to interbreed, they would be expected to produce

A non-viable and non-fertile offspring.

B viable and non-fertile offspring.

C non-viable and fertile offspring.

D viable and fertile offspring.

Question 9

It would be expected that as the number of ecological niches increases, the number of new species would

A significantly decline.

B remain constant.

C decrease.

D increase.

Exam-style questions

Within lesson

Question 10 (8 MARKS)

Currently, 18 known species of Galápagos finches inhabit the Galápagos Islands, which are located 1 000 km off the west coast of South America. Between and within each of these islands, the habitat varies significantly. These finch species are found nowhere else in the world and are thought to have evolved from a single ancestor.

a While some scientists believe that the different species of Galápagos finches are likely to have evolved through allopatric speciation, others have also hypothesised that sympatric speciation may have contributed.

- **i** Outline the process of allopatric speciation in the formation of the Galápagos finches. (3 MARKS)
- **ii** Outline how the process of sympatric speciation may have contributed to the formation of the Galápagos finches. (2 MARKS)

b Explain how scientists could conclude that the populations of Galápagos finches were different species. (2 MARKS)

c When investigating the species that inhabit each island, it was noted that one species may be found on a number of different islands. Suggest how this could have occurred. (1 MARK)

Adapted from VCAA 2001 Exam 2 Section B Q5

Multiple lessons

Question 11 (1 MARK)

Through the formation of the Grand Canyon, a natural barrier was created that divided a squirrel population into two separate populations. As time moved on, the two populations were exposed to different selection pressures and evolved accordingly.

This is an example of

A natural selection.

B bottleneck effect.

C allopatric speciation.

D sympatric speciation.

Question 12 (1 MARK)

There are many similarities and differences between sympatric and allopatric speciation. Which one of the following correctly identifies a similarity between sympatric and allopatric speciation?

A presence of environmental selection pressures

B presence of a geographical barrier

C presence of gene flow

D absence of mutations

Question 13 (9 MARKS)

The Pedra Branca Skink, *Niveoscincus palfreymani*, is a small lizard found only on Pedra Branca Rock, a small craggy island located 26 km off the southeastern coast of Tasmania. Pedra Branca Rock was connected to Tasmania during the ice age, 20 000–15 000 years ago, but has been isolated since as a result of higher sea levels. No living or fossil record of this lizard has been found anywhere else – neither in Tasmania nor on the Australian mainland.

a The Pedra Branca Skink was formed through the process of speciation. Explain how the speciation of the Pedra Branca Skink would have occurred. (3 MARKS)

b Suggest a reason for why the Pedra Branca Skink cannot be found in Tasmania or on the Australian mainland. (1 MARK)

c In 2000, the population size of the Pedra Branca Skink was estimated to be around 476 individuals. Therefore, it has been listed as an endangered species and is at risk of extinction.

i Describe the genetic effect that the ice age would have had on lizard populations. (1 MARK)

ii Explain why a small population size makes the Pedra Branca Skink vulnerable to extinction. (2 MARKS)

iii Describe how new alleles can be introduced into an isolated population. (1 MARK)

iv Suggest what could be done to reduce the chances of extinction of this species. (1 MARK)

Adapted from VCAA 2000 Exam 2 Section B Q6

Question 14 (3 MARKS)

Originally, the North American apple maggot fly would lay its eggs on the fruit of hawthorn trees. However, after European settlement and the introduction of apple trees, it began to inhabit apple trees instead of hawthorn trees. Due to close proximity, individuals that were born in an apple were more likely to breed with other apple-born individuals and individuals that were born in a hawthorn tree were more likely to breed with other hawthorn-born individuals. Over time, the two groups eventually speciated despite the presence of gene flow between each other.

a Identify whether the North American apple maggot fly underwent allopatric or sympatric speciation. Justify your response. (1 MARK)

b Describe the similarities and differences between the processes of sympatric and allopatric speciation. (2 MARKS)

Key science skills and ethical understanding

Question 15 (6 MARKS)

The cattle tick *Rhipicephalus (Boophilus) microplus* are located in tropical and subtropical regions of the world. A study was conducted by a group of scientists to investigate whether *R. microplus* populations in different parts of the world were different species. To do this, they sourced populations from Africa, America, and Australia.

The African population is from Mozambique (MOZ), the American population is from Argentina (ARG), and the Australian population is from Australia (AUS).

By breeding specific individuals from different populations together, the scientists gathered evidence on the viability and fertility of the offspring. The given table shows the results.

Offspring Cross	Viability?	Fertility?
MOZ x MOZ	Yes	Yes
ARG x ARG	Yes	Yes
AUS x AUS	Yes	Yes
MOZ x ARG	Yes	Yes
MOZ x AUS	No	No
ARG x AUS	No	No

a With reference to the data collected, justify whether the Australian and Argentinian populations are the same species. (1 MARK)

b Outline the process of allopatric speciation in the divergence of the Australian population from the Mozambican population. (3 MARKS)

c Scientists conclude that the Mozambican and Argentinian populations are the same species. Suggest one reason why allopatric speciation may not have affected these populations. (1 MARK)

d Suggest one biological implication of introducing a population of Argentinian cattle ticks to Australia. (1 MARK)

9E SELECTIVE BREEDING

There are many different exotic animals around the world including elephants, salamanders, crocodiles, and tigers. Unfortunately, some of these animals express violent tendencies and would be difficult to own safely as pets. But what if there was a way to domesticate them – we've already done it with dogs, which are descended from wolves, so who says we can't do it with these exotic animals? How many years would it take to become best friends with elephants? How many years would it take before you could name your pet tiger Joe?

Image: miroslav chytil/Shutterstock.com

Lesson 9E

In this lesson you will learn how humans can select for particular traits in plants and animals and how this affects a population's genetic diversity.

◀ *Prerequisite knowledge*

Years 7–10
The process of selective breeding involves human-induced selection pressures in breeding for desirable characteristics.

Lesson 9B
Selective breeding shares many similarities with the process of natural selection, including the requirement for variation, the presence of a selection pressure, and the heritability of the trait.

Future applications ▶

Chapter 11
The evolution of hominins, particularly the increase in brain size, allowed for the development and use of selective breeding by the ancestors of modern humans.

Study design dot points

- manipulation of gene pools through selective breeding programs
- biological consequences of changing allele frequencies in terms of increased and decreased genetic diversity

Key knowledge units

Selective breeding	4.2.3.1
The effect of selective breeding on genetic diversity	4.2.2.3

Selective breeding 4.2.3.1

OVERVIEW

Humans can selectively develop desirable traits in plants and animals by altering the breeding process of a population.

THEORY DETAILS

Selective breeding, also known as artificial selection, is the process by which humans can select or remove particular traits from a population by directly controlling the breeding of animals or plants. Selective breeding shares many similarities with **natural selection** including the requirement for variation, the presence of a selection pressure, and the heritability of the trait (Table 1). However, the key difference lies in the origin of the selection pressure (Table 2).

selective breeding the changing of a population's gene pool due to humans altering the breeding behaviour of animals and plants to develop a selected trait. Also known as **artificial selection**

natural selection a mechanism through which organisms that are better adapted to their environment have an increased chance of surviving and passing on their alleles

Table 1 The requirements for selective breeding

Requirement	Description
Variation	Individuals in a population vary genetically, which leads to phenotypic differences.
Selection pressure	Direct human intervention places an artificial selection pressure upon a population of individuals, only allowing certain individuals with **desirable traits** to breed together.
Heritability	The trait selected must be heritable, allowing it to be passed on from the parents to their offspring. Therefore, after the breeding population reproduces, the frequency of the selected allele will increase.

desirable trait a heritable phenotype that humans select for during selective breeding

Table 2 A comparison between selective breeding and natural selection

Mechanism	Selection pressure	Description
Selective breeding	Artificial	Involves human-induced selection pressures in the form of humans directly selecting desirable traits or removing particular traits from a population.
Natural selection	Environmental	Involves naturally occurring environmental selection pressures such as predation, disease, and climate change, which select individuals with a selective advantage within their environment.

An overview of how selective breeding can be used to influence a species is summarised in Figure 1, where a farmer wishes to increase the wool density of sheep.

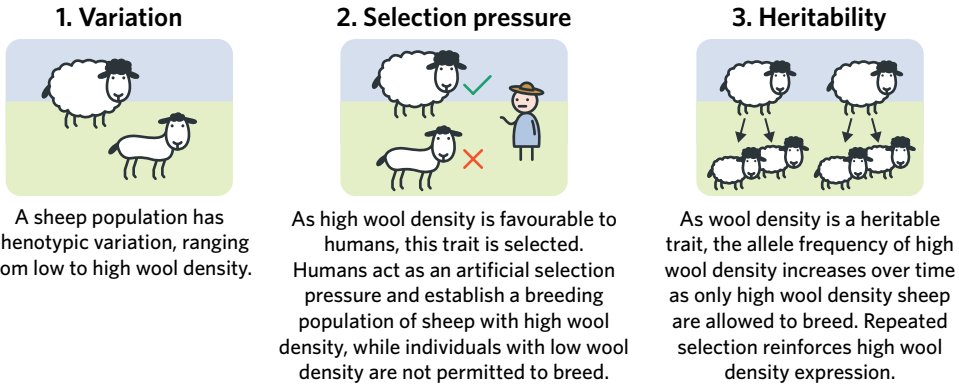


Figure 1 The effect of selective breeding on the wool density of a population of sheep.

Theory in context

RUSSIAN FOXES

Modern dogs are descendants of wild wolves. The evolutionary processes that enabled this transformation have historically been theorised, but rarely demonstrated. The Russian 'domesticated red fox' experiment, ongoing since the 1960s, has gone a long way to developing a deeper understanding of this process. Russian zoologist Dmitry Belyaev argued that behaviour, or tamability, could be selected to domesticate foxes.

To demonstrate this, Belyaev began with a population of wild silver foxes in which he ranked individuals based on traits commonly associated with tameness (e.g. low aggression, affection towards people, etc.). The tamest individuals were bred together, and this was repeated over many generations.

Image: Ondrej Prosicky/Shutterstock.com

Figure 2 Silver foxes *(Vulpes vulpes)*

After ten generations, almost 20% of bred foxes could be categorised as 'domesticated elite', displaying behaviour akin to that of modern dogs. Interestingly, characteristics not selected for were altered in the selection process.
Changes in coat colour, developmental patterns, tail shape, floppy ears, and jaw structure could be seen in some foxes of the population. These phenotypes mirrored some of the traits that distinguish modern dogs from wolves. The exact mechanisms of these shifts have been theorised but not confirmed. It should be noted that effort was taken to ensure inbreeding did not occur.

While the primary method of selective breeding is to simply select for and breed individuals with a desirable trait together, it is also possible to select against an unwanted trait to remove it from the population. An example could be selecting against large body size in a population of fish by overfishing large-bodied fish. Eventually, after many generations, there would only be small-bodied fish left.

Theory in context

MAIZE

Maize (also known as corn) has been a staple crop in agriculture for thousands of years. Nonetheless, the origin of maize puzzled biologists around the world. Relatively recently, it was discovered that maize is a descendant of the wild grass teosinte. Teosinte is generally a poor crop species, but human selection for suitable farming characteristics (soft, large, many kernels, kernel permanence, etc.) over millennia has produced modern maize crops.

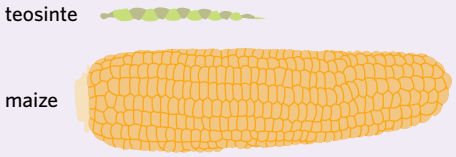


Figure 3 Maize was derived from the ancient teosinte through the process of selective breeding.

The effect of selective breeding on genetic diversity 4.2.2.3

OVERVIEW

Selective breeding can lead to smaller gene pools and overexpression of deleterious alleles, which can reduce adaptability and fitness within a population.

THEORY DETAILS

If poor breeding practices are implemented, selective breeding can cause a human-induced genetic bottleneck. This is because in large populations, only a small percentage of individuals express traits desired by humans. Therefore, by restricting breeding to these individuals, the generational increase in the frequency of the selected allele will decrease genetic diversity as the phenotypes of the population are driven towards a specific allele. For example, in Figure 4, a human selection pressure that favours dark-coloured beetles will increase the prevalence of the dark-coloured allele within the resulting population.

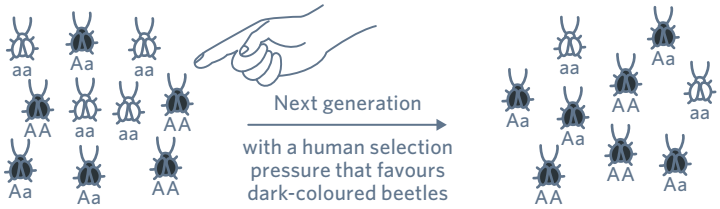


Figure 4 The effect of selective breeding on the gene pool, where the dark colour trait is selected for by humans.

Additionally, reduced genetic diversity can lead to increased inbreeding, which can increase the prevalence of **deleterious alleles**, and a lower **adaptive potential**. These two effects are detrimental to the survival of a population.

Theory in context

DELETERIOUS RECESSIVE ALLELES

If a dominant allele is deleterious, natural selection will act against this allele and remove it from the population over generations. A **recessive allele** can be beneficial, deleterious, or neutral but by its nature is not always expressed. Consequently, a deleterious recessive allele can remain hidden within a population without being selected against. That is until inbreeding leads to high **homozygosity** and expression of these deleterious traits.

It is important to note that the negative effects of inbreeding rarely stem from the expression of one massively deleterious allele, but hundreds of slightly deleterious ones.

Over 100 years, selective breeding has dramatically changed the English bulldog skull shape. Inbreeding has led to the accumulation of deleterious recessive alleles, which have increased the prevalence of respiratory and cardiovascular problems. and poor immune systems in English bulldogs.

Lesson link

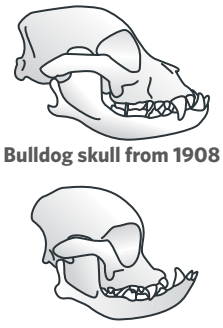
Remember from ***lesson 9B*** and ***lesson 9C*** that natural selection, genetic drift, and emigration can also reduce genetic diversity within a population. Reduced genetic diversity always increases the risk of inbreeding and lowers the adaptive potential of populations.

deleterious allele an allele that has an overall negative effect on individual fitness when expressed

adaptive potential the ability for a population to adjust to new environmental selection pressures

recessive allele a trait that can be masked by a dominant allele on a homologous chromosome

homozygous having identical alleles for the same gene on homologous chromosomes



Bulldog skull from 1908

Bulldog skull from 2008

Figure 5 The change in the English bulldog skull shape over a 100 year period

Theory summary

Historically, humans have altered the genomes of different species to express traits that we find desirable. Inbreeding and heavy selection pressures have resulted in not only a large reduction in overall genetic diversity and, therefore, species fitness, but also the overexpression of previously repressed deleterious alleles.

Through the process of selective breeding, it could be possible to reduce aggression within exotic animals such as elephants and tigers. For example, by establishing a population of tigers with decreased aggression it would theoretically be possible to domesticate them after many generations of breeding. Unfortunately, animals such as elephants and tigers have not been domesticated yet, so you might have to wait a bit longer until you can own one as a pet.

Image: NirmalaARTS/Shutterstock.com

9E QUESTIONS

Theory review questions

Question 1

Selective breeding involves the use of an

A environmental selection pressure.

B artificial selection pressure.

Question 2

Categorise the following examples of evolutionary changes over time as an example of **natural selection** or **selective breeding**.

I Humans killing dogs that exhibit high levels of aggression. _____

II Farmers breeding cows that produce large quantities of milk together. _____

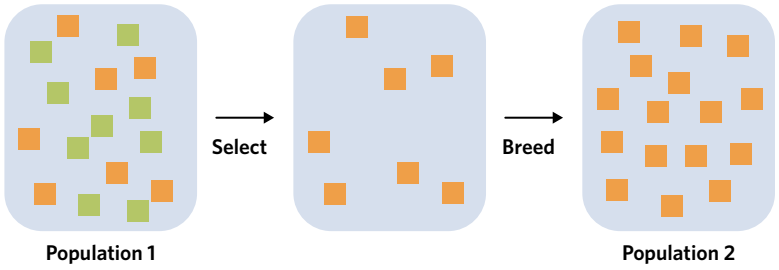
III Due to minimum weight limits within the fishing industry, average fish weights are steadily declining. _____

IV Mussels with thicker shells experience lower rates of predation than thinner-shelled mussels after the intentional human introduction of sea stars that prey on the mussels. _____

V Following the industrial revolution, white peppered moths experienced high rates of predation due to the release of black smog covering trees. _____

Question 3

The following diagram represents a large, randomly reproducing population of wild coloured squares. Humans have isolated a small percentage of the orange squares and allowed them to reproduce.



Which one of the following statements is most likely to be true?

A Population 2 has a higher resistance to environmental change than Population 1.

B Significant inbreeding has affected the gene pool of Population 1.

C Population 1 has greater allelic diversity than Population 2

D Natural selection acted strongly on Population 2.

Question 4

Scientists are trying to replicate the evolution of ancient teosinte to modern corn. Teosinte used to have few and extremely hard kernels, while modern corn has soft kernels. Which one of the following actions would the scientists not have conducted?

A Breeding individuals with alleles associated with the expression of soft kernels together.

B Observed seemingly random changes for which they had not selected.

C Randomly bred individuals with each other over many generations.

D Repeated the selection process over many generations.

Question 5

Which one of the following is not an effect of inbreeding?

A lowered ability to adapt to changes in an environment

B increased genetic diversity within a population

C high prevalence of identical alleles

D expression of deleterious alleles

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 6–10.

The Melbourne Cup is one of Australia's most famous annual horse races, attracting crowds over 80 000, with the winning horse taking eight million dollars in prize money. Preparation for racing at the Melbourne Cup can often take several years, as teams attempt to produce the fastest horses. But are there any special tricks they can use? How do they increase the speed of their horses?

Selective breeding is a technique used by horse breeders to produce the fastest horses. By breeding fast horses together, breeders can produce horses that are even faster. These days, with the rise of genetic testing, a horse's genes are often tested before it is bred with another to ensure that it contains the alleles for features that increase running speed. Through genetic testing, it is even possible to find out how fast a horse will be before it begins its racing career and to determine whether it will be a winner.

Recently, there have been many concerns raised surrounding the use of selective breeding. For example, some argue that it is unethical to breed horses simply for racing as it involves manipulating them for human needs, where the horses are simply considered as property rather than being treated as animals of value. This denigration of their status has been reinforced by the U.S. Supreme Court, which overturned a decision that prohibited the patenting of living organisms, now allowing living organisms to be patented.

Question 6

To produce faster horses, breeders can use the technique of

A selective breeding.

B natural selection.

Question 7

The breeding of racehorses would involve

A genetic testing for specific genes.

B random mating in the wild.

Question 8

Genetic diversity among racehorses is likely to be

A high, due to the presence of genetic drift.

B low, due to the presence of inbreeding.

Question 9

Based on the bioethical concept of respect, selective breeding should be

A banned due to its lack of consideration of the value of animals.

B allowed as harm is not inflicted upon the animals being bred.

Question 10

Based on the bioethical concept of non-maleficence, the patenting of living organisms should be

A banned because it encourages mistreatment and experimentation on animals.

B allowed because it protects the intellectual property of their creators.

Exam-style questions

Within lesson

Question 11 (1 MARK)

Humans selectively bred ancient maize to produce modern corn. Which one of the following would not be a desired trait?

A high number of kernels per cob

B genetically identical plants

C soft kernel covering

D pest resistance

Question 12 (1 MARK)

Which one of the following statements is an example of selective breeding?

A sharks feeding on slow fish, selecting for faster fish

B conservation programs focused on increasing the size of a population

C farmers breeding the sweetest apples together to produce sweeter apples

D whales migrating from cold Antarctic waters to warmer waters near the equator

Multiple lessons

Use the following information to answer Questions 13 and 14.

Native to South America, wild yams are thought to be the ancestor to modern potatoes, after thousands of generations of breeding by farmers. The Great Famine (also known as the Irish Potato Famine) was notorious for demonstrating the potential effect of a single pathogen on a crop species, where virtually all potato crops were destroyed by the pathogen.

Question 13 (1 MARK)

Biologists have suggested that the most likely reason the pathogen had such a large impact is that

A during winter, sunlight availability was reduced, preventing photosynthesis and growth of potatoes.

B cultivated potatoes have a high susceptibility due to low genetic diversity.

C a mutation in the potato genome disrupted B cell production.

D the high pathogen mutation rate facilitated rapid evolution.

Question 14 (1 MARK)

The production of modern potatoes from wild yams is most likely due to

A selective breeding.

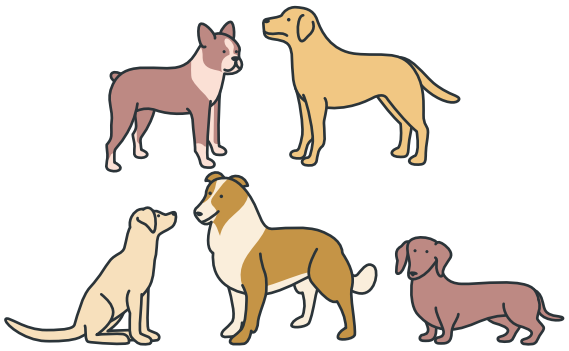
B natural selection.

C founder effect.

D genetic drift.

Question 15 (1 MARK)

The diagram shows five different breeds of dog that now exist within the *Canis lupus familiaris* species.



The development of variant breeds within *Canis lupus familiaris* is an example of

A an increase in the mutation rate due to environmental change.
B genetic drift within isolated *Canis lupus familiaris* populations.
C natural selection in different populations.
D selection acting on desired traits.

Adapted from VCAA 2014 Section A Q38

Question 16 (6 MARKS)

All modern dogs are descendants of ancient wolves. Ancient wild wolf populations were large and genetically diverse. Some of the wolves displayed lowered levels of aggression and a greater ability to become domesticated. Through selective breeding, a domesticated dog population was formed.

a Outline the process of how selective breeding was used to form a domesticated dog population. (2 MARKS)
b Describe the difference between forming a domesticated dog population and randomly mating wolves. (1 MARK)
c While selective breeding is a very useful tool, it can often produce unintended consequences.
  i Describe two potential unintended consequences of selective breeding. (2 MARKS)
  ii Explain the effect of selective breeding on genetic diversity. (1 MARK)

Question 17 (7 MARKS)

Selective breeding is a technique that has been used by farmers for centuries. Farmers discovered that by breeding animals with similar characteristics, they would be able to increase the frequency of those particular characteristics in their animals. For example, to increase milk production, farmers bred cows that produced higher amounts of milk together.

a One requirement of selective breeding is the presence of variation within the initial population.
  i Identify two ways natural variation can exist in a population. (2 MARKS)
  ii Given that the gene coding for milk production is the same for all cows, explain how variation in milk production arises. (1 MARK)
b Over time, selective breeding can lead to speciation. Describe how farmers could determine if a new species is formed. (2 MARKS)
c Farm animals which have been selectively bred are often not suitable for survival in the wild. If a small population of farm animals were released on an isolated island, explain why they would be unlikely to survive. (2 MARKS)

Key science skills and ethical understanding

Question 18 (8 MARKS)

Jean-Baptiste Lamarck proposed that offspring inherit the acquired characteristics of their parents. August Weismann, a prominent 19th-century evolutionary biologist, disagreed with Lamarck and performed an experiment to test Lamarck's theory.

In the experiment, Weismann cut the tails off 68 mice and allowed them to breed. He then cut the tails off all their offspring and bred these mice with each other. After 5 generations, Weismann observed no change in tail size or shape in any of the mice.

a If Weismann wished to form a population of mice with long tails, describe how he could achieve this. (2 MARKS)
b What conclusion can be drawn from the results of Weismann's experiment? (2 MARKS)
c A group of scientists have designed an experiment to generate a population of mice with stunted or missing tails. They use selective breeding to generate this mouse population.
  i State the hypothesis of their experiment. (1 MARK)
  ii This second experiment didn't feature a control group. What would have been the experimental conditions of the mice in a control group? (1 MARK)
d Describe how the bioethical concept of non-maleficence would have influenced the designing of Weismann's experiment. (2 MARKS)

9F EVOLVING PATHOGENS

Every April, just before flu season, people begin rolling up their sleeves to receive the vaccination against the influenza virus. You should remember that after we receive a vaccination, we develop an adaptive immune response against the antigens present in the vaccine, generating memory cells which grant us immunological memory against those antigens. Why then, do we have to receive the flu shot every year? Why can't we just receive the flu shot once and rely on our memory cells?

Image: BEAUTY STUDIO/Shutterstock.com

Lesson 9F

In this lesson you will learn about the development of antibiotic-resistant bacteria and how viruses constantly change through antigenic drift and shift.

◀ *Prerequisite knowledge*

Chapter 8
Vaccinations and antimicrobials are often used to combat pathogens. While vaccines are capable of stimulating the production of immunological memory, antimicrobials such as antibiotics and antivirals can directly target pathogens.

Lesson 9B
Medications used against pathogens can act as an environmental selection pressure, thereby conferring a selective advantage to the pathogens which are resistant to the medication.

Future applications ▶

Lesson 10B
Analysing a pathogen's DNA or amino acid sequences can help determine its relatedness to other known pathogens and help track its origin.

Study design dot point

- consequences of bacterial resistance and viral antigenic drift and shift in terms of ongoing challenges for treatment strategies and vaccination against pathogens

Key knowledge units

Bacterial resistance to antibiotics	4.2.4.1
Viral antigenic drift and shift	4.2.4.2

Bacterial resistance to antibiotics 4.2.4.1

OVERVIEW

The inappropriate use and overuse of antibiotics have led to the emergence of antibiotic-resistant bacteria, which poses a significant public health risk due to the difficulty in eliminating them with existing medications.

THEORY DETAILS

Antimicrobial agents play an important role in protecting us from harmful pathogens. Just as we develop new and improved antimicrobial agents against pathogens, however, pathogens themselves are becoming better at fighting back against us. One of the most problematic areas in modern medicine involves the formation of **antimicrobial resistance** where existing antimicrobials are no longer effective.

antimicrobial agent an agent that kills or slows the growth of microorganisms. Examples include antiseptics, disinfectants, antifungals, antivirals, and antibacterial agents

antimicrobial resistance the ability of a microorganism to survive exposure to an antimicrobial agent

Antibiotic resistance

The formation of antibiotic-resistant bacteria can be attributed to the process of natural selection, where the exposure to antibiotics serves as an environmental selection pressure. If bacteria with resistance to a particular antibiotic are present in the exposed population, then they will be conferred a selective advantage, allowing them to continue living and replicating within their host and increasing the allele frequency for antibiotic resistance (Figure 1). Conversely, those bacteria which are susceptible to the antibiotic are killed by the antibiotic. Additionally, bacteria are also able to exchange genetic material with each other through a process known as **bacterial conjugation**, further spreading the alleles for antibiotic resistance.

bacterial conjugation the process in which bacteria exchange genetic material via direct cell-cell contact

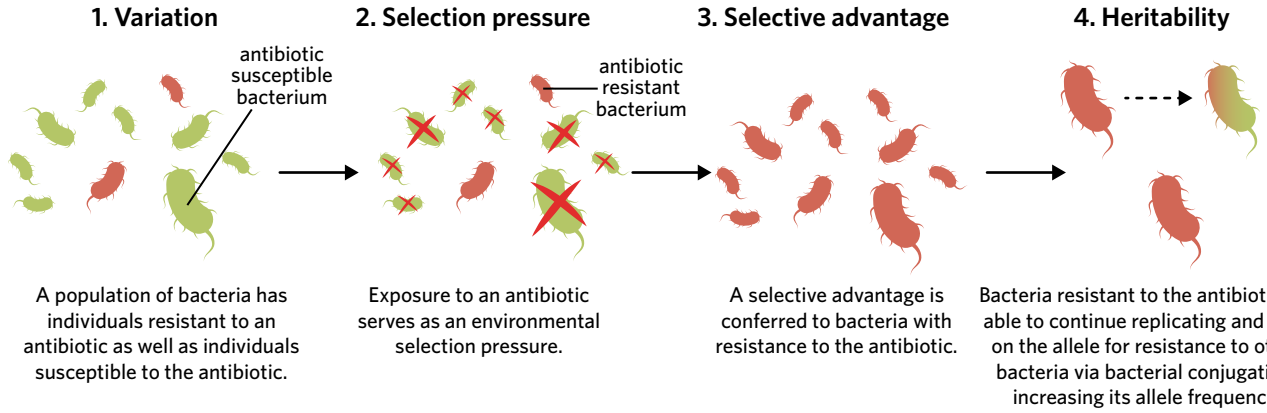


Figure 1 The development of antibiotic-resistant bacteria

Examiners' tip

The evolution and development of antibiotic resistance is an example of natural selection. Antibiotics do not cause bacteria to evolve resistance. Rather, resistance to certain antibiotics already exists within the population. This allows bacteria with genes conferring antibiotic resistance to have a higher chance of surviving in an environment that has exposure to that antibiotic compared to bacteria that don't have those genes.

When asked about the development of antibiotic-resistant bacteria, just like questions regarding the process of natural selection, you simply need to follow the same basic answer structure – (1) outline that variation exists, (2) identify the presence of a new selection pressure (exposure to antibiotic), (3) identify the group that is conferred an advantage, and (4) highlight the increased heritability of the antibiotic-resistant alleles.

Lesson link

From ***lesson 8C***, you should remember that antibiotics are used to treat bacterial infections by targeting specific biochemical pathways or components unique to bacteria.

Variation and the emergence of new alleles conferring resistance against antibiotics is facilitated largely by **mutations**. Through mutations, new alleles can help bacteria develop mechanisms which increase their ability to combat the action of antibiotics. Some of these mechanisms, such as the impermeability of an antibiotic due to a modified cell wall or the active efflux of an antibiotic out of a bacteria, are summarised in Figure 2.

mutation a permanent change to a DNA sequence

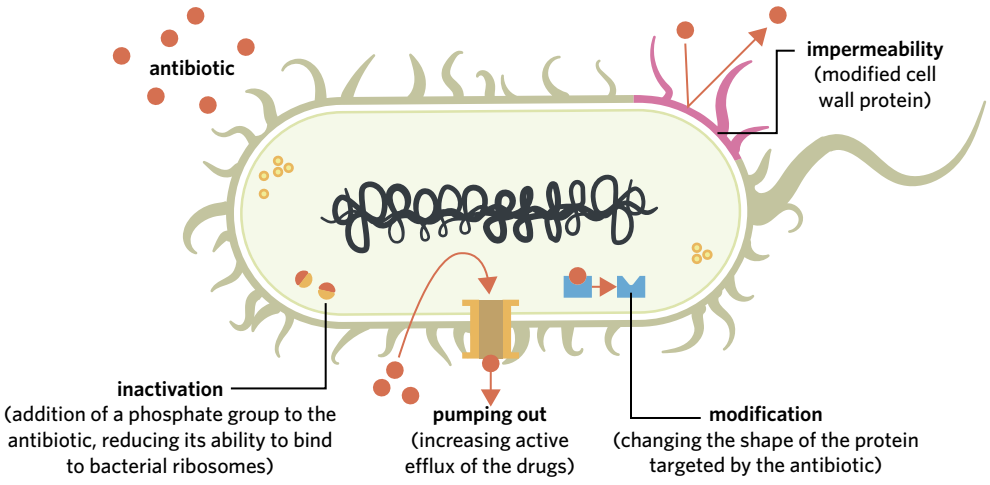


Figure 2 Mechanisms of antibiotic resistance in bacteria

The rapid emergence of antibiotic-resistant bacteria is becoming a significant public health issue, especially when it can take a considerably large amount of time for researchers to develop new antibiotics that fight against these antibiotic-resistant bacteria. When bacteria become resistant, existing antibiotics are no longer effective, which increases the difficulty of treating infections caused by these bacteria. There are many factors which contribute to the formation of antibiotic-resistant bacteria, including the:

- inappropriate compliance with a treatment plan, where a course of antibiotics is prematurely stopped (e.g. when a patient feels better and believes that continued use of their prescribed antibiotics is no longer required). An incomplete course may not sufficiently eliminate all the pathogenic bacteria present, which allows them to continue replicating within the body and provides them with a greater time to accumulate mutations which may confer antibiotic resistance.
- inappropriate use of antibiotics, where antibiotics are prescribed when they are not required. For example, the prescription of antibiotics for treating viral infections (e.g. the common cold or the flu) can expose the **normal flora** inhabiting the body to antibiotics, which can select for antibiotic resistance.
- widespread use of antibiotics, where the general increased use of antibiotics can increase the probability that an individual prescribed antibiotics will be inhabited by antibiotic-resistant bacteria. This subsequently allows for their selection via natural selection.

normal flora naturally occurring, non-pathogenic microbes present in an organism

When treating new highly resistant strains of bacteria, doctors often use a combination of various different antibiotics with differing mechanisms of action, increasing the chances of destroying the bacteria. For example, combinations of antibiotics may target not only bacterial protein synthesis through the inhibition of bacterial ribosomes, but also the replication of bacterial DNA through the inhibition of bacterial DNA polymerases. This ensures that even if some of the bacteria were resistant against one of the antibiotics, the other would be able to destroy them.

Theory in context

EVOLUTION OF ANTIBIOTIC RESISTANCE

Penicillin, the world's first antibiotic, was discovered in 1928 by Alexander Flemming. At the time of its discovery, penicillin was extremely effective against a wide range of bacterial infections by interfering with bacterial cell wall synthesis. However, shortly after its introduction, penicillin-resistant strains of bacteria were beginning to emerge. For example, a gene encoding for an enzyme known as penicillinase began to surface, conferring resistance against penicillin by breaking its structure and inactivating it. In response, a penicillinase-resistant antibiotic called methicillin was developed. However, once again, resistance soon developed, and methicillin was rendered obsolete.

Today, methicillin can no longer be used due to widespread resistance and penicillin use has also been limited to certain populations and diseases. Highly resistant strains of bacteria such as vancomycin-resistant *Staphylococcus aureus* (VRSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) are hard to treat and infections with these bacteria therefore have extremely high rates of death.

Viral antigenic drift and shift 4.2.4.2

OVERVIEW

Viruses constantly adapt and modify their surface antigens through the processes of antigenic drift and shift, thereby increasing the difficulty of forming effective vaccines and medications against viruses.

THEORY DETAILS

Just like bacteria, viruses are constantly adapting and changing, allowing them to increase their **virulence** and resistance against the immune system and existing medications. In particular, the surface antigens of viruses frequently undergo changes in an effort to avoid detection by immunological memory cells developed from past infection or vaccination on subsequent exposure. In doing so, any medications targeting specific surface antigens on the virus are also rendered ineffective. Therefore, it is extremely difficult to develop effective, long-term vaccinations and medications against viruses. The two mechanisms that contribute to the modification of viral surface antigens include:

virulence the potential of a pathogen or disease to cause serious illness or harm

- **antigenic drift**, which involves small and gradual changes in the genes encoding for viral surface antigens (Figure 3). In the beginning, previous memory cells generated will be capable of recognising these mutated surface antigens. However, as the mutations continue to accumulate, a new subtype of virus can form, which will no longer be recognised by previously generated memory cells.
- **antigenic shift**, which involves sudden and significant changes in the genes encoding for viral surface antigens. This commonly occurs when two or more different strains of a virus combine when coinfecting the same host to form a completely new subtype through a process known as **viral recombination** (Figure 3). Natural immunity to this new virus subtype is likely to be uncommon, making it extremely infectious, with the potential to develop into an **epidemic** or **pandemic**.

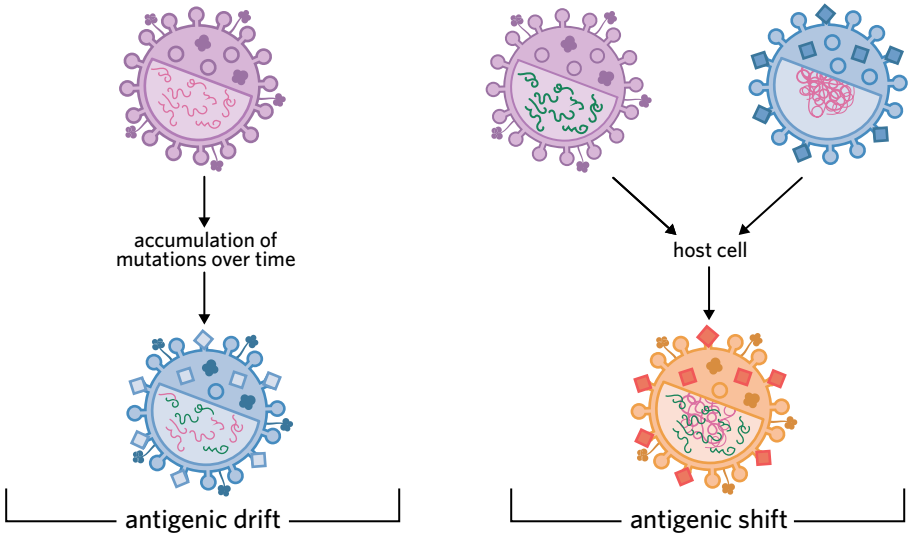


Figure 3 Antigenic drift and antigenic shift

antigenic drift small and gradual mutations in the genes encoding for viral surface antigens

antigenic shift sudden and significant mutations in the genes encoding for viral surface antigens

viral recombination the combination of surface antigens from two or more different strains of a virus to form a completely new virus subtype

epidemic a dramatically increased occurrence of a disease in a particular community at a particular time

pandemic an epidemic that has spread across multiple countries and/or continents

Lesson link

The generation of an adaptive immune response, which involves the formation of immunological memory cells, is explored in ***lesson 7D***.

Theory in context

THE COMMON COLD

While there are many different viruses which can cause the common cold, they are most commonly caused by a group of viruses known as rhinoviruses, which infect the upper respiratory tract. Unfortunately, because of the high ability of the surface antigens on rhinoviruses to constantly mutate, the development of a universal vaccine or antiviral against the common cold is extremely difficult. Therefore, every time you get sick from the common cold, there isn't much you can do except rest and take symptomatic treatment (e.g. cough syrup or paracetamol). This is also the reason why your body is incapable of developing immunological memory against the common cold and why you may get sick every year.

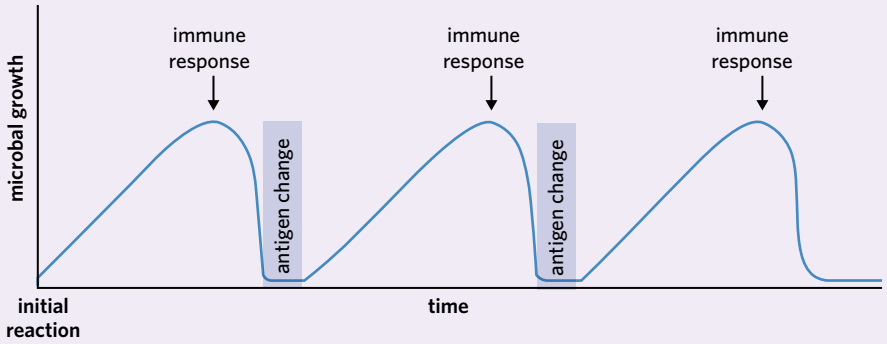


Figure 4 Antigenic drift and antigenic shift

Theory in context

INFLUENZA

In our battle against influenza, also commonly known as the seasonal flu, researchers have identified two surface antigens that can be targeted, haemagglutinin and neuraminidase (Figure 5). When changes occur in either of these surface antigens, the effectiveness of previous vaccinations and existing medications may be reduced, or rendered obsolete.

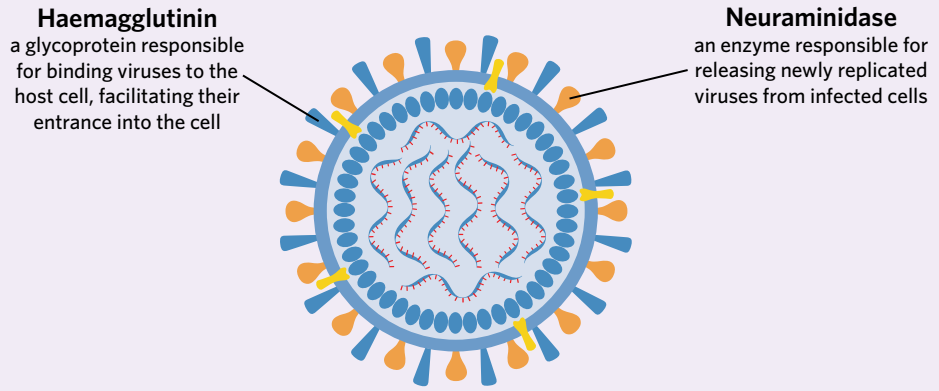


Image: Olga Bolbot/Shutterstock.com

Figure 5 Two surface antigens of the influenza virus – haemagglutinin and neuraminidase

Table 1 The effect of antigenic variation on the influenza virus

Antigenic variation	Consequence
Antigenic drift	When small changes occur in the surface antigens of the influenza virus via antigenic drift, the effectiveness of previously generated memory cells via prior vaccinations will be reduced, until they eventually become ineffective. Therefore, yearly vaccinations against influenza are required in order to maintain immunity.
Antigenic shift	When sudden changes occur in the surface antigens of the influenza virus via antigenic shift, new viral strains can arise through a combination of many different strains. Due to their novel nature, newly created viral strains via antigenic shift are highly infectious. For example, the 2009 swine flu pandemic arose due to the recombination of the classic swine, human, and avian influenza strains (Figure 6).

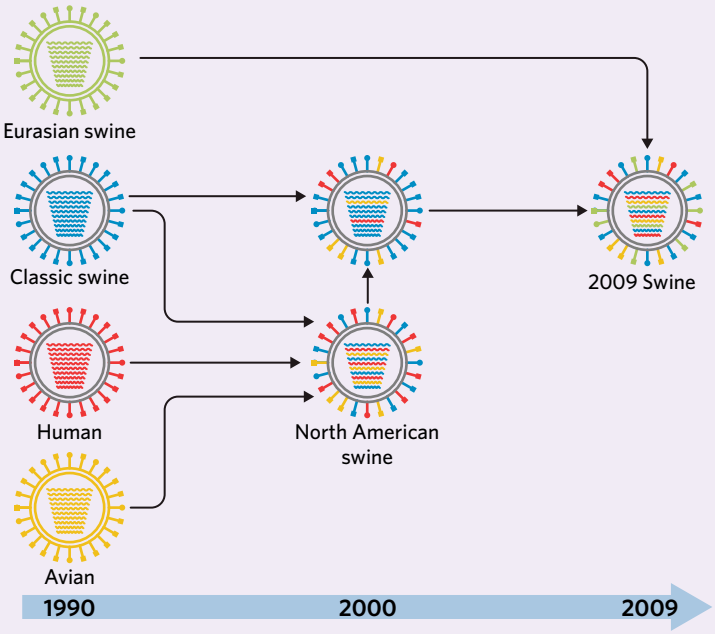


Figure 6 Antigenic shift of influenza virus over time, culminating in the highly dangerous swine flu pandemic in 2009

Theory summary

Antibiotic resistance occurs when exposure to antibiotics serves as an environmental selection pressure and confers a selective advantage to bacteria with existing resistance. Viruses can mutate via antigenic drift and shift, leading to the creation of new surface antigens. Both of these adaptations are significant public health issues, as they make it increasingly difficult to treat bacterial and viral infections.

Unfortunately for us, due to the presence of mutations, the surface antigens present on the influenza virus are able to constantly change from year to year through the process of antigenic drift and occasionally through antigenic shift. This makes previous vaccinations ineffective, requiring yearly vaccinations against the influenza virus.

Image: Mongkolchon Akesin/Shutterstock.com

9F QUESTIONS

Theory review questions

Question 1

Antibiotics

A cause the development of the gene for antibiotic resistance.

B select bacteria with the gene for antibiotic resistance via natural selection.

Question 2

Order the steps to correctly describe the formation of antibiotic resistance.

I The introduction of an antibiotic serves as an environmental selection pressure.

II A selective advantage is conferred upon the bacteria with resistance to the antibiotic.

III A population of bacteria has individuals resistant to an antibiotic as well as individuals susceptible to the antibiotic.

IV Bacteria resistant to the antibiotic are capable of replicating and also passing the allele on via bacterial conjugation, increasing its allele frequency.

Question 3

Changes in the surface antigens of viruses can lead to an increased

A difficulty in developing antivirals.

B effectiveness of vaccinations.

Question 4

Fill in the blanks with the following terms. Terms may be used multiple times or not at all.

- recombination
- antigenic shift
- antigenic drift
- conjugation
- sudden
- small

There are two mechanisms which are involved in modifying the surface antigens of viruses. They include _____, which involves _____ and gradual changes, and _____, which involves _____ and significant changes. _____ can commonly lead to the formation of a completely new strain when multiple viral strains combine in a process known as _____.

SAC skills questions

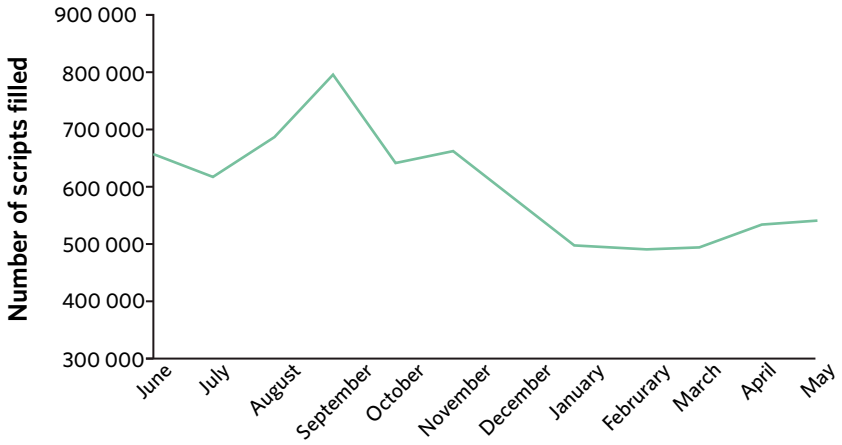
Data analysis

Use the following information to answer Questions 5–9.

In Australia, the overprescription of antibiotics by general practitioners is a significant contributor to the rapid emergence of antibiotic-resistant bacteria. According to Del Mar et al. (2017), some of the more reasonable reasons why a general practitioner may overprescribe antibiotics is that in the early stages of an acute respiratory illness, it can often be difficult to differentiate between a viral or bacterial infection due to the development of similar symptoms, and to determine whether the infection is an indicator of a more serious underlying condition which requires the use of antibiotics.

However, overprescription may also be due to patients' expectations or doctors wanting to speed up the completion of consultations. More often than not, when patients visit their general practitioner, they expect to leave with a prescription or treatment plan to tackle their illness. Therefore, it has been reported that many patients will demand antibiotics even when they've been diagnosed with a viral infection and told that antibiotics are unnecessary. Through the perception that the refusal of antibiotics may be detrimental to the doctor-patient relationship, general practitioners may prescribe antibiotics when they are not required.

The following graph depicts the number of prescriptions for antibiotics monthly between June 2016 and May 2017 from 22 305 general practitioners.



Source: adapted from the Australian Department of Health (2018[b])

Question 5

A reasonable explanation for why a general practitioner may overprescribe antibiotics is that

A in the early stages of infection, it can be difficult to differentiate between a viral or bacterial infection.

B patients demand antibiotics and refuse to leave until they are prescribed.

Question 6

When antibiotics are prescribed for a viral infection

A antibiotic-resistant bacteria may be selected for in an individual's normal flora.

B the replication of the virus can be inhibited.

Question 7

The mechanism by which antibiotic-resistant bacteria are selected for is known as

A natural selection.

B founder effect.

C antigenic drift.

D gene flow.

Question 8

The number of prescriptions for antibiotics in September 2016 is closest to

A 500 000.
B 600 000.
C 700 000.
D 800 000.

Question 9

The increase in antibiotic prescription during the months of June to November can most likely be attributed to

A a sudden outbreak of a bacterial infection.
B an overlap with the yearly flu season.
C a rise in antibiotic-resistant bacteria.

Exam-style questions

Within lesson

Question 10 (1 MARK)

Antigenic shift involves

A the exchange of genetic material with other organisms in a process known as conjugation.
B sudden and significant changes in the genes encoding for viral surface antigens.
C the destruction of previously generated memory cells.
D the development of resistance against antibiotics.

Question 11 (1 MARK)

The rise in antibiotic resistance can be largely attributed to

A the inappropriate use of antibiotics.
B decreasing rates of vaccination.
C antigenic shift.
D antigenic drift.

Question 12 (5 MARKS)

A patient with tuberculosis, which is caused by the bacterium *Mycobacterium tuberculosis*, had a persistent cough, fever, and weakness. The patient was treated with an antibiotic and began to improve after several days. Upon improvement, the patient discontinued use of the antibiotic early.

a After a week of discontinuing the course of antibiotics, the cough and other symptoms returned.
  i Explain the most likely reason why the cough and other symptoms returned. (1 MARK)
  ii Explain how the patient's actions may have contributed to the development of antibiotic-resistant bacteria. (1 MARK)

b Assuming that instead of discontinuing the use of antibiotics prematurely the patient did complete the entire course of antibiotics, explain the most likely reason why the course of antibiotics might not have been successful. (3 MARKS)

Adapted from VCAA 2005 Exam 2 Section B Q6

Multiple lessons

Question 13 (8 MARKS)

Enterobacteriaceae are a family of bacteria that commonly inhabit the gastrointestinal system, including *Escherichia coli*, *Shigella*, and *Salmonella*. When these bacteria become resistant to carbapenem, which is an antibiotic that prevents cell wall synthesis, they become known as carbapenem-resistant enterobacteriaceae.

a Over the past decade, there has been a significant increase in the number of carbapenem-resistant enterobacteriaceae, and in particular, carbapenem-resistant *Escherichia coli* bacteria.

- **i** Describe how the gene for antibiotic resistance could have first arisen in a population of *Escherichia coli* bacteria. (1 MARK)
- **ii** Explain how the increase in prevalence of antibiotic-resistant *E. coli* has occurred. (3 MARKS)
- **iii** Suggest one method to prevent the formation of antibiotic-resistant *E. coli*. (1 MARK)

b Describe how the adaptive immune system could defend against *E. coli*. (3 MARKS)

Question 14 (8 MARKS)

Every year, there is a significant public health campaign in an effort to vaccinate individuals with the influenza vaccine. In order to protect against four different types of influenza, including two influenza A viruses and two influenza B viruses, a quadrivalent vaccine is administered.

a Explain the principles of how vaccinations work. (2 MARKS)

b Explain the purpose of public health campaigns to vaccinate a large proportion of individuals in the community against the influenza virus. (2 MARKS)

c Explain why yearly vaccinations are required against the influenza virus. (2 MARKS)

d Explain how a pandemic caused by the influenza virus may occur. (2 MARKS)

Key science skills and ethical understanding

Question 15 (9 MARKS)

In an effort to investigate the effectiveness of an antibiotic called vancomycin against different strains and species of bacteria, researchers collected nasal swabs from patients with an active infection at a hospital. After collection, they cultured each of the bacterial samples on Petri dishes and applied vancomycin, taking note of the bacteria which were capable of surviving and replicating, and the ones which were destroyed.

a Identify the independent and dependent variables. (2 MARKS)

b Identify two variables which must be controlled in this experiment. (2 MARKS)

c During this experiment, the researchers must take extreme caution to prevent self-infection with the bacteria cultured. Describe two methods of preventing self-infection. (2 MARKS)

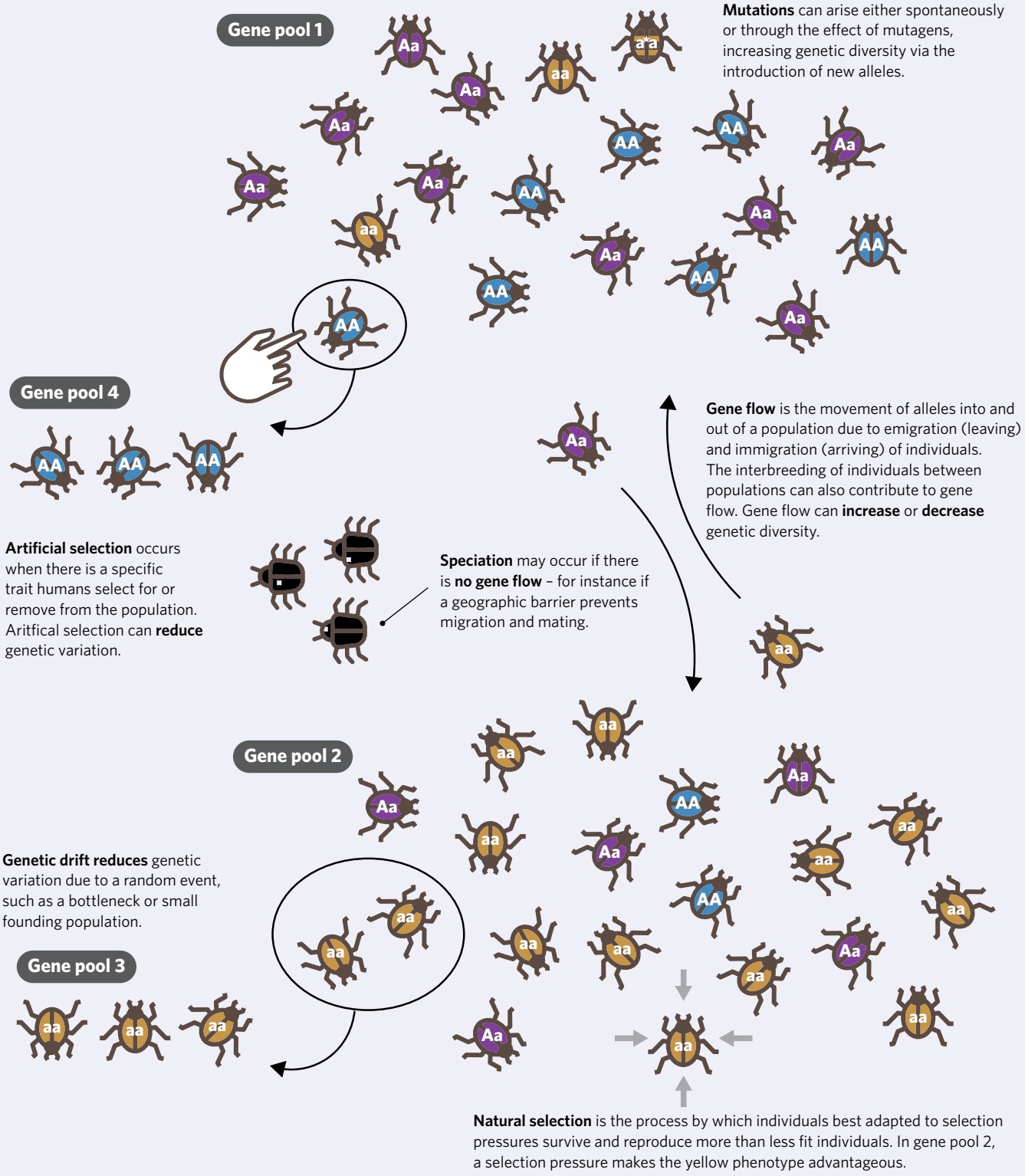
d Suggest a reason why hospitals are often the source of antibiotic-resistant bacteria. (1 MARK)

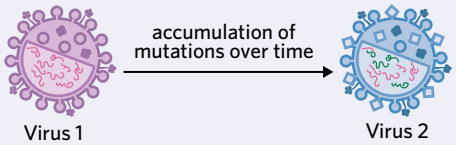
e Describe one potential bioethical issue faced by the researchers in this experiment. (2 MARKS)

CHAPTER 9 SUMMARY

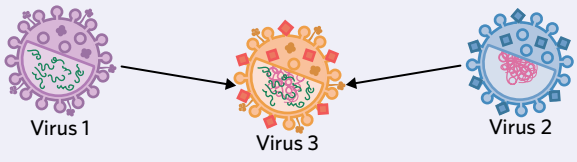
Remember that high genetic diversity means populations have a high **adaptive potential** and a low risk of **inbreeding**.

Factors that affect allele frequencies





Antigenic drift involves small and gradual changes in the genes encoding viral surface antigens.



Antigenic shift involves sudden and significant changes in the genes encoding viral surface antigens.

CHAPTER 9 SAC PRACTICE

SAC skills covered in this section:

✓ Case study analysis ✓ Data analysis ✓ Bioethical deep dive

SALMON GENE POOLS (22 MARKS)

Salmon farming

In Australia, the salmon farming industry primarily occurs in the pristine waters of Tasmania. Although non-native salmon typically live in the cold waters of the North Atlantic Ocean, Tasmanian aquaculturists experience considerable success farming salmon and they argue that the salmon have adapted to the warmer habitat. However, in recent years, environmentalists have begun to question the sustainability and impact on the environment that mass salmon farms have.

For example, in 2021, a debate regarding whether one Australian company should be allowed to build 28 large-scale ocean cages on the eastern coast of Tasmania in Okehampton bay arose. One of the key arguments against constructing these ocean cages relates to its location opposite the heritage-listed Maria Island National Park. Environmentalists and local residents feared that the large quantities of waste produced by the ocean cages (e.g. fish faeces and food) would contaminate the waters surrounding Maria Island.

Additionally, multiple mass death events of salmon within these ocean cages have occurred in recent years. For example, in May 2015, approximately 85 000 salmon suffocated in Macquarie Harbour, Tasmania. During this particular event, a storm surge pushed water that was low in oxygen towards the ocean surface, resulting in the suffocation of salmon within the farms. This led researchers to question the cause of such low oxygen levels, eventually concluding that the huge size of salmon farms and the large number of fish within the harbour were a significant contributing factor.

Image: leo w kowal/Shutterstock.com

1 Identify two arguments against the construction of additional ocean cages in Tasmania. (2 MARKS)

2 Suggest a possible cause of low oxygen levels within Macquarie Harbour. (1 MARK)

3 Describe how the prevalence of Atlantic salmon suited to warmer water may increase over time in Tasmania. (3 MARKS)

4 Describe the effect of mass death events on the genetic diversity of the salmon. (1 MARK)

5 Describe the consequences of mass death events on the survivability of salmon populations in Tasmania. (2 MARKS)

Salmon breeding

In salmon aquaculture, selective breeding is used to increase the growth and size of salmon and increase their resistance to diseases such as amoebic gill disease. In salmon farms, fish spend approximately 10–16 months on land growing in freshwater tanks before being transferred into ocean cages, where they continue to grow before being slaughtered for human consumption.

6 Describe how the process of selective breeding can be used to increase the size of the salmon. (2 MARKS)

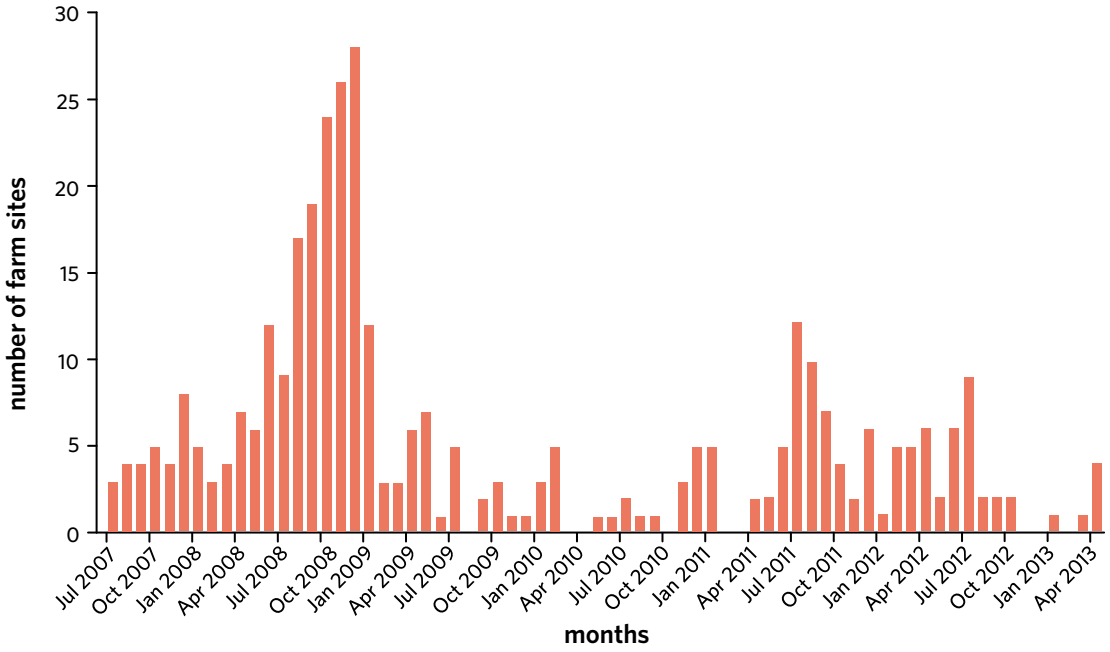
7 Explain the effect of selective breeding on the genetic diversity of the salmon. (1 MARK)

8 Describe how the allele for resistance against diseases such as amoebic gill disease may have first arisen. (1 MARK)

9 Provided that salmon gametes are not able to reach neighbouring cages, suggest whether it is possible for new species of salmon to form, between cages. (2 MARKS)

Salmon diseases

While there are a variety of different pathogens that can infect salmon, one example includes a virus known as the infectious salmon anaemia virus (ISAV), which was first discovered in an Atlantic salmon farm located in Norway. This virus causes anaemia, which involves a deficiency in the number of red blood cells, leading to decreased oxygen uptake and eventually death if untreated. The mortality rate during outbreaks of the infectious salmon anaemia virus varies significantly but can exceed 90% of the total population in severe cases. Unfortunately, there is currently no treatment available against the virus, and therefore, total eradication of a salmon farm is required if an outbreak occurs to prevent the spread of the virus. The following graph depicts the number of confirmed infectious salmon anaemia virus outbreaks in Chilean Atlantic salmon.



Source: Godoy, M et al. (2013)

10 Suggest why a vaccine against the infectious salmon anaemia virus may be extremely difficult to develop. (2 MARKS)

11 Describe the trend within the graph depicting the infection rates of Chilean Atlantic salmon. (3 MARKS)

12 Discuss the relevance of the bioethical concept of non-maleficence with the eradication strategy employed to control the spread of the infectious salmon anaemia virus. (2 MARKS)

CHAPTER 9 EXAM PRACTICE

Section A (10 MARKS)

Question 1 (1 MARK)

New alleles can be introduced into a population by

A artificial selection.
B natural selection.
C genetic drift.
D mutations.

Question 2 (1 MARK)

Which one of the following statements is true about genetic variation?

A Variation decreases a species' chances of survival when affected by an environmental selection pressure.
B Variation increases a species' chances of survival when affected by an environmental selection pressure.
C Variation decreases a species' chances of survival when affected by a random pressure.
D Variation increases a species' chances of survival when affected by a random pressure.

Adapted from VCAA 2014 Section B Q6c

Question 3 (1 MARK)

Random changes in allele frequencies within small populations is known as

A sympatric speciation.
B natural selection.
C genetic drift.
D gene flow.

Adapted from VCAA 2000 Exam 2 Section A Q18

Question 4 (1 MARK)

Which one of the following reasons is unlikely to lead to the development of antibiotic-resistant bacteria?

A overprescription of antibiotics, especially when not required
B discontinuation of a course of antibiotics prematurely
C the use of antivirals against bacteria
D the widespread use of antibiotics

Question 5 (1 MARK)

Selective breeding involves the selection of desirable traits and the breeding of individuals with those desirable traits together. For centuries, farmers have used the process of selective breeding in order to increase the productivity of their farms. Which one of the following statements about selective breeding is incorrect?

A Selective breeding involves the use of an artificial selection pressure.
B Variation must exist in the original population for selective breeding to occur.
C Inbreeding and increased genetic diversity often occur during the process of selective breeding.
D If cows that produce large quantities of milk are bred together, then the average quantity of milk produced will increase over time.

Question 6 (1 MARK)

One of the most detrimental point mutations involves the addition or deletion of a single nucleotide, as this has a significant downstream effect on the gene affected. This type of mutation is known as a

A frameshift mutation.

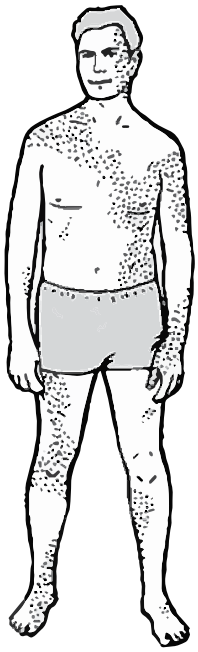
B missense mutation.

C block mutation.

D silent mutation.

Question 7 (1 MARK)

The following diagram shows an individual with 'piebald spotting', a rare autosomal genetic condition. This condition is passed down genetically on an autosomal chromosome.



The mutation must have occurred within the individual's

A germline cells.

B somatic cells.

C skin cells.

D neurons.

Question 8 (1 MARK)

Since the introduction of the poisonous cane toad to Australia in 1935, there has been an increase in the ratio of body length to head size in two species of snakes, the Red-Bellied Black Snake and the Green Tree Snake. A snake with a smaller head cannot consume a large prey item, and also cannot swallow a large cane toad that has sufficient toxin to kill the snake.

Which one of the following statements is false in regards to these two snakes?

A Even small cane toads contain enough toxin to kill large snakes.

B Snakes with larger heads are better at catching and eating cane toads.

C Cane toad toxin acted as a selection pressure on the snake populations.

D Before cane toads were introduced, there was genetic variation in head size in the populations of the two snake species.

Question 9 (1 MARK)

The myxoma virus was introduced to Australia in 1950 to control pest rabbits. The disease is spread by direct contact with infected animals, or by a flea or mosquito vector. In the first two years after release, it reduced the rabbit population from 600 million to 100 million. Now, the virus is less effective, killing only about 50% of infected rabbits.

The cause of increased resistance to the virus is most likely due to

A the virus producing a change in a gene which enhanced the survival of the rabbit.

B the virus producing a change in phenotype which enhanced reproduction of the rabbit.

C a chance mutation in a rabbit gene conferring a survival advantage to some individuals.

D pest controllers successively reducing the levels of virus released into pest rabbit populations.

Question 10 (1 MARK)

Rhinoviruses are a type of virus responsible for causing the common cold. Unfortunately, despite the generation of immunological memory cells, it is possible to be reinfected with rhinoviruses each year, leading to the development of the common cold every year.

This is due to

A genetic shift.

B antigenic drift.

C antigenic shift.

D the Bottleneck effect.

Section B (30 MARKS)

Question 11 (3 MARKS)

The kākāpō (*Strigops habroptilus*) is a species of large, nocturnal, flightless, ground-dwelling parrot native to New Zealand. The total size of the known adult population consists of only around 200 individuals, mostly located on two predator-free islands.

a With reference to selection pressures, suggest why populations of kākāpō are only found on predator-free islands. (1 MARK)

b Suggest how the introduction of a new environmental selection pressure, such as the shortage of a particular food source, may affect the kākāpō. (2 MARKS)

Question 12 (9 MARKS)

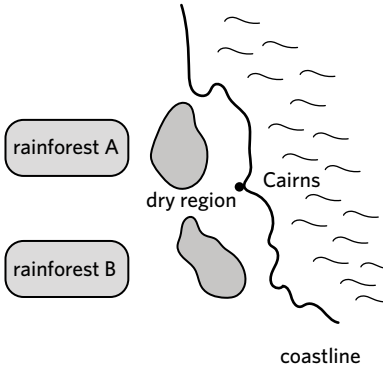
Over the past million years, there have been extended periods where the Earth's climate became cool and dry. During these periods of severe global cooling, the large rainforests broke into smaller, separate rainforests. Populations of plants and animals in these separate rainforests then began to diversify into new species.

a Explain how the separation of rainforests can lead to the diversification of a single species into several new species. (2 MARKS)

b Unfortunately, climate change can also drastically decrease the population size of certain species due to the loss of habitat or food sources.

i Identify the process through which a species' population is drastically reduced by events such as climate change. (1 MARK)

ii Describe the effects of a drastic reduction in population size on the survivability of a species. (2 MARKS)



c During the last extended period of severe global cooling 18 000 years ago, a rainforest located near Cairns was separated into two different rainforests by a dry region.

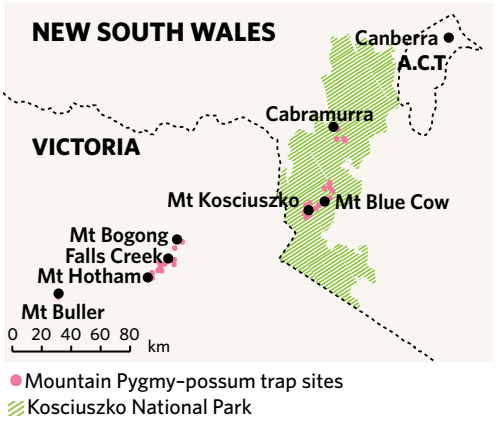
i Assuming that the genetic diversity of a population of lizards inhabiting rainforest B is significantly lower than the genetic diversity of a population of lizards of the same species inhabiting rainforest A, explain how the founder effect may have contributed to this difference. (2 MARKS)

ii Assume that gene flow exists between these two populations of lizards. Explain whether this would prevent speciation from occurring. (2 MARKS)

Adapted from VCAA 1997 CAT3 Article 6

Question 13 (11 MARKS)

The mountain pygmy-possum (*Burramys parvus*) is a highly specialised marsupial with a small population restricted to fragmented subpopulations in the alpine and sub-alpine regions of Victoria and New South Wales (shown on the map). They were thought to be extinct but were rediscovered in 1966.
Since then, many of the known populations have declined.
For instance, on Mt Buller, the population declined from around 350 adults in 1996 to around 40 adults in 2008. Due to ongoing threats, the mountain pygmy-possum was declared 'Critically Endangered' by the International Union for Conservation of Nature (IUCN).

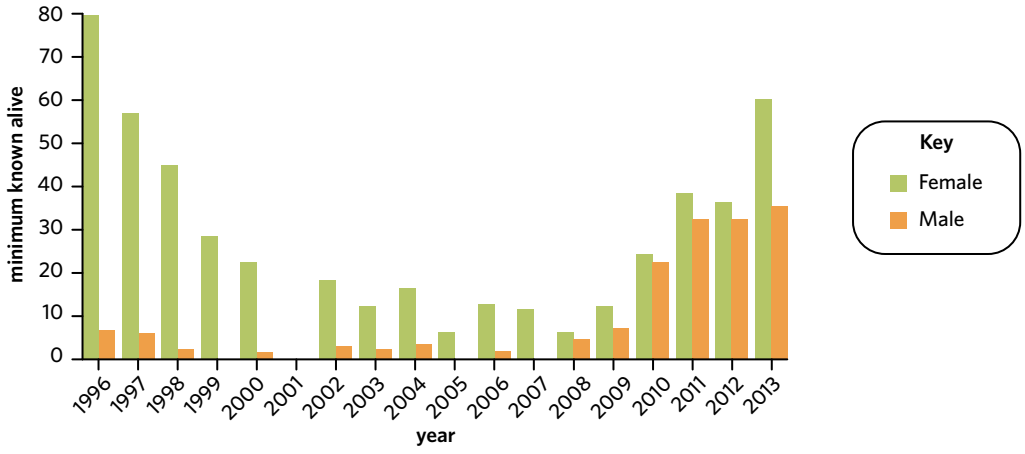


Over the past decades, scientists have surveyed the populations, monitored population size and the number of heterozygous individuals, and established partnerships with nearby ski-resort managers to help raise the profile of the species. Heterozygous individuals are those that contain different alleles at gene loci, increasing genetic diversity.

a Explain why scientists monitored the mountain pygmy-possum population size and determined the number of heterozygous individuals. (3 MARKS)

b Suggest how scientists could tell if gene flow was occurring between the Mt Buller and Mt Hotham mountain pygmy-possum populations. (2 MARKS)

c The figure shows the data that scientists collected from the Mt Buller pygmy-possum population. The figure includes any individuals that were translocated to Mt Buller.



i Describe the trend in male and female pygmy-possum abundance from 1996–2013. (3 MARKS)

ii Over two consecutive years, scientists translocated 12 male pygmy-possums from the Mt Hotham to the Mt Buller population. Identify which years these may have been. (1 MARK)

iii Name the evolutionary process that is being imitated artificially by humans. (1 MARK)

iv Suggest a benefit of the translocation of individuals from the Mt Hotham to the Mt Buller population. (1 MARK)

Question 14 (7 MARKS)

Cephalexin is an antibiotic often prescribed for bacterial infections, preventing cell wall synthesis. Therefore, when bacteria attempt to replicate and divide, due to the inability to properly synthesise a new cell wall, they lyse and are destroyed. However, there are now many bacteria that are resistant to cephalexin.

a Describe how the use of cephalexin contributes to the increase in cephalexin-resistant bacteria. (3 MARKS)

b Just like the prevalence of antibiotic-resistant bacteria is rising, the ability for humans to combat viruses is also becoming increasingly difficult.

i Explain whether cephalexin can be used to combat viral infections. (1 MARK)

ii Describe how new subtypes of viruses can be formed. (2 MARKS)

iii Suggest a possible reason why vaccinations against viruses are incredibly difficult to develop. (1 MARK)

CHAPTER

10

How we are related

10A The fossil record

10B Evidence of relatedness

10C Phylogenetic trees

Key knowledge

- changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transitional fossils, relative and absolute dating of fossils
- evidence of relatedness between species: structural morphology – homologous and vestigial structures; and molecular homology – DNA and amino acid sequences
- the use and interpretation of phylogenetic trees as evidence for the relatedness between species

10A THE FOSSIL RECORD

How might ancient climate change have contributed to the evolution of modern day species? Scientists now believe that the large size of crocodiles and alligators in the 21st century came about as a direct result of the cooling temperatures seen during the Cenozoic era some 60 million years ago! As always, we ask: how can we know this? What evidence have researchers found to support such a wide ranging hypothesis about something that happened so long ago?

Image: vedderman123/Shutterstock.com

Lesson 10A

In this lesson you will learn about the importance of the fossil record, including how it helps us map evolutionary history over time.

◀ *Prerequisite knowledge*

Years 7–10
You learned to interpret different types of evidence for evolution, including a basic introduction to the fossil record and anatomical similarities between different species.

Chapter 9
You learned how species evolve over time, including factors such as selection pressures and genetic drift. This evolutionary process can be understood using the fossil record.

Future applications ▶

Lesson 10B
You will learn how the fossil record can help us demonstrate the relatedness between different species.

Chapter 11
You will learn how the fossil record can be used as evidence of human evolution.

Study design dot point

- changes in species over geological time as evidenced from the fossil record: faunal (fossil) succession, index and transitional fossils, relative and absolute dating of fossils

Key knowledge units

Changes in species over time	4.2.5.1
What is the fossil record?	4.2.5.2
Using the fossil record: relative dating	4.2.5.3
Using the fossil record: absolute dating	4.2.5.4

Changes in species over time 4.2.5.1

OVERVIEW

Life on Earth has changed a lot over the last 4 billion years. Beginning with the emergence of prokaryotes around 3.8 billion years ago (bya) – evolving into eukaryotes 2 bya – to multicellular life around 1 bya – before finally reaching our genus, *Homo*, two million years ago (mya).

THEORY DETAILS

Over the course of Chapter 10 and Chapter 11, we will be travelling back across time and taking a closer look at different trends throughout evolutionary history and the relatedness between different species. This is really an incredible thing to think about. How is it that we are able to sit here today and make assumptions about different organisms and groups of species that lived and died tens of thousands of years ago? The answer, in part, is due to the **fossil record**.

fossil record the information derived from fossils. The fossil record is arranged in chronological order and helps us map the history of life on Earth, placing species in the appropriate geologic time frame

However, before we jump into the fossil record and consider how it is used, it is important to become familiar with the general trajectory of the Earth's evolutionary history and come to terms with a few key events in our geological timeline. Some of these key moments, which are represented in Figure 1, include:

- the emergence of prokaryotes (3.8 bya)
- widespread photosynthesis (2.4 bya)
- the first eukaryotes (2 bya)
- the first multicellular organisms (900 mya)
- the **Cambrian explosion** (535 mya)
- animals on land (530–400 mya)
- mammals (251 mya)
- flowering plants (140 mya).

Cambrian explosion a period (~535 mya) of rapid diversification of multicellular life, characterised by the evolution of hardened body parts such as shells or bones

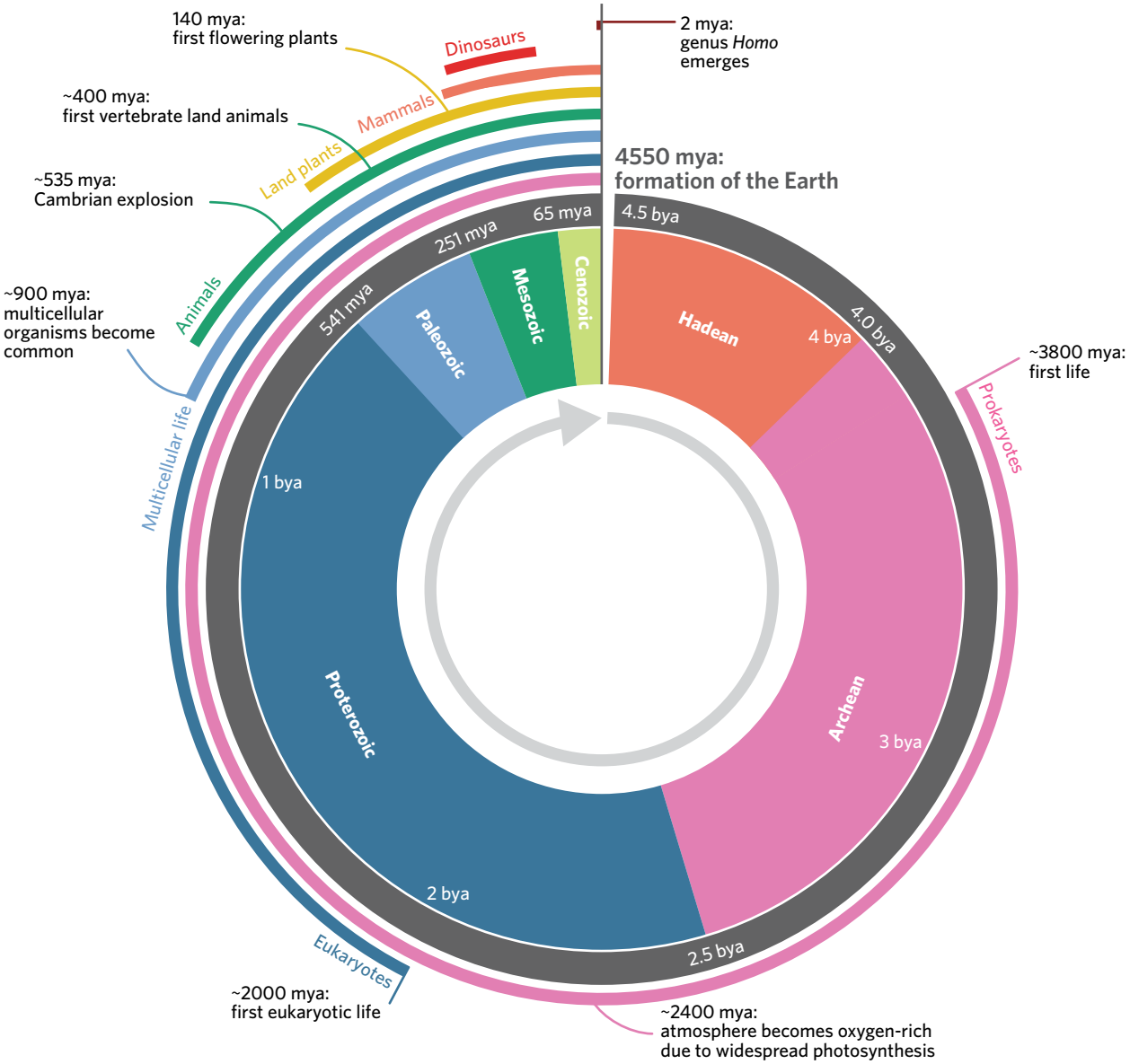


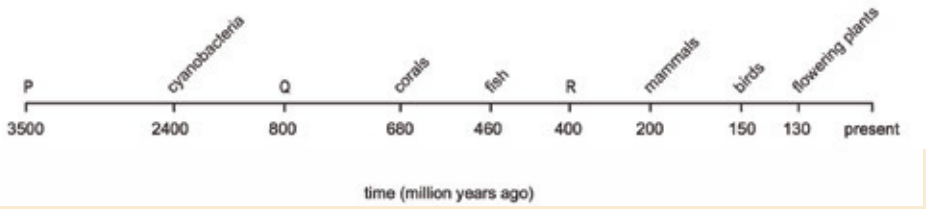
Figure 1 A timeline of the key biological events in the Earth's history, including the rise of multicellular organisms, animals on land, the first flowering plants, and mammals. Note: for the purposes of VCE Biology, it is not necessary to memorise the scientific names of different eras. Instead, this figure is provided to give context to the fossil record by showing when different life forms evolved. *mya = millions of years ago, bya = billions of years ago.

All living things on Earth evolved from a single-celled prokaryote that existed around 3.8 bya. This prokaryote had generations of offspring, some of which evolved the ability to photosynthesise. In turn, the ability to photosynthesise oxygenated the atmosphere, which allowed for organisms that respire aerobically (such as simple eukaryotes) to survive. From here, multicellularity arose and the Cambrian explosion occurred, marking a massive rise in the diversity of living things. It is during this period that almost all of the major animal groups began appearing, including those with hard shells and skeletons. This widespread explosion of complex species across the Earth's surface is where the fossil record comes from, and we are still uncovering more evidence to this day.

Examiners' tip

The VCAA has asked some general questions regarding the overall trajectory of life over geological time. While it is not necessary to memorise the dates and names of species, an understanding of these key biological events can be useful. For example, consider the following prompt from Question 27 (Section A) of the 2018 VCE Biology Exam:

The timeline below summarises the first appearance of some major groups of organisms in the evolution of life on Earth, as indicated by the fossil record. Three major groups are missing from the timeline and are represented by the letters P, Q and R.



Students were asked to correctly identify the groups of organisms labelled P, Q, and R from a list of options. The answer is: P = bacteria; Q = multicellular organisms, and R = insects. Only 58% of students got this answer correct.

What is the fossil record? 4.2.5.2

OVERVIEW

The fossil record provides information about the history of life on Earth. However, fossils are hard to come by as they require certain conditions in order to be created and to last over time.

THEORY DETAILS

Let us now turn our attention to fossils more specifically. Usually, when an animal dies, it is either consumed or its body decomposes completely, leaving no evidence that it ever really existed. But sometimes, given the right set of conditions, the remains of the body can be preserved and form a **fossil**. Ultimately, it is all of these fossils, both discovered and undiscovered, that we refer to as being the fossil record, and it is from this that we are able to study evolutionary changes by comparing living descendants to their long-dead ancestors.

Typically, the process of **fossilisation** occurs when:

1. Remnants of an organism are rapidly covered by **sediment**, meaning that the dead organism is not exposed to oxygen, microorganisms, and other disturbances that would increase its rate of decomposition.
2. Over time, sediment layers build upon each other and compact, layer by layer, until pressure cements them together to form **sedimentary rock** (Figure 2).
3. Within this rock, the fossilised remains can take many forms, including a **permineralised** (Figure 3), **mould**, or **cast fossil**.

fossil the preserved body, impressions, or traces of a dead organism

fossilisation the process by which an organism becomes a fossil

sediment naturally occurring solid material, such as earth and rock, that is broken down into very fine pieces and typically settles at the bottom of liquid

sedimentary rock rock that has formed through the accumulation of sediment that hardens under pressure

permineralised fossil fossil formed when mineral-rich groundwater deposits minerals like silica and calcite into organic material, creating a mineral relic

mould fossil fossil formed when a living thing decomposes underneath sediment, creating a cavity in the shape of the dead organism

cast fossil fossil formed when a mould fossil is filled with sediment

There are also other fossil types like **trace fossils**, which are indirect evidence of an organism's existence, such as their footprints or nests (Figure 4). Additionally, there are examples of fossils that still contain soft tissue yet to decompose, such as human mummies, mammoths frozen in ice, and insects stuck in amber. No matter the fossil type, the conditions that increase the likelihood of fossilisation include:

- physical protection from scavengers and decomposers (e.g. fungi, bacteria)
- areas of rapid sediment accumulation
- constant cool temperatures
- low oxygen availability
- low light exposure.

trace fossil fossil or structure indicating the presence of organisms, rather than the organisms themselves (e.g. nests, footprints, and burrows)

Examiners' tip

In light of these conditions, can you think of an environment likely to facilitate a higher rate of fossilisation? Consider Question 8B (Section B) of the VCAA 2020 Biology Exam:

> Fossils of species of fish are more likely to be found than fossils of land-dwelling animals.
>
> Explain why this is the case with reference to **two** conditions required for the fossilisation of an organism.

As aquatic systems regularly deposit large amounts of sediment, many aquatic animals and plants are preserved more readily than land-dwelling animals. To achieve the two marks on this question, students need to reference two of the conditions we've mentioned earlier. For example:

1 less oxygen, leading to reduced decomposition
2 lower temperatures, leading to reduced decomposition
3 more sediment accumulation, so remains are covered more quickly and are less likely to decompose or be disturbed by scavengers.

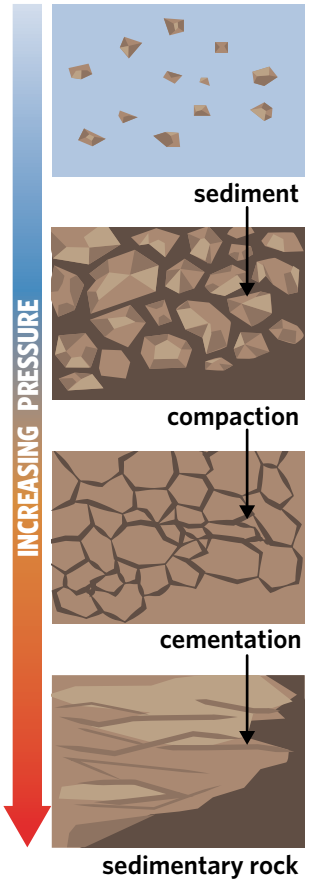


Figure 2 The process of creating sedimentary rock. It is inside this sedimentary rock where fossils are most likely to form.

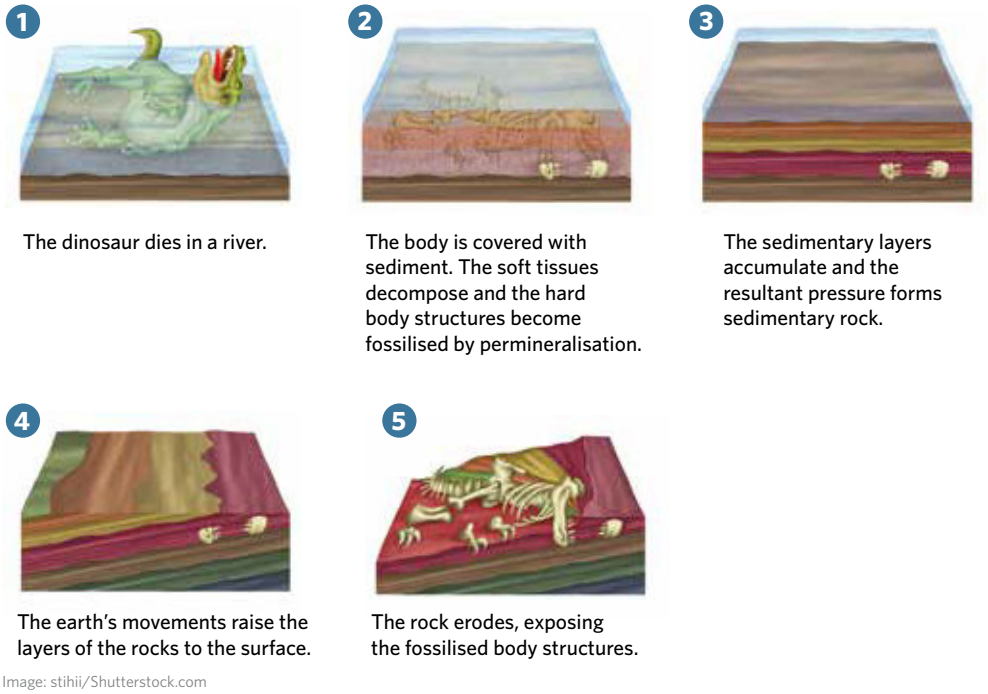


Image: stihii/Shutterstock.com

Figure 3 The process of a dinosaur becoming fossilised via permineralisation after being covered in sediment.

Image: GOLFX/Shutterstock.com

Figure 4 A trace fossil (that is also a cast fossil) showing ancient dinosaur footprints

Using the fossil record: relative dating 4.2.5.3

OVERVIEW

One way in which fossils can be dated is via relative dating, which uses the law of fossil succession to assign a fossil a relative age in relation to other fossils and rock strata (layers). Within the geological timescales that are created, researchers might also use index and transitional fossils, which are particular classes of fossils that help with relative dating techniques.

THEORY DETAILS

Now that we understand what the fossil record is and how fossilisation is facilitated, you might be wondering what it is that the fossil record is actually used for. While this is covered in the next couple of chapters when we look at evolving species over time, at its simplest, the fossil record is used to date fossils. By determining the age of fossils, researchers are able to compare fossils across time and put together a picture of evolution that maps changes to species. Ultimately, there are two main techniques for dating fossils that we will explore in VCE Biology – **relative dating** and **absolute dating**. Let's start by looking at relative dating, which is based on the law of **fossil succession**.

relative dating a dating technique used to determine the relative age of a fossil by comparing its position to other fossils or rock in surrounding rock strata (layers)

absolute dating a dating technique used to determine the absolute age of a fossil by measuring the relative amounts of radioisotopes to their products. Also known as **radiometric dating**

fossil succession the principle that fossils of the same age will be in the same layer of sedimentary rock, and fossils found in a higher or lower sedimentary layer will be younger or older, respectively. Also known as **faunal succession**

relative age the age of a fossil as determined by relative dating techniques. Describes the age of a fossil compared to other fossils, instead of a fossil's exact age in years

The law of fossil succession

The law of fossil succession states that because sedimentary rock is formed by the accumulation of sedimentary layers on top of each other, the fossils closer to the surface must be younger than those that are found below them (Figure 5). This means that we can assign fossils a **relative age** – an approximate age based on the position of the fossil compared to other fossils.

Figure 5 Relative dating is used to assign fossils' approximate ages relative to their positions within different sedimentary strata (layers).

Geological timescales

The law of fossil succession also means that we don't necessarily need to have another fossil to compare against in order to assign our new fossil a relative age. Instead, we only need to be able to determine the age of the rock **stratum** in which the fossil is found. Because the law of fossil succession tells us that sedimentary rock layers develop in chronological order, scientists are able to assign each separate stratum to a particular period of time. As we can see in Figure 6, lower layers are older and known to exist from period X to period Y, before new layers formed on top and existed from period Y to period Z, and so on. We call this aging of rock strata a geological time scale (Figure 6).

stratum (pl. strata) a layer of sedimentary rock

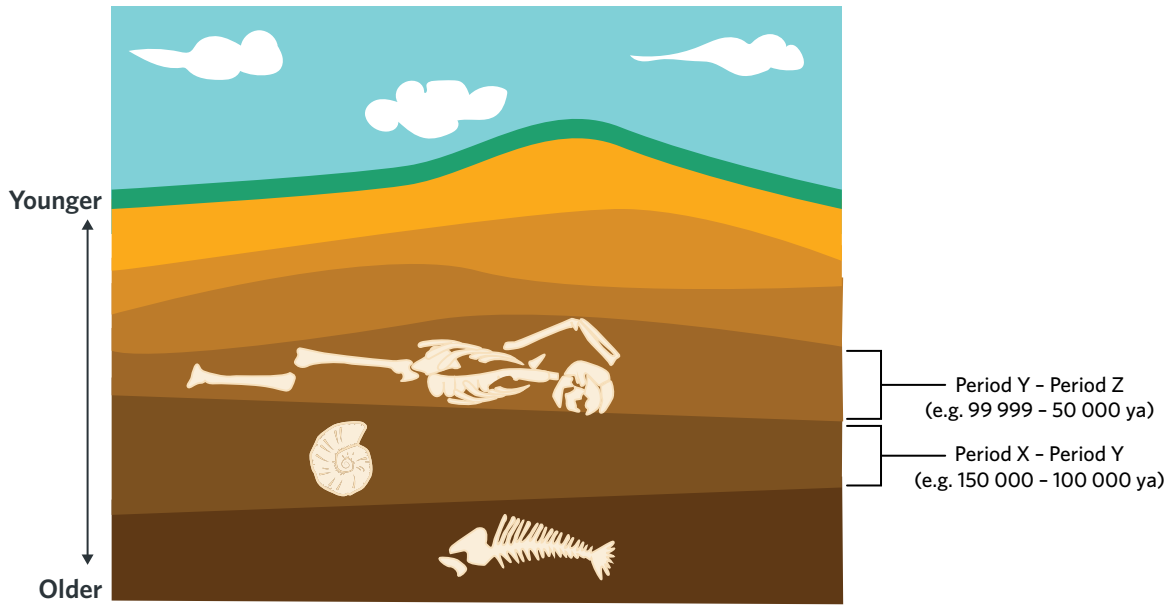


Figure 6 Relative dating makes use of a geological time scale, where different rock strata are given a general period of time and fossils are dated depending on their position in these strata.

Index fossils

Besides geological time scales, researchers can use particular fossils known as **index fossils** to help them determine the relative age of a new fossil. These are fossils that come from an organism that was geographically widespread and existed for a short, but precisely known, period of time. They are useful because they enable researchers to quickly and easily define the relative age of a target fossil. For example, in Figure 7 assume we have found a number of specimens of Fossil X, which existed across a range of countries and has been known to exist between 100 000 to 150 000 years ago. If we found a different fossil, Fossil Y, in the same stratum as Fossil X, researchers could assume that the two fossils are from species that lived within a similar time period and can assign a relative age to Fossil Y of 100 000 to 150 000 years old. If a new fossil is found in strata above or below Fossil X, then scientists can assign a relative age to the fossil.

index fossil a group of widespread fossils which existed for a short period and have a known age. Can be used as a reference to easily determine the age of unknown fossils

For the best index fossils, the species must be:

- physically distinctive
- have had a large population
- have existed in many geographical areas
- only lived within a known short period of time.

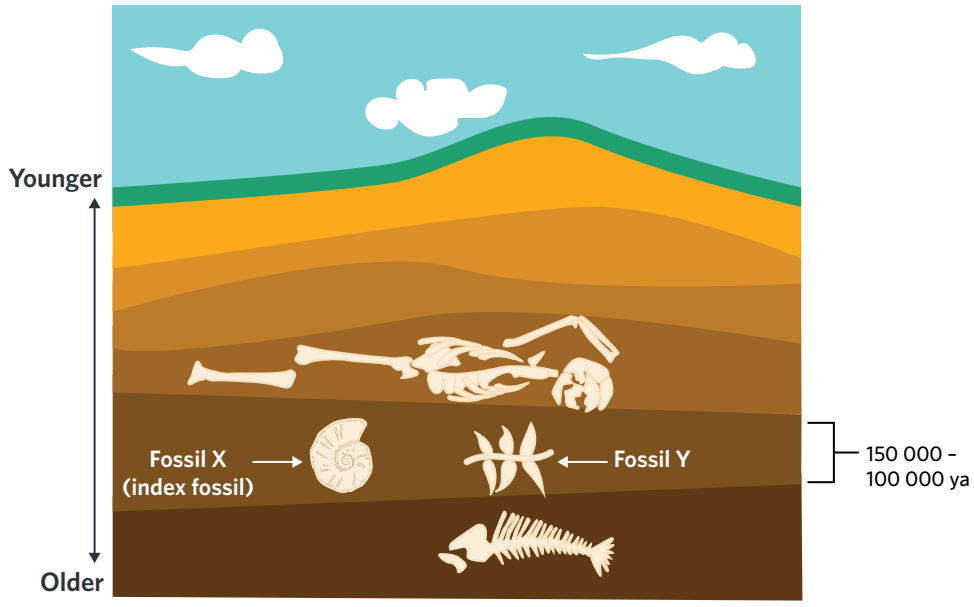


Figure 7 As well as the law of fossil succession and geological timescales, researchers can also use index fossils as a means of establishing the relative age of a fossil. In this case, researchers may infer the relative age of Fossil Y given that it is found in the same stratum as a known index fossil – Fossil X.

Image: Merlin74/Shutterstock.com

Figure 8 Trilobites, which are a class of ancient marine arthropods, are excellent index fossils as each species tended to exist across a large geographical range and over a known time period.

Transitional fossils

Finally, researchers can also use **transitional fossils** to assist with relative dating. Essentially, these are fossils that can be thought of as 'intermediaries' between an ancestral species and a descendant species and are especially useful when these two species are morphologically very distinct. To put it another way, transitional fossils demonstrate the different forms of a particular genus that occurred along that genus' evolutionary pathway.

transitional fossil a fossil that shows traits that are common to both its ancestral group and its descendant group. They are particularly important when the descendant species is physically very distinct from the ancestral species, such that the transitional fossil can help demonstrate evolutionary changes between the two

They are called transitional fossils because they exhibit traits that are common to both an ancestor and its descendants. For example, assume you have a known species – Species X – and another species – Species Z – and you hypothesise that the two species are related. However, in this hypothetical example, the two species look very different and you can't see how Species X might have evolved to look like Species Z. In this case, a transitional fossil – Species Y – is needed, as it can show the link between both of the species due to the fact that it shares similarities to both and shows how certain features might have evolved progressively over time.

Theory in context

THE *TIKTAALIK ROSEAE* – A TRANSITIONAL FOSSIL BETWEEN SEA AND LAND

One terrific example of a transitional fossil is the *Tiktaalik roseae*, which lived around 375 million years ago and demonstrates the transition of vertebrate life from water to land. Discovered in 2004, the Tiktaalik fossil was key in demonstrating how four-legged (tetrapod) creatures first developed the ability to move on land. It was found that the creature shared many similarities with earlier sea-dwelling relatives, such as gills, scales, and fins, but that it also had some key characteristics that linked it to its later land-dwelling descendants – such as bones inside its fins (indicating weight bearing capabilities), a mobile neck, and a strong ribcage. This is an example of how transitional fossils demonstrate the linking characteristics between earlier ancestors and later descendants, especially when the morphological changes are not always immediately apparent.

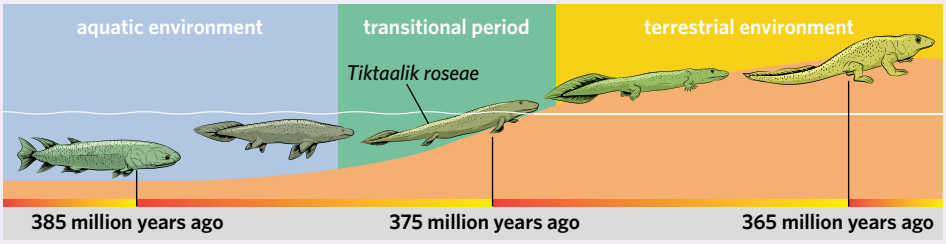


Image: EreborMountain/Shutterstock.com

Figure 9 *Tiktaalik roseae* is an example of a transitional fossil. It shared characteristics with both earlier aquatic descendants and later terrestrial ancestors.

Using the fossil record: absolute dating 4.2.5.4

OVERVIEW

Researchers can also use absolute dating techniques, where the known half-lives of different radioisotopes can be used to measure the absolute age of a fossil.

THEORY DETAILS

Half-life of radioactive isotopes

In contrast to relative dating, absolute dating techniques can be used to calculate the **absolute age** of a fossil in years. While there are many absolute dating techniques, such as luminescence and electron spin resonance, the VCAA generally only assess radiometric dating methods, which is what will be discussed here.

absolute age an estimate of the age (in years) of a fossil or rock

radioactive isotope a radioactive atom of a specific element. This atom breaks down into a predictable and stable product. Also known as a **radioisotope**

Radioactive dating is one of the most commonly used methods for determining the age of fossils and involves comparing the ratio of **radioactive isotopes** (radioisotopes) found inside the fossil to the relatively stable amount found in the atmosphere. For the purposes of VCE Biology, it is enough to know that absolute dating is based on the following three principles:

1 Radioisotopes are unstable elements that will break down over time into a more stable product. For instance, carbon-14 (a radioisotope) will break down into nitrogen-14.

2 While these radioisotopes can break down at any point, on average the rate of breakdown is constant and can be modelled. One of the ways in which we model this breakdown is by calculating the **half-life** of that radioisotope.

half-life the time taken for half the mass of a radioisotope sample to break down into its products

3 Half-life describes the amount of time before half of the mass of a radioisotope is broken down into predictable and stable products. For example, carbon-14 is a radioisotope that has a half-life of 5 730 years. This means that after 5 730 years of an organism's death, half of its carbon-14 atoms will have broken down into nitrogen-14 atoms (Figure 10).

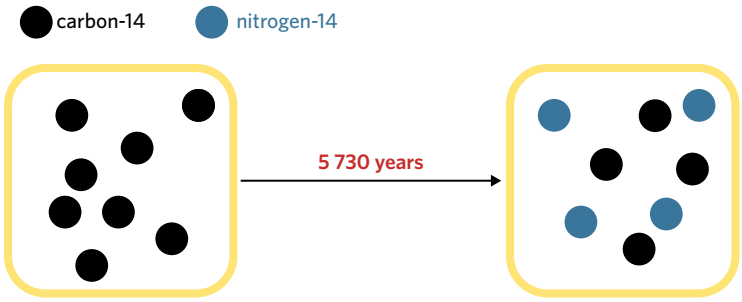


Figure 10 Radioactive dating works on the principle of half-life decay. Here we see that after one half life (5 730 years), half of the amount of radioactive carbon-14 has decayed into its stable product – nitrogen-14.

Scientists can use different radioisotopes to date a fossil. Regardless of the radioisotope used, scientists will measure how much of it is present in the fossil versus how much of its breakdown product is present. This ratio can be used to work out the time that would have needed to have passed to facilitate this amount of breakdown, which is the age of the fossil.

The half-life varies between radioisotopes, as shown in Table 1. Each radioisotope series will have a different **dating period**, simply because some have shorter half-lives than others. This means that some radioisotopes will have broken down completely after a certain period of time, and may not be as useful as others when trying to date much older fossils. Dating period, therefore, refers to the period of evolutionary time for which each radioisotope series is useful. For example, you would not use the carbon-14 dating technique to measure the age of a fossil that is millions or even billions of years old.

dating period the range of time since fossilisation in which a particular radioisotope series can be used. Beyond this period, most of the radioisotope will have broken down into its products, meaning that it is too difficult to estimate the fossil's age

Table 1 Summary of radiometric dating techniques commonly assessed by the VCAA.

Radioisotope series	Half-life	Dating period	Dating of
Carbon-14 – nitrogen-14	5 730 years	1 000 – 50 000 years	Organic materials
Uranium-235 – lead-207	700 million years	1 million – 4.5 billion years (used together with U-238 – Pb-206 dating)	Uranium-containing materials (shells, corals)
Uranium-238 – lead-206	4.5 billion years	1 million – 4.5 billion years (used together with U-235 – Pb-207 dating)	Uranium-containing materials (shells, corals)
Potassium-40 – argon-40	1.3 billion years	100 000 + years	Igneous (volcanic) rocks

Radiocarbon dating

Let's look at a specific type of radioactive dating known as **radiocarbon dating**, which uses the radioisotope series carbon-14 (^{14}C) to nitrogen-14 (^{14}N). The general principles of radiocarbon dating are as follows:

radiocarbon dating a form of absolute dating used to determine the age of a fossil by measuring the properties of radiocarbon, a radioactive isotope of carbon. Also known as **carbon dating** and **radioactive carbon dating**

1 All living things contain carbon. This carbon exists as a ratio of two isotopes – ^{12}C (a stable isotope) and ^{14}C (a radioactive isotope). The ratio of these two isotopes will be the same as the ratio in the atmosphere, given that carbon is constantly being cycled between the organism and its environment while it is alive.

2 When the organism dies, its ^{14}C will begin to decay. This is because ^{14}C is radioactive and breaks down into ^{14}N (a stable isotope). While this decay occurs, the carbon in the dead organism will not be replaced by existing carbon in the atmosphere. As such, levels of ^{12}C (a stable isotope) will remain the same, while ^{14}C decays at a known rate – causing the ratio between the two isotopes to change.

3 At any point, scientists can measure the amount of ^{14}C present in the fossil and determine how long ago it died. This is done by comparing the $^{14}C : {}^{12}C$ ratio in the fossil to the ratio of $^{14}C : {}^{12}C$ in the atmosphere. The longer ago the organism died, the less ^{14}C will be present (having broken down into ^{14}N).

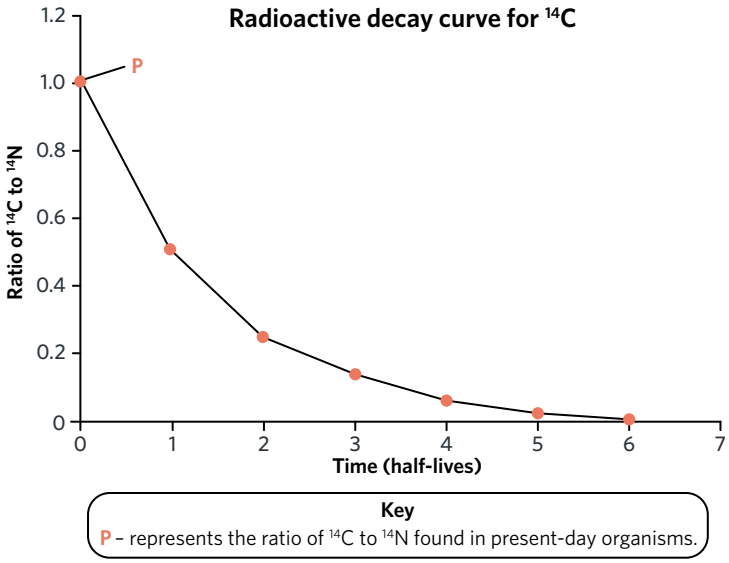


Figure 11 The age of a fossil can be determined based on the ratio of carbon-14 (the radioisotope) to nitrogen-14 (its stable product).

If we consider Figure 12, we see that when an organism dies, its levels of ^{12}C remain constant, while levels of ^{14}C decay at a steady and known rate. The ratio of both can be measured at any time and assessed against the corresponding levels in the atmosphere to determine the fossil's age.

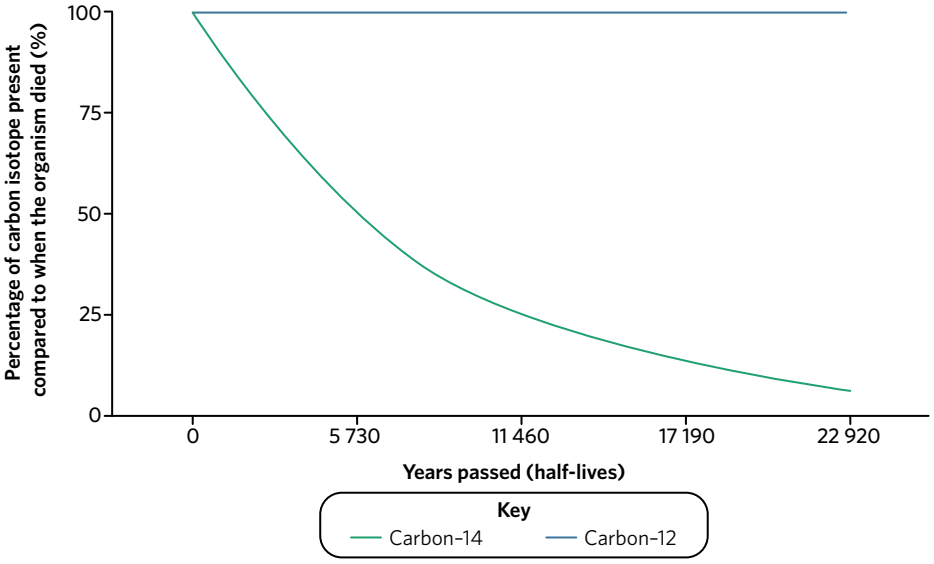


Figure 12 How ^{14}C and ^{12}C decay over time. Note: on the x-axis, half-lives are given in years.

Theory summary

We know that life has developed over billions of years, from early single-celled prokaryotes to us modern day humans in the 21st century. However, exactly how this occurred, and the details of this development, are still being uncovered today as we find more and more fossils from the fossil record. But finding fossils isn't always easy, as not all dead organisms fossilise. The conditions that increase the likelihood of fossilisation include:

- physical protection from scavengers and decomposers (e.g. fungi, bacteria)
- areas of rapid sediment accumulation
- constant cool temperatures
- low oxygen availability
- low light exposure.

Being able to date fossils is important as it allows us to map different changes to species over geological time. Two ways we date fossils include relative dating techniques, which assign the fossil a relative age, and absolute dating techniques, which assign the fossil an absolute age in years. Some of the key principles and tools that go into these two techniques are summarised in Figure 13.

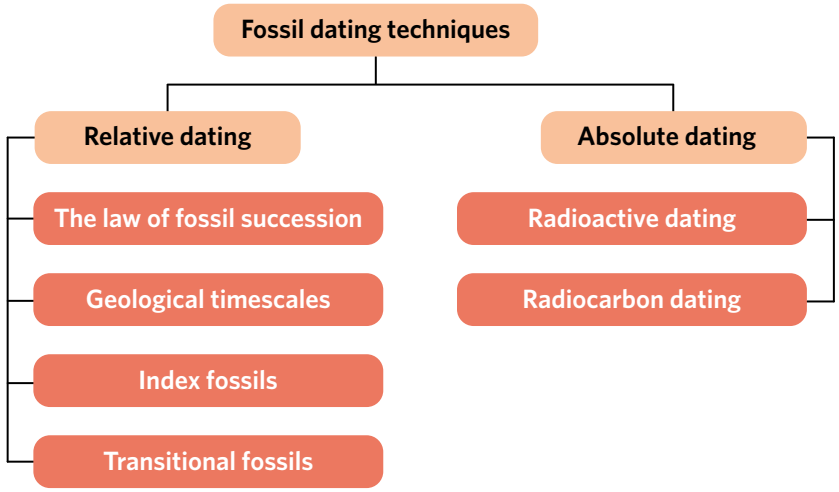


Figure 13 A comparison of the two fossil dating techniques: relative and absolute dating

In the case of our crocodiles, researchers measured fossil skulls from museum collections around the world and matched them up with climate conditions from when the specimens lived. They found that, for Crocodylia*, larger skull sizes tended to be found with lower temperatures, especially during the cooling period of the Cenozoic era. This is an example of how the fossil record can be used to provide evidence to evolutionary narratives and shows that in the end, the large size of modern day crocs, which we take as fierce and powerful, might actually tell a much more nuanced story – one of ecological fragility and a past riddled with difficulty and extinction.*

10A QUESTIONS

Theory review questions

Question 1

Match the key term from the lesson with the correct definition.

Key term

- half-life
- index fossil
- permineralisation
- sedimentary rock
- transitional fossil
- Cambrian explosion
- geological timescales
- radioisotope
- radiocarbon dating
- law of fossil succession

Definition

I _____ The time taken for half the amount of a radioisotope to break down into its products.

II _____ An unstable atom of a specific element that breaks down into a predictable and stable product.

III _____ Rock that has formed through the accumulation of sediment and hardened under pressure.

IV _____ A fossil that exhibits characteristics that are common to both its ancestor group and its descendant group.

V _____ A process for determining the age of a fossil by measuring the changing ratio of two different carbon isotopes.

VI _____ A system for chronologically dating different sedimentary rock strata using known time frames, such as periods, eras, or eons.

VII _____ A fossil that, due to the short existence and wide geographic distribution of its species, is used to define and quickly identify particular geographical timeframes.

VIII _____ A key principle for the use of the fossil record, which states that fossils of the same age will be in the same layer of sedimentary rock, and fossils found in a higher or lower sedimentary layer will be younger or older respectively.

IX _____ A key biological event in Earth's history approximately 535 million years ago when practically all major animal phyla started appearing in the fossil record.

X _____ A process of fossilisation where mineral deposits, typically carried by water, fill the spaces within organic tissue and form rock-like relics of an organism.

Question 2

Order the following key events in the Earth's biological history.

I emergence of eukaryotes

II emergence of prokaryotes

III first vertebrate land animals

IV appearance of multicellular life

V appearance of the *Homo* genus

Question 3

Which of the following factors is likely to increase the chances of fossilisation?

A exposure of the organism to increased light

B exposure of the organism to increased oxygen

C exposure of the organism to warmer temperatures

D exposure of the organism to areas of high sediment accumulation

Question 4

Which of the following statements is true of relative dating techniques?

A Geological timescales can be used as a relative dating technique in that they outline the exact age (in years) of each new fossil found.

B Index fossils can be used as a relative dating technique in that they demonstrate morphological links between an ancestor species and a later descendant group.

C The law of fossil succession underpins relative dating techniques, and suggests that fossils closest to the surface must be younger than those that are found below it.

D Radioactive carbon isotopes can be used as a relative dating technique in that they decay at a known constant rate and their ratio does not change an organism's death.

Question 5

Which of the following statements is true of absolute dating techniques?

A Radiocarbon dating is the only absolute dating technique.

B Levels of carbon-14 found in a dead organism will increase after its death.

C The ratio of carbon-12 to carbon-14 will depend on the amount of carbon-12 decay.

D The rate of decay of certain radioisotopes is known and can be measured accurately.

Question 6

Consider the modelling of ^{14}C to ^{12}C radioisotopes. Which of the following statements correctly explains the importance of atmospheric carbon levels?

A It is assumed that when the organism was alive, its proportion of ^{14}C to ^{12}C was relatively constant and mirrored that of the atmosphere. As the fossil's ^{14}C levels change post-death, its ^{12}C levels remain unchanged, which is key for determining age.

B It is assumed that when the organism was alive, its proportion of ^{14}C to ^{12}C was relatively constant and mirrored that of the atmosphere. As the fossil's carbon levels change post-death, its levels of ^{12}C will increase while its levels of ^{14}C will decrease, which is key for determining age.

SAC skills questions

Data analysis

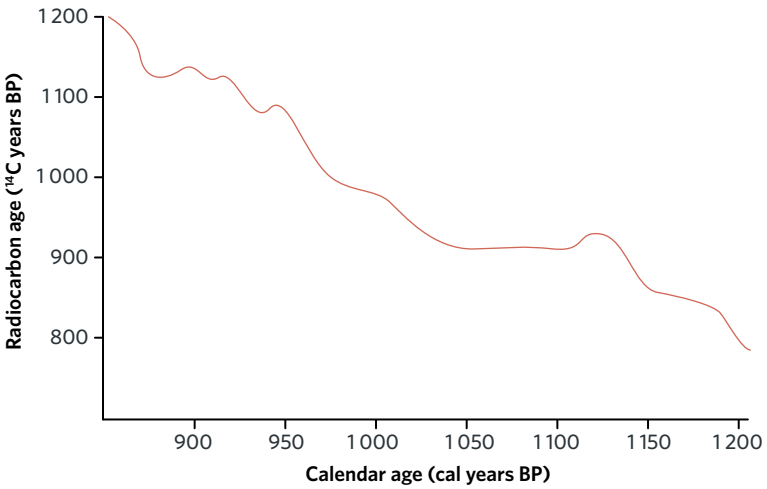
Use the following information to answer Questions 7–11.

Radiocarbon dating has been used for nearly 80 years now, after Nobel Prize-winning chemist Willard Libby first discovered the following:

- Small amounts of radioactive carbon are created in the Earth's upper atmosphere.
- This radioactive carbon is converted to carbon dioxide and taken up by plants during regular photosynthesis. It then travels up the food chain through organisms.
- These organisms will have the same proportion of this ^{14}C as their external environments while alive. However, once dead, the organisms will no longer take in any more carbon, and existing levels of ^{14}C in the fossil will decay at a steady rate over time.
- Therefore, by measuring the level of ^{14}C in a specimen, we can deduce how long ago that organism died.

An implicit rule that this finding rests on is that the level of ^{14}C in the atmosphere remains constant over time. This is not the case. Factors like climate change, the widespread use of fossil fuels, cosmic radiation, and nuclear testing have all caused large variations in the amount of environmental ^{14}C. As a result, in order to ensure the accuracy of radiocarbon dating, researchers need to work to accurately adjust/calibrate existing radiocarbon dates into more accurate calendar ages. Without these adjustments, it has been found that some dates could be inaccurate by as much as 10–15%.

Thus, a radiocarbon age needs to be converted to a more accurate calendar age, which takes into account these changes to atmospheric carbon levels over time. To do this, researchers must find a sequence of accurately dated samples which can be tested for their radiocarbon age, so as to create a 'calibration curve' with which to plot new samples. The samples that are often used are tree-rings of ancient wood bark, as researchers can directly tie these to a known age. An example of one such calibration curve is shown in the following graph.



The main elements of this plot are:

- the original radiocarbon determination is shown on the y-axis, where BP refers to 'before present' (where present is 1950)
- the calibrated calendar age is shown on the x-axis, where cal years BP refer to 'calibrated date before present'
- the red line represents the calibration curve, inclusive of a standard error range.

Question 7

Which of the following best summarises the main concern presented in the text regarding the accuracy of radiocarbon dating?

A The ratio of atmospheric ^{14}C to ^{12}C has not remained constant over time, meaning that the traditional calculation of radiocarbon ages may be inaccurate.

B Dead organisms continue to replace their levels of internal ^{14}C , meaning that the ^{14}C to ^{12}C ratio between the organism and its external environment may be inaccurate.

Question 8

According to Libby, what is the relationship between atmospheric radiocarbon and living organisms?

A Radiocarbon from the atmosphere is transferred throughout the food chain, meaning that living organisms have the same ratio of radiocarbon elements as their external environment.

B Radiocarbon from the atmosphere serves as an input for photosynthesis. By measuring the amount of this radiocarbon in living plants, researchers can plot the rate at which photosynthesis occurs in different environments.

Question 9

Which of the following statements is not true of radiocarbon calibration?

A Calibration curves only account for the changes in overall radiocarbon levels due to the increased usage of fossil fuels.

B Calibration increases the relative accuracy of radiocarbon dating techniques by converting ages from 'radiocarbon years' to updated calendar ages.

C Calibration is performed by comparing existing radiocarbon measurements of a sample to measurements made on organic material (typically tree rings) with a known age.

Question 10

If a sample has a radiocarbon age of 1 000 ^{14}C years BP, its calibrated calendar age will be closest to

A 900 cal years BP.

B 950 cal years BP.

C 975 cal years BP.

D 1 050 cal years BP.

Question 11

Recent updates to calibration curves are based on as many as 15 000 radiocarbon measurements, taken largely from tree stumps and coral. Using the bioethical concept of integrity, how might we best support the work that has gone into establishing a new calibration curve?

A In testing living organic material, rather than long-dead fossils, researchers avoid unnecessary harm that may arise in removing important fossils and historical artefacts from their original communities.

B The new radiocarbon calibration curve provides increased accuracy when dating, which can bring to light new information, or correct misleading preconceptions, about how organisms have evolved over time.

Exam-style questions

Within lesson

Question 12 (1 MARK)

Radiometric dating (absolute dating) is used to determine the age of fossils and their surrounding rocks. Which statement about radiometric dating is true?

A Radiometric dating calculations assume that the rate of breakdown of a radioactive isotope changes with age.

B All radiometric dating techniques are only reliable when dating fossils that were formed less than 50 thousand years ago.

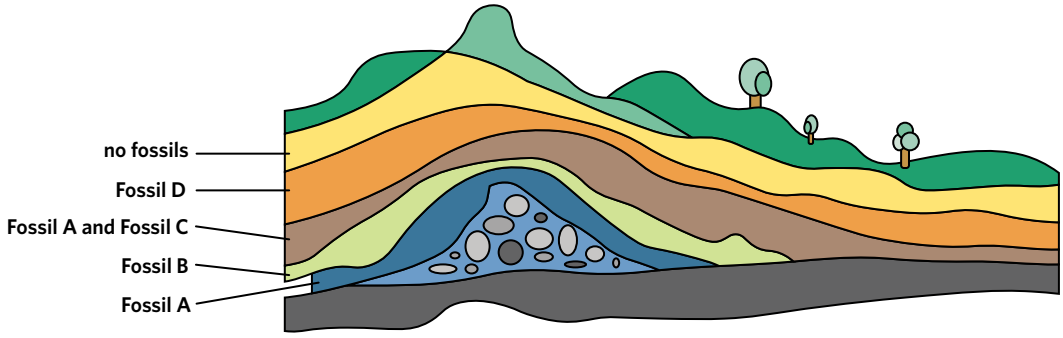
C Potassium is the best element to provide useful radiometric evidence for calculating the age of all fossils due to its long half-life.

D Radiometric dating techniques can be used to compare if one fossil is older than another, by providing an estimate of how many years ago each fossil was formed.

Adapted from VCAA 2017 Northern Hemisphere Section A Q34

Use the following information to answer Questions 13 and 14.

The fossils present in different rock strata in a particular location are shown in the diagram.



Question 13 (1 MARK)

From the diagram, it can be concluded that

A all of these species are extinct.

B Fossil A is a permineralised fossil.

C Fossil A is the oldest fossil present.

D Fossil A and Fossil C belong to the same species.

Adapted from VCAA 2017 Sample Section A Q31

Question 14 (1 MARK)

Using radiometric dating techniques, the age of Fossil C was calculated to be 60 million years old.

This suggests that

A Fossil B is an ancestor of Fossil C.

B Fossil B is less than 60 million years old.

C very little carbon-14 could be found in Fossil A.

D less carbon-14 would be found in Fossil D than Fossil C.

Adapted from VCAA 2017 Sample Section A Q31

Use the following information to answer Questions 15 and 16.

Image (left to right): Mark Brandon, Catmando/Shutterstock.com

The photograph shows a fossil of the bird-like dinosaur *Archaeopteryx*, which is thought to have lived around 150 million years ago. The image on the right is a 3D reconstruction of the complete dinosaur. Evidence suggests that the dinosaur was fossilised at the bottom of an ancient riverbed. Two stratigraphically younger fossils that had been found previously at a nearby site are closely related to *Archaeopteryx*, though exhibit stark morphological differences when compared to *Archaeopteryx's* most recent ancestors from the clade Maniraptora. These ancestors had fewer feathers, a longer, bonier tail, and a full set of teeth.

Question 15 (1 MARK)

It is most probable that the two stratigraphically younger fossils would have been found in a layer of rock that

A was formed from hot ash sediment.

B was located at a depth less than 2.3 m below the ancient riverbed.

C was closer to the present-day ground surface than the rock surrounding the *Archaeopteryx* fossil.

D contained a smaller quantity of potassium-40 than the rock surrounding the *Archaeopteryx* fossil.

Adapted from VCAA 2018 Section A Q25

Question 16 (1 MARK)

From the information provided, *Archaeopteryx* can most likely be thought of as

A an index fossil useful in defining the geological time frame between 130–160 mya.

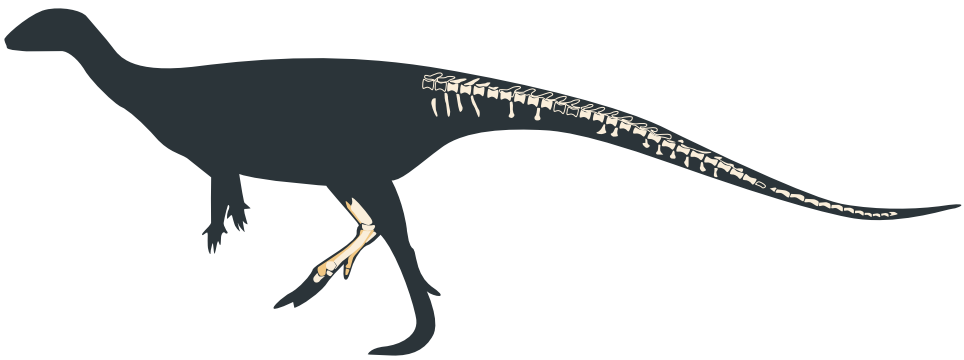
B a transitional fossil useful in defining the geological time frame of approximately 150 mya.

C a permineralised fossil with relatively higher levels of carbon-14 than current atmospheric levels.

D a transitional fossil useful in demonstrating the morphological changes between ancient reptiles and more modern bird species.

Question 17 (1 MARK)

The diagram is a reconstruction of a fossil of the ornithopod dinosaur *Diluvicursor pickeringi* found in a 113 million year old rock in western Victoria. Evidence suggests that the dinosaur was fossilised at the bottom of an ancient riverbed, where all the organic materials in the bones of *D. pickeringi* had been replaced with inorganic materials. The fossil consisted of a tail, a partial hind limb and some vertebrae.



From the information given, what can be said about the *D. pickeringi* fossil?

A *D. pickeringi* is an example of a trace fossil.

B *D. pickeringi* is an example of a transitional fossil.

C *D. pickeringi* is an example of a permineralised fossil.

D Only the tail, partial hind limb, and vertebrae were covered in sedimentary layers.

Adapted from VCAA 2018 Section A Q25

Question 18 (1 MARK)

Which one of the following statements correctly explains why 50 000 years is the upper limit of the dating period for radiocarbon dating?

A The half-life of carbon-12 is short and so, by 50 000 years, there is too little carbon-12 left to measure.

B After 50 000 years, almost all carbon-14 has radioactively decayed into its breakdown products, and accurate age estimations cannot be made.

C Fossils older than 50 000 years have accumulated too much carbon contamination from the surrounding rock and minerals for accurate measurements.

D Carbon-14 is produced when high energy light penetrates the ozone layer. As the ozone layer was too thick over 50 000 years ago to let this high energy light through, carbon-14 was rare.

Adapted from VCAA 2017 Sample Section A Q29

Question 19 (3 MARKS)

The image shows two thylacines (*Thylacinus cynocephalus*, also called Tasmanian tigers) at Hobart zoo in the early 1900s. Declared extinct in 1986, thylacines were large, dog-like marsupials. A fossilised carcass of a thylacine was found in a cave on the Nullarbor Plain. The carcass was dated to about 5 000 years old and the cave itself hosted a stable, cool, and dry internal environment with very low levels of light. Researchers believe these conditions were why the thylacine carcass was found in the open, with little evidence of serious decomposition.

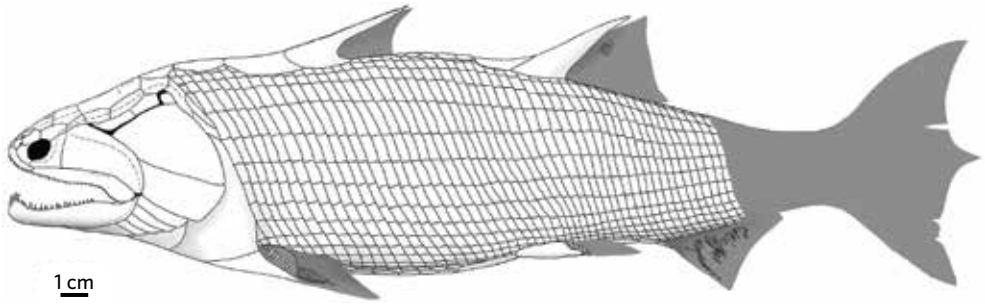
a Briefly describe the method that was most likely used to date the thylacine carcass. (1 MARK)

b One of the members of the research team suggested that the fossilised carcass was actually an example of a mould fossil. Explain whether or not this researcher is correct with reference to your knowledge of mould fossils. (2 MARKS)

Multiple lessons

Question 20 (8 MARKS)

Osteichthyans are a group of fish with hard, bony skeletons, rather than the softer, cartilaginous skeletons found in earlier fish species. The diagram below shows the body structure of perhaps the oldest Osteichthyan to exist, where an almost complete fossil was found in China in 2009.



The scientists calculated the relative age of the fossil by referring to fossils of several ancient molluscs (shellfish). The molluscs used are only found in a small region off the Chinese coastline and were calculated to live 400 million years ago.

a Explain how scientists would have calculated the absolute age of the ancient mollusc fossils. (2 MARKS)

b How can the absolute age of the ancient mollusc fossils be used to estimate the relative age of the ancient Osteichthyan fossil? (2 MARKS)

c The Osteichthyan fossil was found in a sedimentary layer deeper than those of the ancient molluscs. What is the relative age of the Osteichthyan fossil? (1 MARK)

d From the information given, suggest whether these ancient molluscs could be used as an index fossil in the future. Explain. (1 MARK)

e Describe one important step in evolution from ancient unicellular eukaryotes to Osteichthyans. (1 MARK)

f Suggest a selection pressure that could have made it advantageous for Osteichthyans to develop a hard, bony skeleton. (1 MARK)

Adapted from VCAA 2018 Northern Hemisphere Section B Q7

Key science skills and ethical understanding

Question 21 (8 MARKS)

Scientists studying the fossils in an ancient lake bed have an almost perfect fossil record ranging more than 25 000 years. In this fossil record, there are two forms of a fish called a 'stickleback'. One form of the fish has large spines on its back (dorsal spines) and large pelvic bones. The other form has smaller or no spines and smaller pelvic bones. Modern-day stickleback fish with large dorsal spines are common in the ocean, while stickleback fish with small spines are more common in freshwater.

Image: Liliya Butenko/Shutterstock.com

Scientists are trying to gain an understanding of the evolutionary pathway of the stickleback fish. The table contains a summary of some structural features of the fossils found in different sediment layers in the ancient lake bed.

Sediment layer	Proportion of carbon-14 relative to ancient atmospheric levels (%)	Pelvis	Dorsal spines
top	50	small	small or absent
middle	25	large	large
lower	6.25	small	small or absent

a The scientists want to accurately describe key time periods in stickleback evolution. Suggest whether the scientists should calculate the relative or absolute ages for the stickleback fossils. Justify your response. (2 MARKS)

b Using your understanding of the half-life of carbon-14, calculate the absolute ages of stickleback fossils within the top, middle, and lower sedimentary layers. (3 MARKS)

c Using evidence from the scenario, describe the changes in the lake environment over the past 25 000 years, particularly in relation to levels of saltwater. (3 MARKS)

10B EVIDENCE OF RELATEDNESS

An angry cat doesn't even need to look at you to tell you to back off. Just a simple flex of its ear muscles and you know it wants to be left alone. We love a subtle Queen! Cats regularly move their ears to detect sounds or express their emotions. When they are angry or defensive, they tend to push their ears backwards, and when they are alert or interested, they move their ears upright. Why can't we do this?

Image: DreamBig/Shutterstock.com

Lesson 10B

In this lesson you will learn about the evidence that can be used to determine the relatedness between different species.

◀ *Prerequisite knowledge*

Chapter 2
The similarities and differences in amino acid and DNA sequences are used to determine how related different organisms are.

Lesson 10A
Fossils are used to determine the relatedness between different species.

Future applications ▶

Lesson 10C
The relatedness between different species can be visually represented using phylogenies.

Chapter 11
This chapter considers the evolutionary pathway of modern humans and examines the relatedness between us and our recent ancestors.

Study design dot point

- evidence of relatedness between species: structural morphology – homologous and vestigial structures; and molecular homology – DNA and amino acid sequences

Key knowledge units

Homologous structures	4.2.7.1
Vestigial structures	4.2.7.2
Amino acid sequence similarity	4.2.7.3
DNA sequence similarity	4.2.7.4

Homologous structures 4.2.7.1

OVERVIEW

Homologous structures are physical similarities between two species which indicate that they have a common ancestor.

THEORY DETAILS

In chapter 9, you learned how a single species can evolve and eventually become two separate species. This means that there's some level of relatedness between different species. One method to assess this relatedness is through **structural morphology**, which involves looking for similarities between the physical features (e.g. skeletal structures) of different species.

structural morphology the study of physical structures to establish relatedness

Homologous structures are features found in different species that may look and function very differently from one another but can be shown to have been derived from a common ancestor. An example of this is the upper limb of humans, cats, whales, and bats (Figure 1). They have different shapes and functions – humans carry things with their arms, cats walk with their legs, whales swim with their flippers, and bats fly with their wings – yet we can see that they all share a similar bone structure. This suggests that each of these species diverged from a common ancestor which had this limb structure.

homologous structures features present in two or more species that may look and function very differently in each species, but are derived from a common ancestor

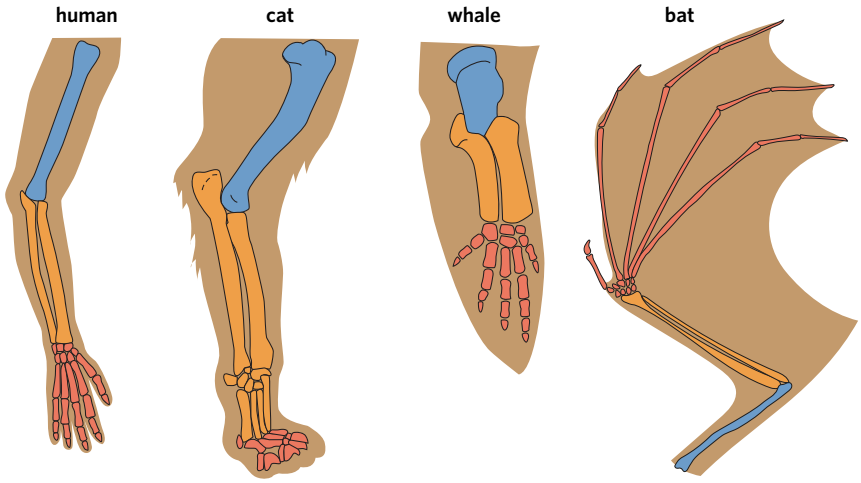


Image: Usagi-P/Shutterstock.com

Figure 1 The upper limbs of humans, cats, whales, and bats are homologous structures.

Homologous structures are physical evidence of **divergent evolution**, the evolutionary process where two or more populations of a single species accumulate enough genetic differences to be classified as different species. This process typically occurs as a result of individual populations adapting to different selection pressures or genetic drift which alters population genomes over extended periods of time.

divergent evolution the process in which a common ancestor evolves into two or more descendant species

analogous structures features present in two or more species that fulfil the same function but do not originate from a common ancestor

convergent evolution the process in which distantly related species evolve similar traits over time due to the action of similar selection pressures

Analogous structures

Analogous structures are structures that serve similar biological functions but are not derived from a common ancestor. Analogous structures are evidence of **convergent evolution** in which two or more distantly related species (without a recent common ancestor) can be seen to have independently evolved similar traits to adapt to similar environments and selection pressures. For example, the wings of birds and insects are analogous structures because they are used to fly. They are very different structures, however, as birds and insects evolved independently from one another (Figure 2).

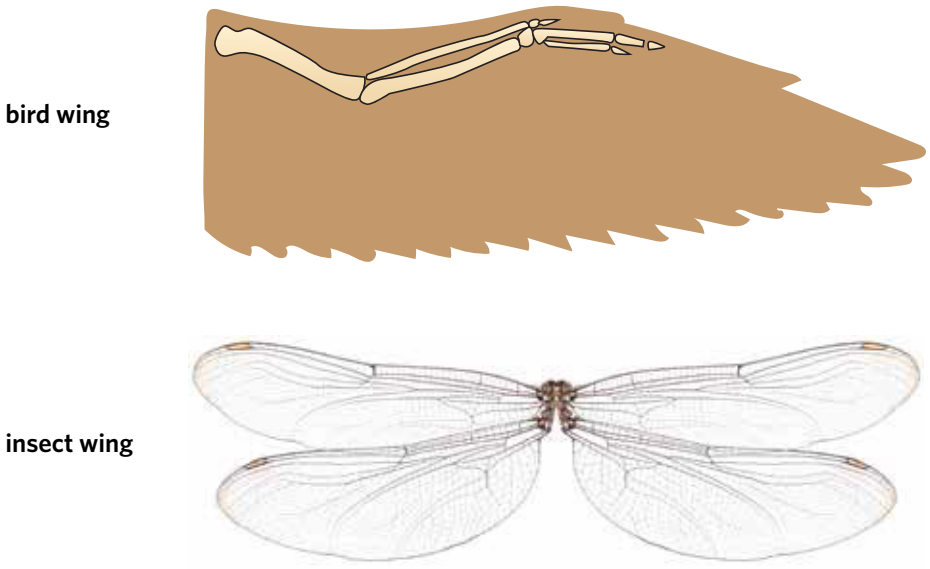


Image (top to bottom): Usagi-P, Mr. SUTTIPON YAKHAM/Shutterstock.com

Figure 2 A bird's wing and an insect's wing are analogous structures. A bird's wing consists of several bones, while an insect's wing consists of a thin membrane without any bones.

Lesson link

In ***lesson 10A*** you learned about the fossil record and how it can be used to demonstrate the evolutionary pathways of species. We also learned that various structures of organisms can be preserved and discovered in these fossils. In this lesson, we will consider how many of these preserved structures can be used to help determine the level of relatedness between different species.

Memory device

'*Homo*' means 'same' in Greek, so homologous structures originate from the same ancestor.

Vestigial structures 4.2.7.2

Overview

Vestigial structures with no apparent function can be used to determine species' ancient ancestors.

THEORY DETAILS

Vestigial structures are structures found within organisms that once served a purpose for an organism's ancestors but, due to changing selection pressures, have lost their original function and are no longer required for survival. Despite having no function, these structures often remain in a species as they are not selected against, and therefore are an easy method to infer relatedness between species.

vestigial structures features that have lost all or most of their usefulness as a result of evolution by natural selection

The human coccyx (also commonly known as the tailbone) does not serve a significant function in modern humans (Figure 3). It is a vestigial structure, a remnant of our ancestors' tail that was used to balance their bodies when they lived in trees. Over time, more advanced features such as the cerebellum and inner ear evolved in humans to help us with balance, meaning that the tail was no longer necessary for survival. However, we have still retained a small tailbone given that it was not directly selected against.

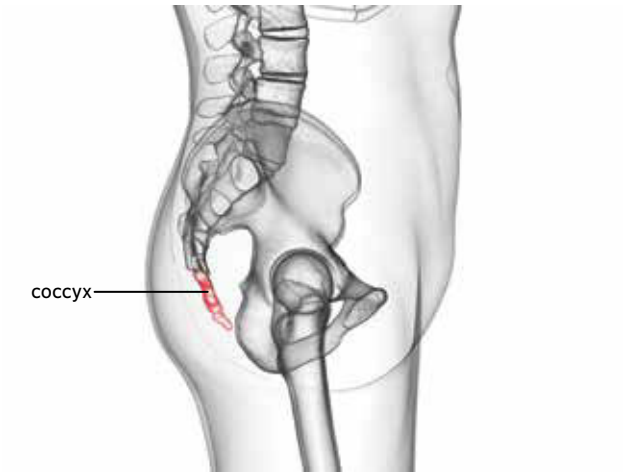


Image: SciePro/Shutterstock.com

Figure 3 The coccyx is a remnant of our ancestors' tails.

Vestigial structures can also be found in other animals, such as pelvic bones in snakes and whales despite them not having legs (Figure 4). We now know that this is because snakes are descendants of reptiles that had legs, while whales are descendants of earlier mammals that had legs.

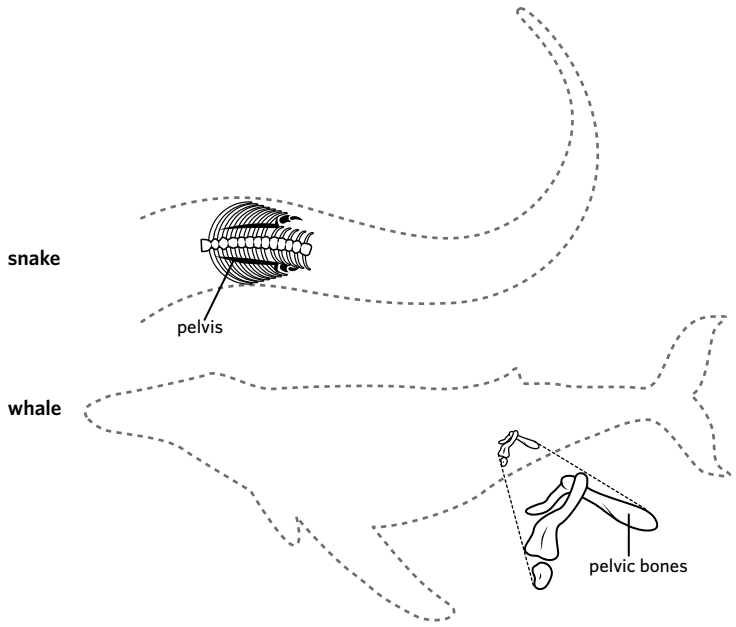


Figure 4 Pelvic bones in snakes and whales

Amino acid sequence similarity 4.2.7.3

OVERVIEW

Comparing amino acid sequences is another means of determining how related different organisms are.

THEORY DETAILS

Molecular homology is the study of the similarities between organisms at a DNA and amino acid level. There are many different types of proteins present in the body, but when studying amino acid sequence similarities, we analyse proteins from **conserved genes** which are found in a number of different species.

molecular homology the study of the similarities in the nucleotide sequences of DNA or amino acid sequences in proteins between organisms to establish relatedness

conserved genes genes that have remained largely unchanged throughout evolution, and are found across the genome's of many different species

haemoglobin (Hb) a protein found in red blood cells that is responsible for the transport of oxygen in the body

Haemoglobin

Haemoglobin (Hb) plays an important role in carrying oxygen from the lungs to the cells around the body of many different species. It is composed of up to 4 polypeptide chains: 2 alpha chains consisting of 41 amino acids and 2 beta chains consisting of 146 amino acids. Researchers can assess the number of amino acid differences between the chains of different organisms and come to a conclusion regarding their degree of relatedness.

Figure 5 shows a portion of the amino acid sequence of haemoglobin in humans and four other vertebrates. It can be seen that, compared to humans, chimpanzees have the highest level of similarity in their amino acid sequence for haemoglobin (no difference), followed by gorillas, and finally kangaroos. This suggests that chimpanzees are the most closely related to humans (and, by extension, share the most recent common ancestor) and kangaroos are the most distantly related.

Human	*Arg*	*Leu*	*Leu*	*Gly*	*Asn*	*Val*	*Leu*	*Val*	*Cys*	*Val*	*Leu*	*Ala*	*His*
Chimpanzee	*Arg*	*Leu*	*Leu*	*Gly*	*Asn*	*Val*	*Leu*	*Val*	*Cys*	*Val*	*Leu*	*Ala*	*His*
Gorilla	*Lys*	*Leu*	*Leu*	*Gly*	*Asn*	*Val*	*Leu*	*Val*	*Cys*	*Val*	*Leu*	*Ala*	*His*
Kangaroo	*Lys*	*Leu*	*Leu*	*Gly*	*Asn*	*Ile*	*Ile*	*Val*	*Ile*	*Cys*	*Leu*	*Ala*	*Glu*

Figure 5 A section of the amino acid sequence of haemoglobin in humans, alongside four other vertebrates.

> **Lesson link**
>
> In ***lesson 2A***, you learned that proteins are made from amino acids joined together via peptide bonds. Proteins have a diverse range of functions in the human body.

Cytochrome c

Cytochrome c is an enzyme present in mitochondria that consists of 104 amino acids which are encoded by a conserved gene in **mitochondrial DNA (mtDNA)**. Figure 6 shows a segment of the amino acid sequence in cytochrome c of humans, rats, and yeast. In this particular segment, rats show more similarity to humans than yeast does. Table 1 shows how amino acid sequences can map our relatedness to many other organisms.

cytochrome c an enzyme found in mitochondria that carries electrons in aerobic and anaerobic respiration reactions

mitochondrial DNA (mtDNA) circular DNA found in mitochondria

Human	*Ser*	*Tyr*	*Thr*	*Gly*	*Ala*	*Ala*	*Asn*	*Lys*	*Asn*
Rat	*Ser*	*Tyr*	*Thr*	*Gly*	*Asp*	*Ala*	*Asn*	*Lys*	*Asn*
Yeast	*Ser*	*Tyr*	*Thr*	*Gly*	*Asp*	*Ala*	*Asn*	*Lys*	*Lys*

Figure 6 A section of the amino acid sequence of cytochrome c in humans, rats and yeast.

Table 1 Comparison of the whole amino acid sequence of cytochrome c between humans and other species.

Species pairing	Number of differences in amino acids
Human – chimpanzee	0
Human – rhesus monkey	1
Human – horse	12
Human – pigeon	12
Human – rattlesnake	14
Human – snapping turtle	15

cont'd

Table 1 Continued

Species pairing	Number of differences in amino acids
Human – tuna	21
Human – fruit fly	29
Human – wheat	43

DNA sequence similarity 4.2.7.4

OVERVIEW

DNA sequences of different species can be compared to determine the evolutionary relatedness between them.

THEORY DETAILS

As with amino acid sequences, **DNA** sequences can also be used to determine the relatedness between different organisms. Just like amino acid sequences, a higher similarity in DNA sequence implies a closer level of relatedness between different organisms.

In Year 11, you learned that different **nucleotides** have different nitrogenous bases – adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U). We can compare the differences in the DNA sequences of different species simply by looking at the order of these bases at corresponding gene regions. Figure 7 shows a comparison of the DNA sequence of humans, rats, and yeast at a particular segment of corresponding DNA. In this particular segment of the DNA sequence, humans and rats have three nucleotide differences, whereas humans and yeast have seven nucleotide differences, indicating that rats are more closely related to humans than yeast.

Human	5′ -	AGA	ATA	TGA	CGG	CGA	TTG	TTC	TTA	- 3′
Rat	5′ -	AGA	ATA	TGA	CTG	CGT	TTG	TTT	TTA	- 3′
Yeast	5′ -	AGA	ATG	TGG	CTG	CGT	TTG	TAT	TTC	- 3′

Figure 7 Comparison of a section of the cytochrome c DNA sequence between humans, rats, and yeast. Orange highlights indicate nucleotide differences.

DNA (deoxyribonucleic acid) a double-stranded nucleic acid chain made up of nucleotides. DNA carries the instructions for proteins which are required for cell and organism survival

nucleotide the monomer subunit of nucleic acids. Made up of a nitrogen-containing base, a five-carbon sugar molecule (ribose in RNA and deoxyribose in DNA), and a phosphate group

Lesson link

The process of protein production from DNA is explained in ***lesson 2D.*** DNA is transcribed into mRNA which is then translated into protein.

Theory in context

DETERMINING RELATEDNESS USING MITOCHONDRIAL DNA

Mitochondria, like chloroplasts, contain their own **genome**. This is because they once existed as free-living organisms before being engulfed by our eukaryotic ancestors. Their DNA is referred to as mitochondrial DNA (mtDNA). mtDNA is almost 17 000 nucleotides long in humans and contains 37 genes, compared to the over 24 000 genes in our nuclear DNA. Mitochondrial DNA is maternally inherited, meaning that it is only inherited from your mother.

There are two main advantages of using mtDNA instead of **nuclear DNA** when comparing the relatedness between species:

1. The mutation rate in mtDNA is much higher than in nuclear DNA. This means that for very closely related species, the mutation rate still ensures that there are enough differences in DNA sequences for us to compare.
2. There is no recombination (mixing of DNA between homologous chromosomes during meiosis) in mtDNA because it's only inherited from the mother. This means that mtDNA remains unchanged from generation to generation, which makes it easier to trace past ancestors.

For these reasons, mtDNA is incredibly useful for tracing human lineages. Researchers have been able to trace maternal lineages using mtDNA and have shown that modern humans share a common female ancestor that lived in Africa about 160 000 years ago.

genome the complete set of DNA housed within an organism

nuclear DNA DNA that is located in the nucleus of a cell

Analysing amino acid sequences vs DNA sequences

Both amino acid and DNA sequence analysis help determine the relatedness between different organisms. So which technique should we use? It depends on how related the species are.

A limitation to analysing amino acid sequences is that closely related species are likely to share very similar sequences for certain proteins. In these instances, scientists determine relatedness by comparing nucleotide sequences, looking for silent mutations that, due to the redundancy of the genetic code, may have accumulated without altering the amino acid sequence. Amino acid sequences, however, are easier to interpret and are therefore used to determine relatedness in more distantly related species.

Besides DNA and amino acid sequences, we can also compare whole genomes of different species. The higher the degree of similarity between the genomes of different species, the more related they are and the more recent they diverged from a common ancestor. We can see in Figure 8 that humans and chimpanzees have very similar genomes.

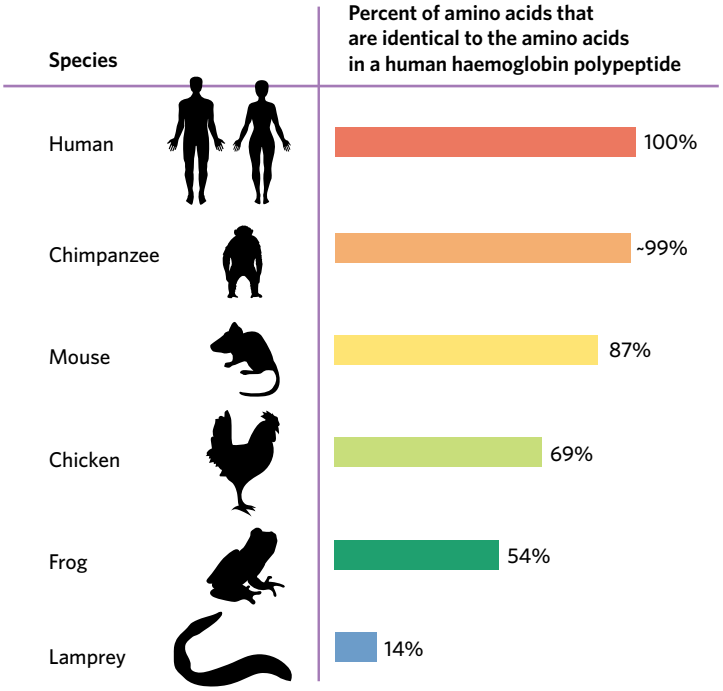


Figure 8 Comparison of the human genome with other animals

Theory summary

Table 2 Summary of structural and molecular evidence used to determine the relatedness between different organisms

Structural morphology	Molecular homology
Homologous structures: these are features that have the same underlying structure, but different functions. The presence of homologous structures in different species suggests they share a common ancestor.	Amino acid sequences: researchers can examine shared proteins between species and the degree of amino acid similarity between them to demonstrate relatedness. A higher degree of similarity suggests the species being compared are more closely related.
Vestigial structures: these are structures that remain within a species despite losing their function and necessity. Like homologous structures, their presence suggests the organisms being compared share a common ancestor.	DNA sequences: researchers can consider corresponding gene regions (or entire genomes) between species, and demonstrate degrees of relatedness based on nucleotide differences. A higher degree of similarity suggests the species being compared are more closely related.

Interestingly, humans do actually have three external auricular (ear) muscles that once helped our ears move. However, most of us no longer use them – they are an example of a vestigial structure. Our ancestors in the past moved their ears to detect sound and predators to survive in the wild. During the evolutionary process, humans did not need these functions anymore so we stopped using these muscles, eventually losing the ability to move our ears completely! Given that humans and cats both have these ear muscles, it seems we share a common ancestor along our evolutionary lines – in fact, current estimates suggest this ancestor existed around 90 million years ago. Now, go and hug your cat!

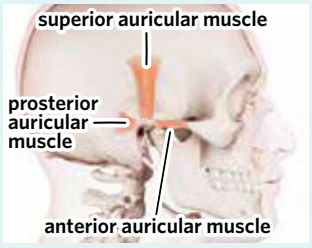


Image: SciePro/Shutterstock.com

10B QUESTIONS

Theory review questions

Question 1

Fill in the blanks in the following sentences.

Homologous structures are structures found in different species that may look and function very _____ from one another but can be shown to have been derived from a _____. _____ are features that have lost all or most of their usefulness as a result of evolution by natural selection.

Question 2

Which of the following options shows the most homology to the given amino acid sequence?

Met – Lys – Arg – Ala – Ala – Asn – Ser

A *Met – Thr – Arg – Ala – Ala – Asn – Ser*

B *Met – Lys – Arg – Thr – Ala – Asn – Tyr*

C *Met – Lys – Gly – Pro – Ala – Asn – Ser*

D *Met – Lys – Ser – Ala – Pro – Lys – Ser*

Question 3

Which of the following DNA sequences shows the least homology to the given DNA sequence?

5′ – ATG GCC GAA AGA TGG TCA – 3′

A 5′ – ATG GCC GGA AGA TGT TCC – 3′

B 5′ – ATG GAC GAA AGA TGG TCA – 3′

C 5′ – ATG GCA GGA AGA TGG TCA – 3′

D 5′ – ATG GCA GGA AGA TGA ACA – 3′

Question 4

Categorise the following as **true** or **false**.

I Analogous structures suggest that two different species share a common ancestor.

II The more similarity between amino acid sequences, the more related two species are.

III Similarity in DNA sequences between different species is assessed by comparing the level of glucose present.

IV Vestigial structures used to be useful for ancient ancestors but do not serve any significant functions in recent descendants.

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 5–8.

In 2013, frozen meat pies in the UK were found to contain more than 1% horse DNA – out of 24 280 tests performed, 47 tests showed the presence of horse DNA. Horse DNA was also detected in some beef burgers – out of 27 beef burgers analysed, 10 of them were found to contain horse DNA. What's more, a trace amount of a particular veterinary drug used on horses was found in one of the beef burgers analysed, causing serious concern for consumers regarding the safety and purity of the meat products they were purchasing.

How was this found to be the case? One technique used to detect horse meat in the beef samples was to compare cytochrome b genes of horses and cows. The cytochrome b gene is found in mitochondria and is specific to each species. If the sample was pure beef, only cow cytochrome b gene would be found. If the sample contained both beef and horse meat, both cow and horse cytochrome b genes would be found. A portion of the cytochrome b DNA sequence of cows, pigs, and horses is shown.

Cow	5′ - CTA GAA AAG TGT AAG ACC CGT AAT AT - 3′
Pig	5′ - CTA TGA ATG CTG TGG CTA TTG TCG CA - 3′
Horse	5′ - CTC AGA TTC ACT CGA CGA GGG TAG TA - 3′

Question 5

Within the given DNA segment, how many nucleotides do cows and horses have in common?

A five

B six

Question 6

Based on the information provided, we can assume that a pig is more closely related to a

A cow.

B horse.

Question 7

Based on the research conducted, the company responsible for the meat pies recalled all stock and decided to report the information publicly, both through a government regulator of goods and services, as well as through their website and mailing list. Which of the following bioethical concepts is likely to have informed this decision?

A respect

B integrity

C beneficence

D consequences-based approach

Question 8

When conducting the investigation, the researchers decided to compare their samples with existing DNA data housed in a relevant genomic database. The team explained that this decision was based on a specific set of bioethical considerations, including respect, non-maleficence, and their own interpretation of a duty/rule-based approach to bioethics. In light of this information, which of the following statements provides the best justification for this decision?

A 'Using information housed in a genomic database removes the need to extract new tissue for investigation.'

B 'Using information housed in a genomic database ensures that all data is accurate, freely reported, and easily accessible for public scrutiny.'

Exam-style questions

Within lesson

Question 9 (1 MARK)

The American *Trichocereus macrogonus* and the South African *Euphorbia pentagono* are plants belonging to different families. Both plants live in dry regions.

They both have thick, succulent stems to store water and spines for protection. These are examples of

A divergent evolution.

B random genetic drift.

C analogous structures.

D homologous structures.

Trichocereus macrogonus

Euphorbia pentagona

Adapted from VCAA 2014 Section A Q27

Question 10 (5 MARKS)

The Australian platypus (*O. anatinus*) and short-beaked echidna (*T. aculeatus*) are both members of the order Monotremata. They are known to share a common ancestor. Platypuses are endemic to Australia, while short-beaked echidnas are found in Australia and New Guinea. Echidnas live in a terrestrial environment, have spines and lay eggs. Platypuses also lay eggs, but live in an aquatic environment and do not have spines.

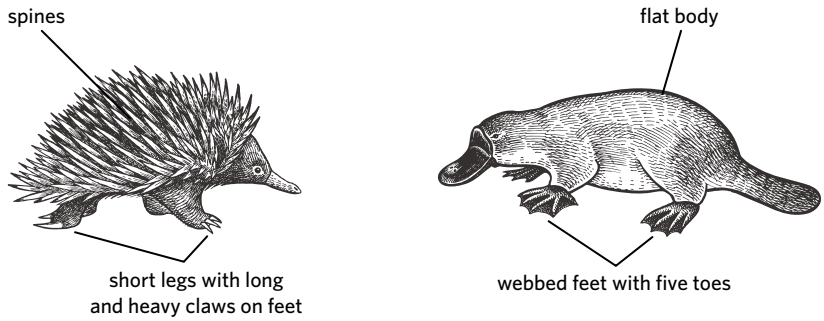


Image: mamita/Shutterstock.com

a Explain whether the legs of short-beaked echidnas and platypuses are an example of homologous or vestigial structures. (1 MARK)

b Suggest whether the short legs or spines evolved first in the order Monotremata. Justify your answer. (2 MARKS)

c A fossil of an extinct species of Monotremata was found in a cave. Describe how scientists could determine whether the fossil is more closely related to a short-beaked echidna or a platypus. (2 MARKS)

Multiple lessons

Question 11 (1 MARK)

Which of the following options best explains how amino acid differences in proteins can indicate evolutionary relationships?

A Mixing proteins of two species will cause them to bind together. The higher the temperature the proteins separate, the more closely related the two species are.

B Over time, mutations accumulate that may change the sequence of amino acids. The more differences in the amino acid sequence, the less related the two species are.

C Over time, silent mutations accumulate in DNA that don't change the amino acid sequence. The resultant proteins look the same and this similarity indicates closely related species.

D Mutations in amino acids accumulate over time, resulting in metabolic enzymes with different structures. The rate at which each enzyme catalyses reactions may be compared between species to determine relatedness.

Question 12 (1 MARK)

Which of the following is an example of a vestigial structure?

A Pharyngeal slits in terrestrial mammals and birds, which form the bones in the inner ear and provide structure.

B The pelvic bones in modern-day whales and dolphins, which serve no observable purpose.

C Similar bone structures in the forelimbs of bats and humans, used for locomotion.

D Similar limb shapes in penguins and fish, which serve similar functions.

Question 13 (1 MARK)

Research has shown that the sequence of nucleotides found in a number of particular genes is unique to humans and is not found in chimpanzees.

Gene with sequence unique to humans	Functional role of gene with sequence unique to humans
HAR1	active in the brain necessary for development of the cerebral cortex
FOXP2	facilitates formation of words by the mouth
AMY1	facilitates digestion of starch
ASPM	controls brain size
LCT	permits digestion of milk sugar in adulthood
HAR2	drives gene activity in the wrist and thumb during development

Using the information in the table, it is reasonable to conclude that chimpanzees

A are unable to digest starch.

B are able to digest milk sugar in adulthood.

C have a similar ability to form words by mouth as humans.

D process and remember more complex information than humans.

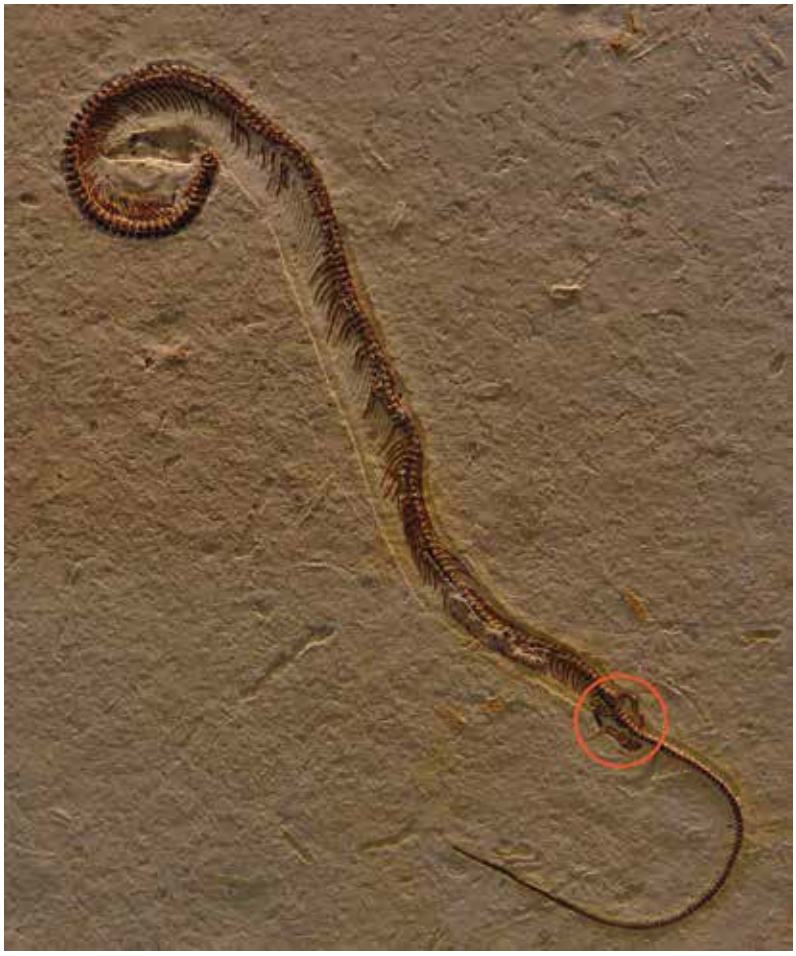
Question 14 (10 MARKS)

Members of the suborder Serpentes (modern snakes) are limbless, carnivorous reptiles. Originally thought to be the precursor to modern lizards, the presence of certain vestigial limbs suggests that this theory is incorrect. Rather, members of the suborder Serpentes are actually descendants of lizards.

a With reference to the information provided, explain what is meant by the term 'vestigial limbs'. (1 MARK)

b How did vestigial limbs arise in modern snakes? (3 MARKS)

c Scientists recently found a fossil, *Tetrapodophis amplectus*, surrounded by ash within sedimentary rock. The fossil shown possesses skeletal hindlimbs and is an intermediary species between ancient lizards and modern snakes.



Scientists have calculated the relative age of *Tetrapodophis amplectus* to be 100 million years old, using the absolute age of the igneous sedimentary layer.

i Explain how the scientists calculated the age of the igneous sedimentary rock. (2 MARKS)

ii Outline why the scientists did not calculate the age of the rock using carbon-14? (1 MARK)

iii How did the scientists approximate the age of *Tetrapodophis amplectus* from the surrounding rock? (2 MARKS)

iv How can this be used to determine when modern snakes began to diverge from lizards? (1 MARK)

Question 15 (5 MARKS)

Mitochondria contain their own genome, as they were once unicellular prokaryotes. This DNA, known as mtDNA, is maternally inherited, meaning all offspring will only possess their mother's mtDNA. The Mitochondrial Eve Hypothesis suggests that the mitochondrial DNA of all living people can be traced back to a few women in Africa.

mtDNA from five modern-day humans of different backgrounds were sequenced and their sequences at seven sites are shown.

Individual	Site A	Site B	Site C	Site D	Site E	Site F	Site G
Individual 1	ATT	GA**C**	CCA	TGG	AAG	CCC	TTG
Individual 2	ATT	GA**C**	CCA	TGG	AAG	CC**T**	TTG
Individual 3	ATT	GAG	C**A**A	TGG	**C**AG	C**G**C	TT**A**
Individual 4	ATT	GAG	CC**T**	TG**A**	AAG	CCC	TTG
Individual 5	ATT	**T**AG	C**A**A	TGG	**C**AG	CCC	T**CA**

a Based on the data, which two individuals are the most closely related? Explain your reasoning. (2 MARKS)

b The sequence TGA found in individual 4 at site D encodes a stop triplet, whereas TGG encodes a Trp triplet.

Name this type of point mutation and explain the consequences this would have on gene expression if the mutation was found in a protein-coding gene. (3 MARKS)

Key science skills and ethical understanding

Question 16 (5 MARKS)

Albumin is a protein found in blood plasma. It consists of a single polypeptide chain with 584 amino acids. A group of students conducted a scientific investigation to test the similarity in the amino acid sequence of albumin between humans and gorillas, gibbons and kangaroos. It is well known that humans are most closely related to gorillas, followed by gibbons and are least related to kangaroos. A rabbit was injected with human albumin. As a result, it produced human albumin antibodies in its blood. There were 30–40 different antibodies produced, each specific to a particular antigenic site on the human albumin molecule. The antibodies were collected and divided into 4 samples. Sample 1 was mixed with human albumin. Sample 2 was mixed with kangaroo albumin. Sample 3 was mixed with gibbon albumin. Sample 4 was mixed with gorilla albumin.

The observations were recorded in the given table. Precipitate refers to albumin-antibody complexes.

Trial number	Albumin mixed with the antibodies
1	Human
2	Kangaroo
3	Gibbon
4	Gorilla

a Predict which trial will have the least amount of precipitate formed. Explain your answer. (2 MARKS)

b Identify the control of the experiment. (1 MARK)

c To collect antibodies from the rabbits, the students used needles to take their blood instead of cutting parts of their bodies and letting them bleed. The students then purified the antibodies from the collected blood. Identify the bioethical concept that has been applied by the students. Explain your response. (2 MARKS)

10C PHYLOGENETIC TREES

It has been suggested that there are more than one trillion different organisms on Earth. Each of these organisms is related to other organisms to varying degrees. But how do we show this relatedness between them? How do we represent the tree of life?

Image: Chayasit Fangem/Shutterstock.com

Lesson 10C

In this lesson you will learn about phylogenetic trees and how to use them to illustrate and understand the relationships between groups of organisms.

◀ *Prerequisite knowledge* | *Future applications* ▶

Lesson 10A

Information gathered from fossil evidence is crucial in determining relatedness between different species, allowing for researchers to build phylogenetic trees.

Lesson 10B

There are many different pieces of evidence used to determine relatedness between different species, including homologous structures, vestigial structures, and molecular homology, which help us create phylogenetic trees.

Chapter 11

Phylogenetic trees can be used to represent the relatedness between modern humans and their ancestors as well as their evolution from other ancestral species.

Study design dot point

- the use and interpretation of phylogenetic trees as evidence for the relatedness between species

Key knowledge units

What is a phylogenetic tree?	4.2.8.1
Interpreting phylogenetic trees	4.2.8.2

What is a phylogenetic tree? 4.2.8.1

OVERVIEW

A phylogenetic tree is a diagram that shows the evolutionary relationships between different species.

THEORY DETAILS

In lesson 10B, we explored how structural morphology and molecular homology can be used to determine relatedness between different species. While understanding that different species are related is beneficial, we need a method of representing or mapping out how all of these different species are related to each other. How do we achieve this? **Phylogenetics**!

Phylogenetics is the study of the evolutionary history of an organism or group of organisms, with the goal of describing an organism's relationships to other organisms, both past and present. This information can be represented in a **phylogenetic tree**, which is a diagram used to illustrate **evolutionary relationships** between species. They can be useful in displaying:

- the timeline of **lineages**
- relatedness between **taxa**
- shared characteristics of different taxa.

phylogenetics the study of the relatedness between organisms

phylogenetic tree a diagram used to show the relatedness between organisms

evolutionary relationship the relatedness of organisms based on shared ancestry

lineage a direct sequence of species that evolved from a common ancestor

taxon (pl. taxa) a unit of biological classification into which related organisms are classified. Taxa are arranged in a hierarchical rank from kingdom down to species, where members of a specific taxon typically share certain morphological characteristics

The structure of a phylogenetic tree

There are many different components of a phylogenetic tree, including a **root**, **branches**, **nodes**, and **leaves**. These are summarised in Table 1.

root represents the most recent common ancestor for all members of a phylogenetic tree

branch a line on a phylogenetic tree that represents an evolutionary path

node the splitting point between two branches on a phylogenetic tree, representing a speciation event

leaf the end of a branch that shows the current (or final) form of a species

Table 1 The components of a typical phylogenetic tree

Component	Description
Root	A line at the origin, representing the earliest common ancestor
Branch	Each line on the phylogenetic tree
Node	A point where the branches split from each other, representing a divergence between those two taxa
Leaf	The end of a branch, representing where the present-day or extinct species are found. They are labelled with the species or taxa name

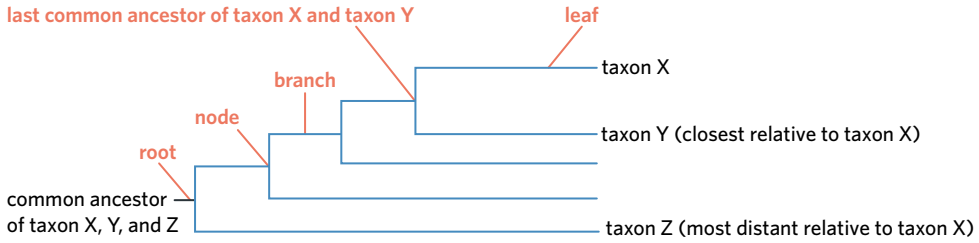


Figure 1 The basic structure of a phylogenetic tree

Examiners' tip

Phylogenetic trees can have several different shapes (Figure 2). You will notice, for example, that the second phylogenetic tree is simply the first phylogenetic tree turned by 90°. All three phylogenetic trees represent the same information. It is important to be aware of these different depictions and to be able to read the key information, as the VCAA will often present phylogenetic trees in a range of different formats.

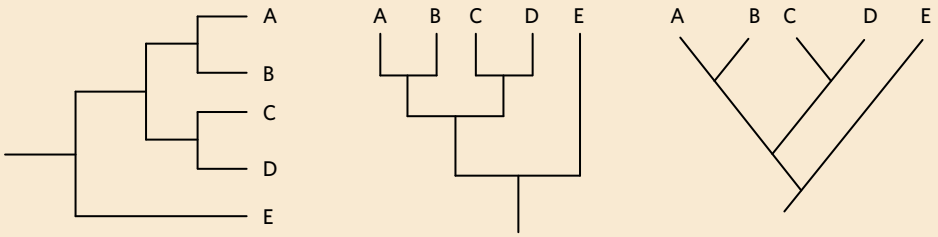


Figure 2 Different shapes of phylogenetic trees

Interpreting phylogenetic trees 4.2.8.2

OVERVIEW

Evolutionary relationships between different organisms can be determined by interpreting phylogenetic trees.

THEORY DETAILS

Reading phylogenetic trees

Phylogenetic trees can be read backwards to determine the most closely related species to a particular taxon. Using Figure 3, you can trace back from the 'human' leaf to reach the next node (node A), which splits humans from monkeys, showing that monkeys are the closest relative to humans on this tree. You can trace the tree further back to the next node (node B), which separates humans and monkeys from dolphins and sheep, showing that humans are more closely related to monkeys than they are to dolphins and sheep. It's important to note that the most recent common ancestor of humans and monkeys occurs at node A.

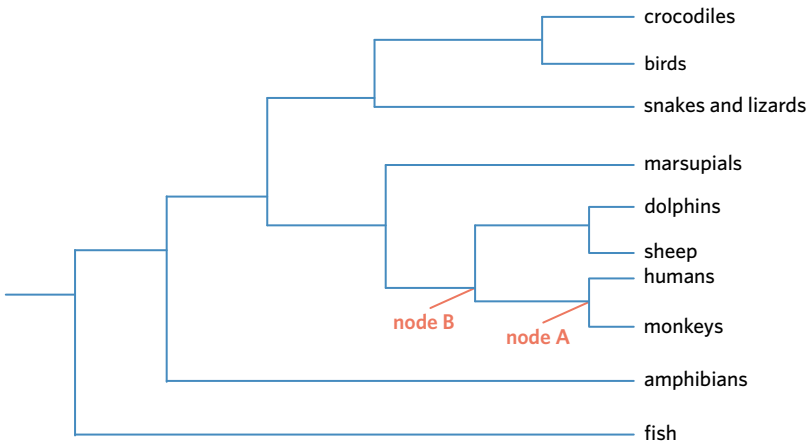


Figure 3 Phylogenetic tree of vertebrates. Node A represents the most recent divergence for humans and node B represents the second most recent divergence for humans.

Phylogenetic trees may include a timescale in order to show the time points of divergence events. When a phylogenetic tree includes a timescale, the branch length represents time. You can see in Figure 4 that Ethiopian wolves diverged from dogs four million years ago.

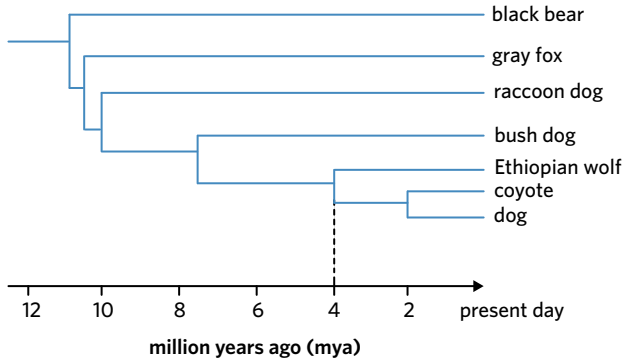


Figure 4 Phylogenetic tree of dogs and some of their relatives, including a timescale

Constructing phylogenetic trees

To understand how to construct a phylogenetic tree, we will look at an example based on the presence or absence of particular homologous structures. In Table 2, the traits of different animals are shown ('+' indicates the presence of a feature and '–' indicates the absence of a feature).

Table 2 The presence or absence of particular homologous structures used to construct a phylogenetic tree

Trait	Bony fish	Amphibians	Marsupials	Placental mammals
Four legs	–	+	+	+
Fur	–	–	+	+

A phylogenetic tree of these animals can be constructed using the following steps (Figure 5):

1. We first figure out the trait that is shared by the largest number of animals. In Table 2, most of the animals have four legs, except for bony fish. Therefore, we can draw the bony fish lineage branching off from the rest of the animals.
2. Then we look for the trait shared by the second-largest number of animals, which in this case is the fur. Among the remaining animals, only amphibians do not have this trait, so we draw the amphibians lineage branching off from the rest of the animals before the 'fur' label.
3. Finally, it can be noticed that both marsupials and placental mammals have fur, so the marsupials and placental mammals lineages are branching directly from the trait fur.

Lesson link

In ***chapter 11***, phylogenetic trees will help you understand the evolution of human species. They are used to model human ancestry and show species which are closely related to humans.

Lesson link

Recall from ***lesson 10B***, relatedness between different organisms can be determined based on structural morphology and molecular morphology, which can be used to construct phylogenetic trees.

Theory in action

Check out scientific investigation 10.1 to put this into action!

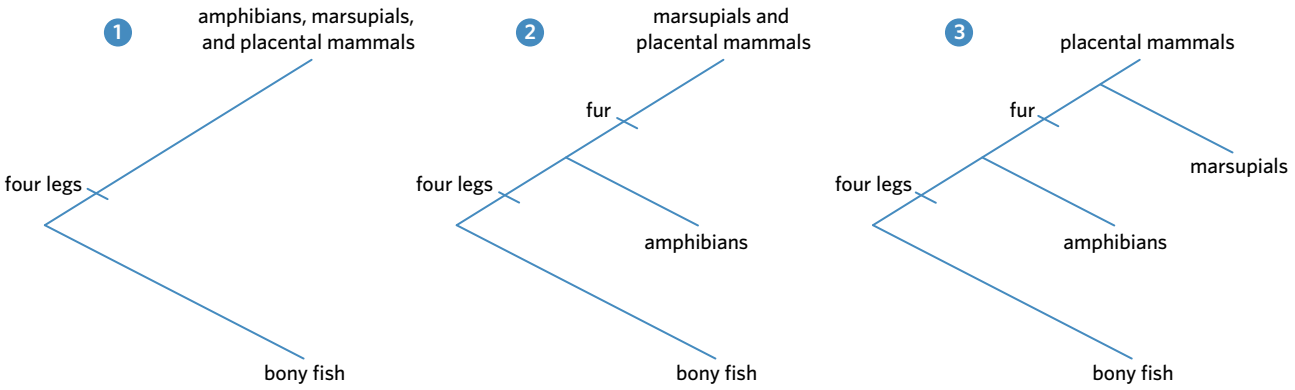


Figure 5 Steps of building a phylogenetic tree

Depicting uncertainties in phylogenetic trees

Often uncertainties may occur when using fossil evidence, since dating techniques are not always completely accurate and fossils are not typically perfectly preserved. These uncertainties can be expressed using phylogenetic trees in several ways (Figure 6).

The lack of a node between species Y and Z means that the exact divergence point is unknown. The break between species W and X means that W is possibly an ancestor of X but there is no evidence of transitional fossils between the two species to support this hypothesis. The branch with species S does not reach the end of the tree, indicating that it is extinct.

Nodes usually only split into two lineages, but sometimes they can split into three or more, as seen between species T, U, and V. This means that it's unclear which species diverged from the others first. This occurs if there is insufficient data or if two speciation events occurred closely together – such as with **adaptive radiation**.

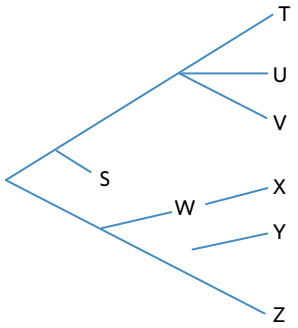


Figure 6 Phylogenetic tree showing the evolution of species. This tree is based on fossil evidence and is full of uncertainties.

adaptive radiation the rapid divergent evolution of a species, thereby producing a wide array of species/forms

Exchanging genetic material between groups

Sometimes genetic material is passed between groups after they have diverged. For example, there is strong evidence to suggest that certain groups of modern humans (*Homo sapiens*) interbred with Neanderthals (*Homo neanderthalensis*), causing parts of their genomes to be passed between each other. This is depicted using a line between branches (Figure 7).

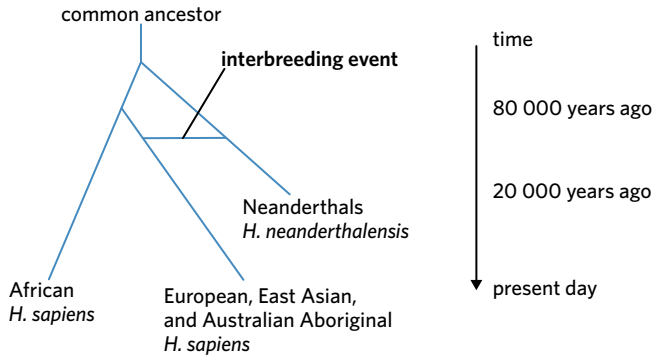


Figure 7 Phylogenetic tree depicting interbreeding between modern humans and Neanderthals

Theory summary

Phylogenetic trees are diagrams used to show the relatedness between organisms. By interpreting phylogenetic trees, we can understand how related some organisms are to others as well as trace back the lineage of different groups of organisms. A phylogenetic tree can be constructed by looking at the traits shared by a large group of species.

Trees hold a special place in our hearts, providing us with not only fresh oxygen and beautiful landscapes, but now also phylogenetic trees for us to represent complex relationships between different species. We can use phylogenetic trees, which are tree-shaped diagrams, to represent the relatedness between different organisms. Uncoincidentally, the structures of a phylogenetic tree are named after the structures of a tree – root, branch, node, and leaf.

Image: gidl/Shutterstock.com

10C QUESTIONS

Theory review questions

Question 1

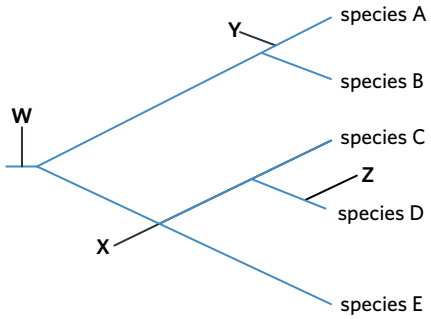
Fill in the blanks in the following sentences.

In a phylogenetic tree, a _____ is a line of species that evolved from a common predecessor. A _____ is a group of related organisms (e.g. class, order, kingdom).

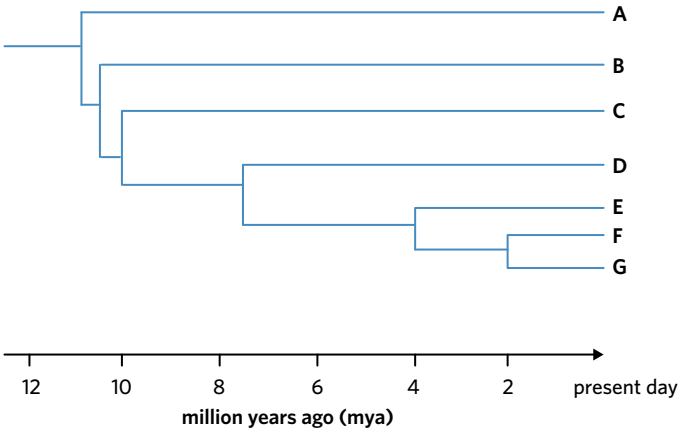
Question 2

Label the parts of the phylogenetic tree from the list of terms.

- branch
- leaf
- node
- root



Question 3



According to the phylogenetic tree, Species F and Species G diverged from their most recent common ancestor

A ~4 million years ago.

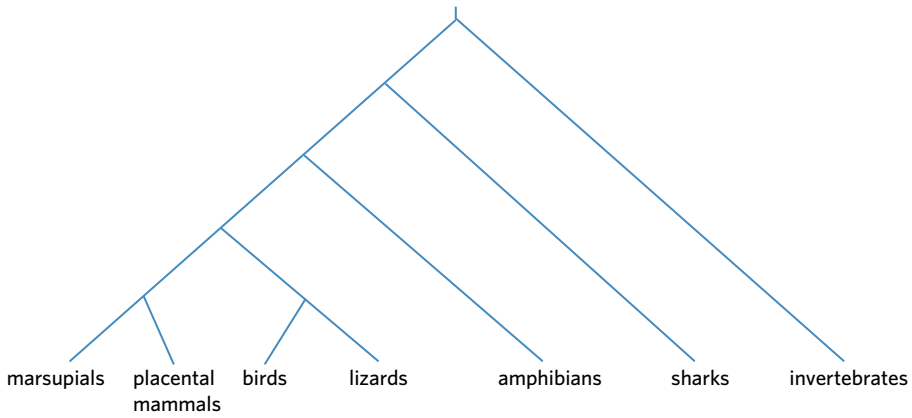
B ~2 million years ago.

C ~6 million years ago.

D more than 7 million years ago.

Question 4

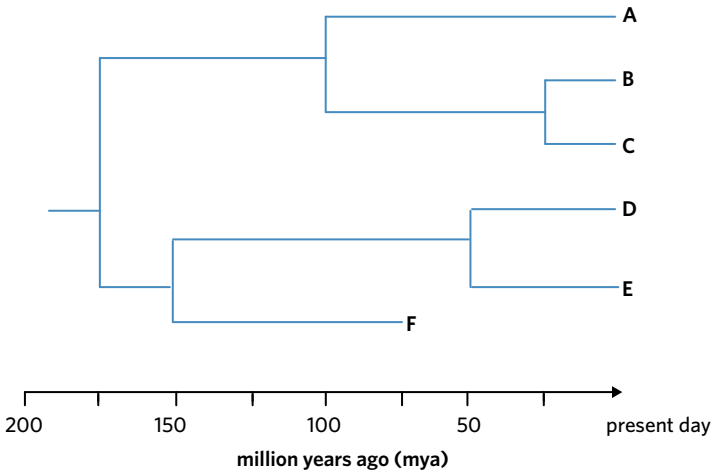
According to the phylogenetic tree, which of the following species is the most closely related to lizards?



A placental mammals
B amphibians
C sharks
D birds

Question 5

Which of the following is correct regarding the phylogenetic tree shown?



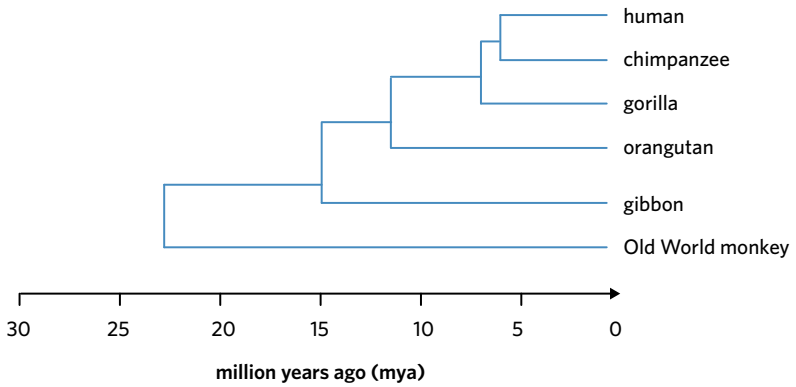
A Species F is extinct.
B Species F diverged from Species D around 50 mya.
C The split between Species D and E is the most recent divergence.
D Species E is more closely related to Species A than it is Species F.

SAC skills questions

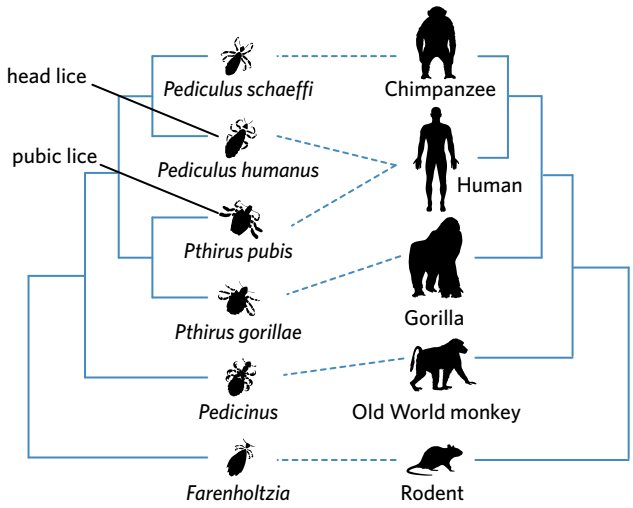
Case study analysis

Use the following information to answer Questions 6–9.

Lice are highly specialised blood sucking parasites that live on specific host species. Human head lice and chimpanzee lice belong to the same genus, but they diverged into two separate lineages millions of years ago. Scientists hypothesise that human head lice and chimpanzee lice diverged at the same time that humans and chimpanzees diverged (Hypothesis 1). To test the hypothesis, they compared the DNA sequences of two genes found in both human head lice and chimpanzee lice: the mitochondrial *cytochrome c oxidase subunit I (Cox1)* gene and the *elongation factor 1-alpha (eEF1a1)* gene. The test results indicated that the most recent ancestor of human head lice and chimpanzee lice lived around 6 million years ago. These findings were compared against a known phylogenetic tree of human evolution.



Following this experiment, the scientists further hypothesised that human head lice and human pubic lice diverged from a common ancestor around 6 million years ago on a human ancestor host (Hypothesis 2). They also tested the hypothesis by again comparing the DNA sequences of the *Cox1* gene and *eEF1a1* gene in human head lice and human pubic lice. The test results showed that the most recent common ancestor of human head lice and human pubic lice lived around 12 million years ago.



Question 6

When did humans and chimpanzees diverge from their most recent common ancestor?

A ~5 million years ago

B ~6 million years ago

C ~15 million years ago

D ~10 million years ago

Question 7

Was Hypothesis 1 supported by the findings?

A Yes, because there was similarity between *Cox1* gene and *eEF1a1* gene around 6 mya.

B No, because human head lice existed much earlier than humans and chimpanzees diverged from their most recent ancestor.

C Yes, because human head lice and chimpanzee lice diverged from their most recent ancestor around 6 mya and so did humans and chimpanzees.

D No, because human head lice and chimpanzee lice diverged from their most recent ancestor around 6 mya, while humans and chimpanzees diverged from their most recent ancestor around 5 mya.

Question 8

Based on the DNA test results, what could the scientists conclude about Hypothesis 2?

A Human head and pubic lice diverged ~6 million years ago on a human ancestor host.

B Human head and pubic lice diverged ~12 million years ago on a human ancestor host.

C Human head and pubic lice diverged ~6 million years ago but not on a human ancestor host.

D Human head and pubic lice diverged ~12 million years ago but not on a human ancestor host.

Question 9

Based on the given phylogenetic tree, which species is the most closely related to human pubic lice?

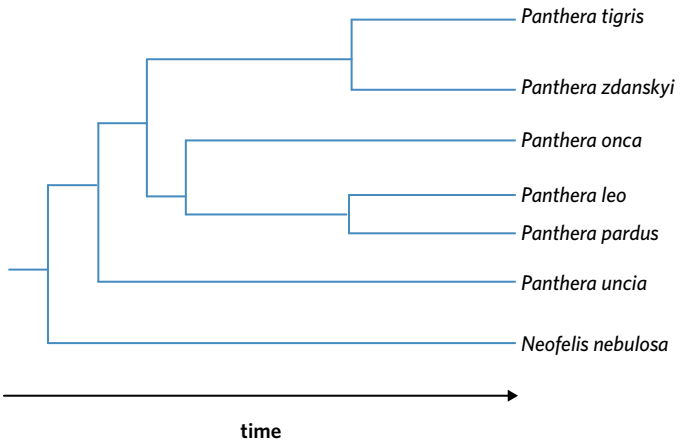
A Old World monkey lice
B human head lice
C chimpanzee lice
D gorilla lice

Exam-style questions

Within lesson

Question 10 (1 MARK)

The following phylogenetic tree shows the evolutionary relationship between wild cat species.

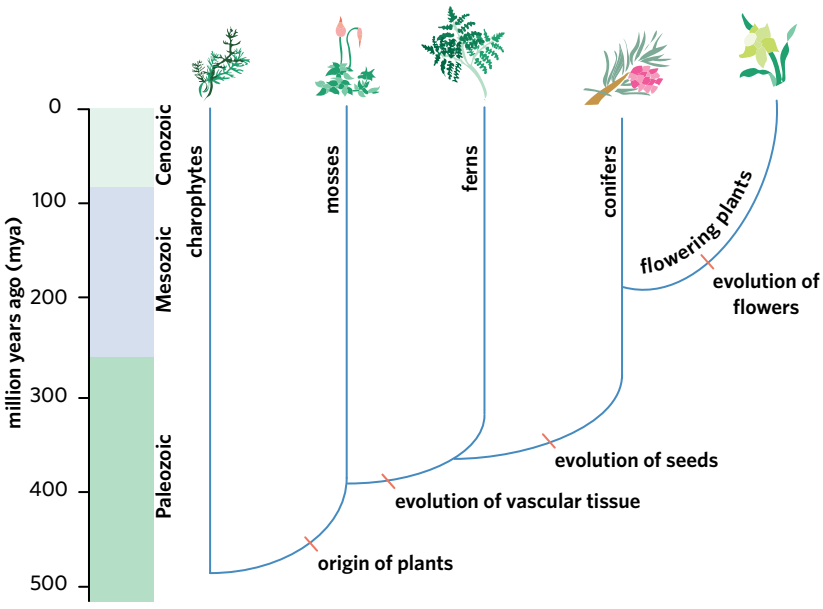


The information in the phylogenetic tree suggests that

A *Panthera leo* shares a more recent common ancestor with *Panthera tigris* than with *Panthera onca*.
B *Panthera uncia* is more closely related to *Panthera pardus* than it is to *Panthera zdanskyi*.
C *Panthera tigris* is more closely related to *Panthera pardus* than it is to *Panthera uncia*.
D *Neofelis nebulosa* is an ancestor of the other six species.

Use the following information to answer Questions 11 and 12.

In the evolution of plants there have been several major adaptations. These are shown in the image of the phylogenetic tree.



Question 11 (1 MARK)

Based on the diagram, which of the following statements is false?

A Ferns are more closely related to conifers than they are to mosses.

B Flowers evolved from charophytes around 160 million years ago.

C Seeds evolved in plants around 350 million years ago.

D Mosses do not contain vascular tissue.

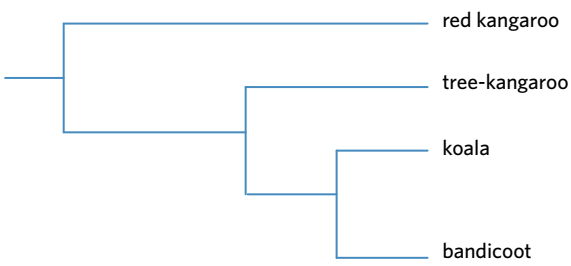
Question 12 (1 MARK)

The table shows a summary of three traits in four marsupials.

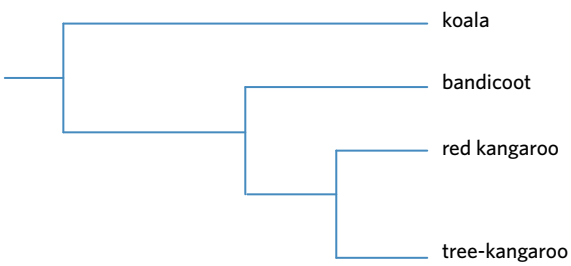
Animal	Pouch	Diet	Locomotion
red kangaroo	present	herbivore	bipedal
bandicoot	present	omnivore	quadrupedal
tree-kangaroo	present	herbivore	bipedal
koala	present	herbivore	quadrupedal

Using this information, which of the following phylogenetic trees shows the most likely evolutionary relationship between these four marsupials?

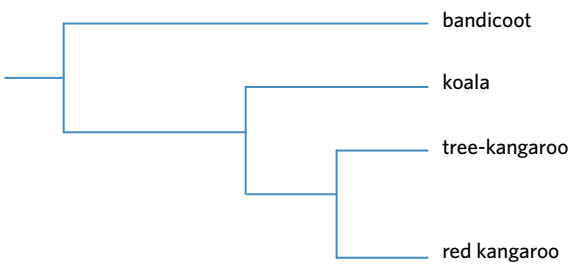
A



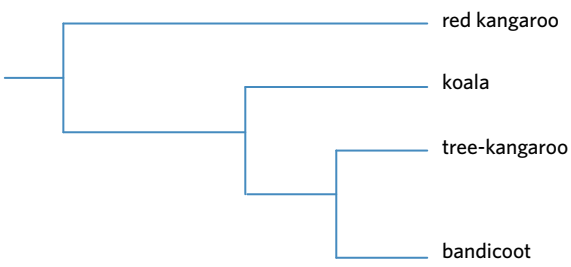
B



C



D



Question 13 (1 MARK)

Scientists found a fossil impression of a now-extinct species of plant. This species was identified as a clubmoss, a class of vascular plants that do not contain seeds or flowers. Instead, clubmosses reproduce using spores found at the base of their leaves.

Based on this information, what time period would clubmosses most likely have first appeared in the fossil record?

A 470–400 mya

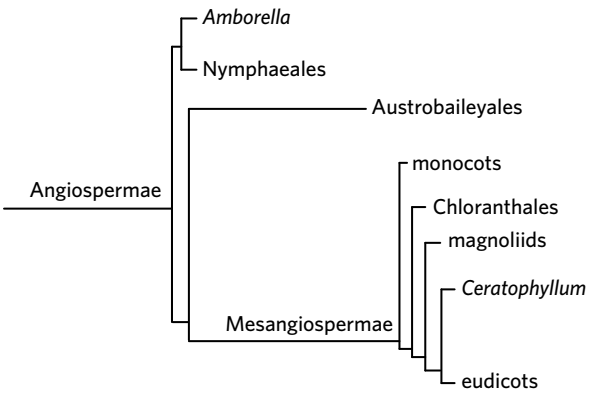
B 390–350 mya

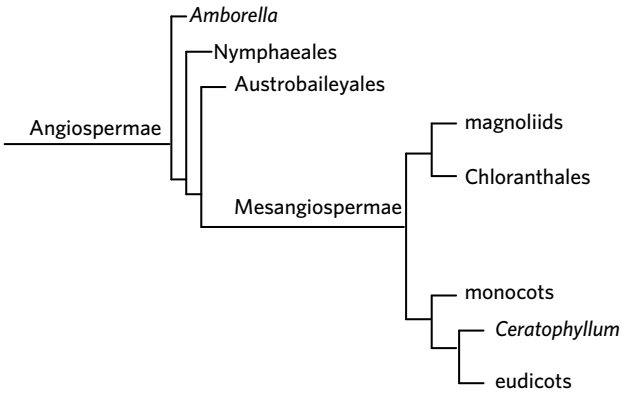
C 350–160 mya

D 160–50 mya

Question 14 (4 MARKS)

Two possible phylogenetic relationships between eight groups of flowering plants are shown in the two diagrams. Each diagram represents the relationships between the species slightly differently.





a Identify two similarities between the evolutionary relationships depicted in both alternatives. (2 MARKS)

b Identify two differences between the evolutionary relationships depicted in both alternatives. (2 MARKS)

Adapted from VCAA 2011 Exam 2 Section A Q23

Question 15 (3 MARKS)

The traits of five animals are shown in the table.

Trait	Lamprey	Antelope	Alligator	Seabass	Bald eagle
Lungs	–	+	+	–	+
Jaws	–	+	+	+	+
Gizzard	–	–	+	–	+

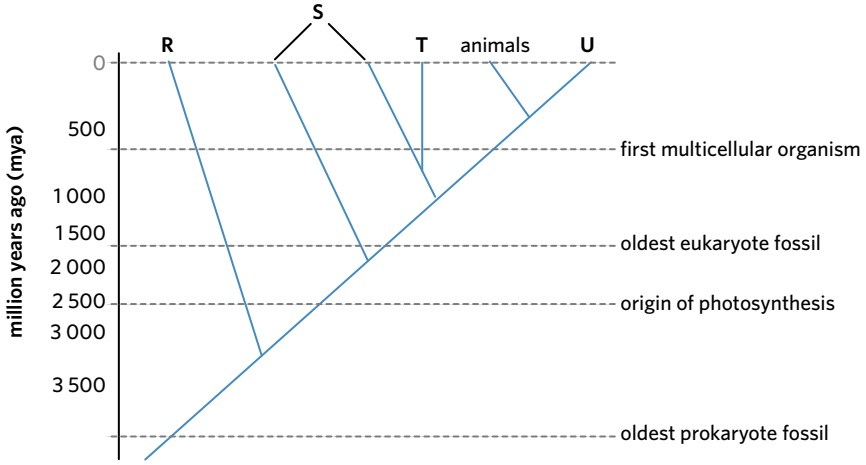
a Draw a phylogenetic tree of the animals. (2 MARKS)

b Using the phylogenetic tree you constructed, identify the animal that is the most closely related to alligators. (1 MARK)

Multiple lessons

Question 16 (1 MARK)

The phylogenetic tree represents the approximate order and time of appearance of the major groups of living organisms. It includes four groups represented by the letters R, S, T, and U.



Which of the following shows the correct placement of the organisms on the phylogenetic tree?

A R – bacteria, S – protists, T – plants, U – fungi

B R – bacteria, S – protists, T – fungi, U – plants

C R – protists, S – bacteria, T – plants, U – fungi

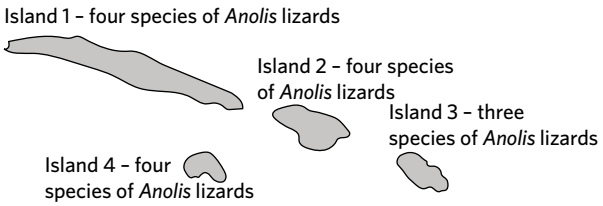
D R – bacteria, S – plants, T – protists, U – fungi

Adapted from VCAA 2017 Section A Q32

Question 17 (4 MARKS)

Scientists studied 15 species of lizards of the genus *Anolis* on four islands in the Caribbean Sea. On each island, there were up to four different species of these lizards. The lizards on each island tended to show similar morphology to other lizards on that island, including tail length and egg colour.

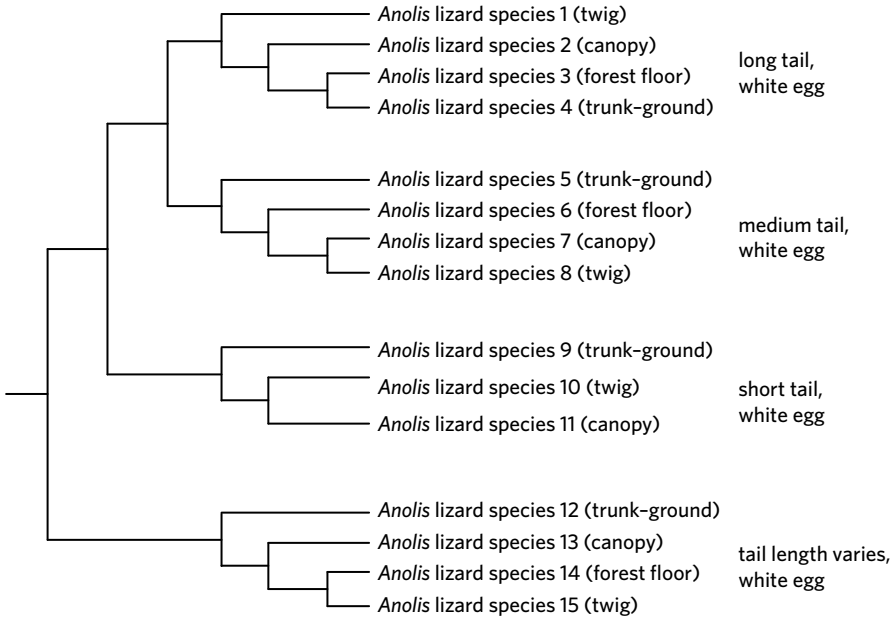
Habitat	Characteristics common to *Anolis* species living in each habitat
canopy (leafy section at the top of the tall trees)	large body, large toe pads, able to climb on the surface of broad leaves
twig (part-way up the trees on thin branches)	small body, very short legs, moves slowly, able to cling to twigs
trunk-ground (the trunks of the trees and the ground around the trunks)	chunky body, long legs, able to move very fast
forest floor (grassy areas and bushes between the trees)	long tail, slender body, short legs, able to cling to grasses and thin branches



Each island is covered with large areas of forest. In the forests, there are four distinct habitats, as described in the table. The habitats correspond to different layers of the forest, from the canopy down to the forest floor. The scientists noticed that each of the habitats on each island was inhabited by just one of the species of lizards found on that island.

a Explain how morphological data of various lizard species could be used to construct a phylogenetic tree. (1 MARK)

b The following phylogenetic tree was constructed by scientists for the *Anolis* genus of lizards based on tail length and egg colour. In brackets is the habitat type of each species.



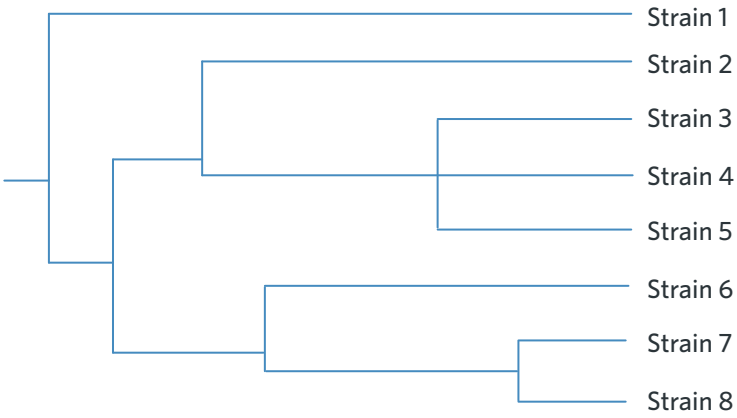
i Use the phylogenetic tree to justify whether *Anolis* lizard species 11 is more closely related to *Anolis* lizard species 3 or *Anolis* lizard species 12. (1 MARK)

ii Explain how the phylogenetic tree supports the hypothesis that trunk-dwelling lifestyles evolved separately on four occasions. (2 MARKS)

Adapted from VCAA 2018 Northern Hemisphere Exam Section B Q8

Question 18 (9 MARKS)

In humans, severe acute respiratory syndrome (SARS) is a serious form of pneumonia. SARS is caused by a coronavirus that was first identified in 2003. Scientists suspected that the virus had been transmitted to humans from another animal. Testing was completed on several animal species. Strains of the coronavirus similar to those found in humans were identified in different species of horseshoe bats (genus *Rhinolophus*) and palm civets (*Paguma larvata*). Morphological data, such as protein coat composition, was used to determine relatedness between strains of the virus.



a How can molecular evidence be used to assess the relatedness between two strains of the virus? (2 MARKS)

b The molecular data enabled the scientists to draw an evolutionary tree for different strains of the virus. The branch length indicates time since divergence.

i Which two strains would be expected to be the most molecularly similar? Justify your response. (2 MARKS)

ii The branches for strains 3, 4, and 5 diverge at the same point. Explain how this can occur with reference to uncertainties in the data. (2 MARKS)

c Strains 3 and 4 are found in palm civets and strains 5 and 6 are found in humans. All other strains are found in different species of horseshoe bats.

i What conclusion can be drawn about the origin of the strains of virus that cause SARS in palm civets? (1 MARK)

ii What conclusion can be drawn about the origin of the strains of virus that cause SARS in humans? (2 MARKS)

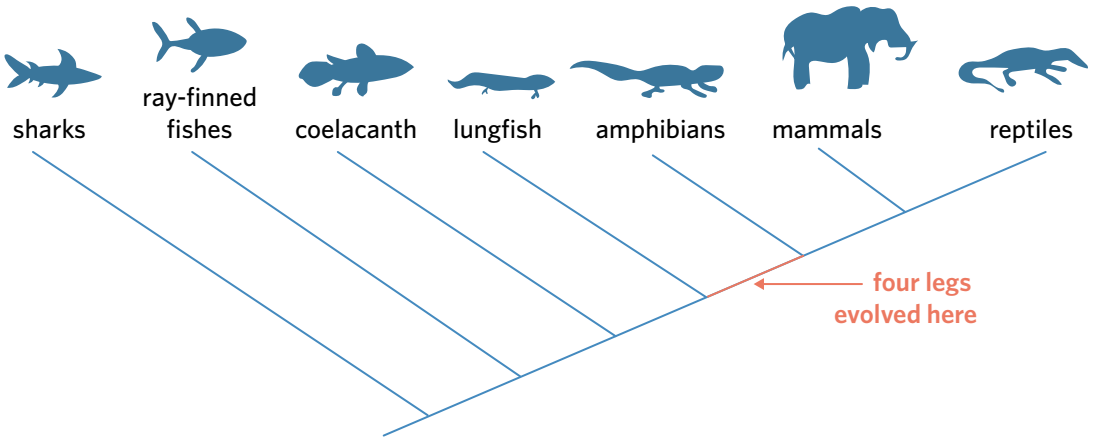
Adapted from VCAA 2013 Section B Q10

Key science skills and ethical understanding

Question 19 (6 MARKS)

Tetrapods (meaning 'four legs') are a group of animals that have four limbs and include amphibians, mammals, and reptiles. Tetrapods are predominantly terrestrial animals but evolved from a fish ancestor that lived in marine environments.
The closest living marine relatives of tetrapods are lungfish, which have highly specialised lungs that allow them to take gulps of air for oxygen.

The phylogenetic tree summarises the evolutionary relationships of tetrapods among vertebrates.



a Based on the phylogenetic tree, what is the most recent divergence among vertebrates? (1 MARK)

b A fossil named *Tiktaalik*, that remains to be dated, was found by a group of scientists. The scientists hypothesise that it is a transitional fossil between ancestral fish and tetrapods.

i Predict two structural features of the *Tiktaalik* fossil that would provide evidence to support the hypothesis that it is a transitional fossil and suggest a survival advantage on land of each feature. (2 MARKS)

ii Describe a piece of evidence that might refute the hypothesis that *Tiktaalik* is a transitional fossil between a fish ancestor and present-day tetrapods. (1 MARK)

iii Another group of scientists suggested that it is not a transitional fossil because it does not have features that are shared between ancestral fish and tetrapods. In this case, which bioethical concept should be followed by both groups of scientists? Explain your answer. (2 MARKS)

Adapted from VCAA 2018 Section B Q9b

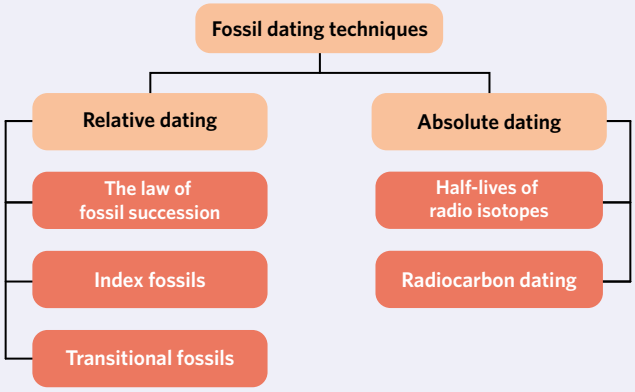
CHAPTER 10 SUMMARY

Fossils

Fossils are the preserved body, impressions, or traces of a dead organism

Good conditions for fossilisation

- areas of rapid sediment accumulation
- constant cool temperatures
- low oxygen availability
- low light exposure
- physical protection from scavengers and decomposers (e.g. fungi, bacteria)



Relative dating determining a fossil's approximate age based on the position of the fossil compared to other fossils

The law of fossil succession fossils of the same age will be in the same layer of sedimentary rock, and fossils found in a lower or higher sedimentary layer will be older or younger respectively

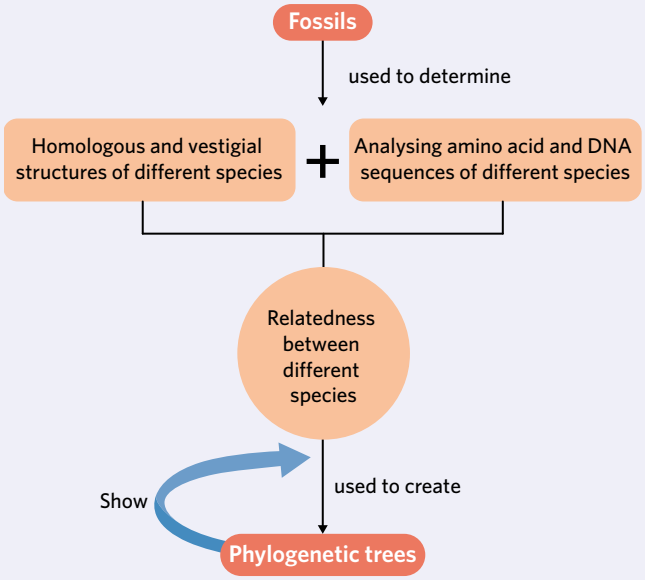
Index fossils widespread fossils which existed for a short period and have a known geological age

Transitional fossils show traits that are common to both its ancestral group and its descendant group

Absolute dating determining the absolute age (in years) of a fossil

Half lives of radioactive isotopes the time taken for half the mass of a radioisotope sample to break down into its products which is used to calculate the absolute age of a fossil

Radiocarbon dating using the decay of carbon-14 (^{14}C) radioisotope to nitrogen-14 (^{14}N) to estimate the absolute age of a fossil



Structural morphology

1 **Homologous structures** features that have the same underlying structure but different functions. The presence of homologous structures in different species suggests they share a common ancestor.

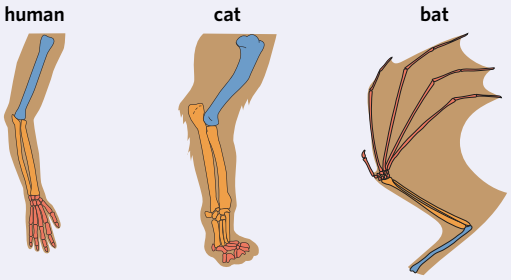


Image: Usagi-P/Shutterstock.com

2 **Vestigial structures** structures that remain within a species despite losing their function and necessity. Like homologous structures, their presence suggests the organisms being compared share a common ancestor.

Image: SciePro/Shutterstock.com

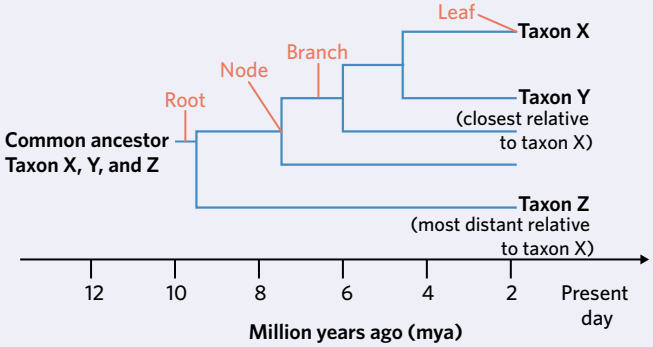
Molecular homology

1 **Amino acid sequences** researchers can examine shared proteins between species and examine the degree of amino acid similarity between them to demonstrate relatedness. A higher degree of similarity suggests the species being compared are more closely related.

2 **DNA sequences** researchers can consider corresponding gene regions (or entire genomes) between species, and demonstrate degrees of relatedness based on nucleotide differences. A higher degree of similarity suggests the species being compared are more closely related.

Phylogenetic trees

Phylogenetic trees are diagrams used to show the relatedness between different species.



Component	Description
Root	A line at the origin, representing the earliest common ancestor
Node	A point where the branches split from each other, representing a divergence between those two taxa
Branch	Each line on the phylogenetic tree
Leaf	The end of a branch, representing where the present-day or extinct species are found. They are labelled with the species or taxa name

CHAPTER 10 SAC PRACTICE

SAC skills covered in this section:

✓ Case study analysis ✓ Data analysis ✓ Scientific methodology comparison

EUCALYPTUS FOSSILS (20 MARKS)

Eucalypts, commonly known as gum trees, are dominant trees in Australia that can be found in most terrestrial environments, except for a small region of rainforests, acacia, and some deserts. With over 800 species, the trees contribute significantly to Australian biodiversity. Researchers suggested they started spreading around Australia when the continent started drying up around 20 million years ago.

Although eucalypts are native to Australia, the oldest known eucalypt fossils of fruits, flowers, buds, and leaves were discovered in Patagonia, South America. It has been a challenge for researchers to determine the absolute ages of eucalypt fossils in Australia due to the size of Australia and the small number of research groups looking for fossils. Some researchers estimated that they date to the Late Cretaceous period (approximately 66–100.5 million years ago). However, a recent study indicates that fossils first existed in the Early Eocene period (33.9–56 million years ago).

1 Describe three conditions that increase the likelihood of fossilisation. (3 MARKS)

2 Briefly describe the absolute dating of a fossil. (1 MARK)

3 According to the text, what are the factors that make identifying the absolute ages of eucalypt fossils so difficult? (2 MARKS)

4 Should ^{14}C radioactive isotopes be used to estimate the absolute age of the oldest known eucalypt fossils in South America? Explain your answer. (2 MARKS)

5 Different research groups have assigned different ages to eucalypt fossils. In this case, identify a bioethical concept that should be followed by both research groups. Explain your response. (2 MARKS)

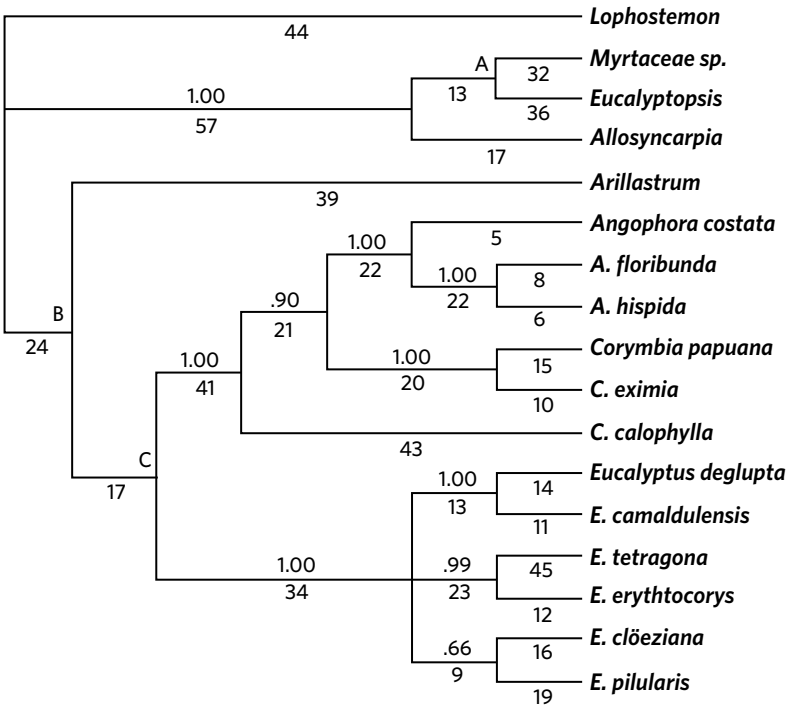
The eucalypt group includes seven genera: *Eucalyptus*, *Corymbia*, *Angophora*, *Eucalyptopsis*, *Stockwellia*, *Allosyncarpia*, and *Arillastrum*. Living in Australia where bushfires frequently occur, trees in the *Eucalyptus* genus have adapted to their harsh environments by developing structures called epicormic buds. These buds, present on both branches and the trunk, are well protected beneath thick bark which allows them to be insulated from the intense heat of the bushfires. When the top or crown of trees is severely damaged following bushfires, the buds sprout to regenerate branches from the trunks. Along with epicormic buds, many species in the *Eucalyptus* genus also develop thick and fibrous bark to protect the buds. Eucalypts undergo bark shedding which can initiate and prolong fires. Shed bark is low in nutrients and breaks down very slowly, which provides significant fuel load for bushfires.

6 Explain the difference between homologous structures and analogous structures. (2 MARKS)

7 A group of scientists have discovered a new genus called X that has a common ancestor with the *Eucalyptus* genus. Trees in the X genus have epicormic buds that sprout in response to fungal infections. In this case, are these epicormic buds considered homologous or analogous structures? Justify your response. (3 MARKS)

8 According to the text, what is a potential structure apart from epicormic buds that can be used to look for species that have close evolutionary relationships with the *Eucalyptus* genus? (1 MARK)

Udovicic & Ladiges (2000) analysed the sequences of a number of different nuclear and chloroplast DNA regions to assess the relatedness between the genera in the eucalypt group. Using their results, they constructed a phylogenetic tree.



9 Explain why the scientists analysed DNA sequences instead of amino acid sequences to examine the evolutionary relationships between the genera in the eucalypt group. (2 MARKS)

10 Using the phylogenetic tree, state which species is the most closely related to *Angophora floribunda* (1 MARK)

11 Using the phylogenetic tree, state which genus is the most closely related to *Corymbia* (1 MARK)

CHAPTER 10 EXAM PRACTICE 60 min

Section A (12 MARKS)

Question 1 (1 MARK)

Fossils found in Australia include representatives from across the geological time scale on Earth. The table shows some of the types of fossils found in Australia and their ages.

Type of fossil	Location	Geological time	Age
Stromatolites	Arkaroola, South Australia	Precambrian era	770 mya
Jellyfish	Flinders ranges, South Australia	Ediacaran period	645–542 mya
Dinosaurs	Many places, including Queensland and Victoria	Jurassic and Cretaceous periods	200–65 mya
Megafuana (large marsupials and flightless birds)	Naracoorte, South Australia	Cainozoic era	65–7000 ya

Note: mya = million years ago; ya = years ago

Which of the following statements is true?

A Jellyfish were not likely dated using carbon-14 dating.
B Stromatolites were likely dated using carbon-14 dating.
C Dinosaurs are one of the earliest examples of multicellular eukaryotes.
D Stromatolites are one of the earliest examples of multicellular eukaryotes.

Adapted from VCAA 2017 Sample Exam Section B Q7a

Question 2 (1 MARK)

The chances of an animal becoming fossilised are increased by

A direct exposure to sunlight.
B the presence of soft tissues.
C rapid burial within sedimentary layers.
D the death of the animal in a predator-rich environment.

Question 3 (1 MARK)

Radioisotopic dating (radiometric dating) is used to determine the age of fossils and surrounding rocks.

Which statement about radioisotopic dating is true?

A Radioisotopic dating can be used to accurately calculate the age of sedimentary rock that existed more than 50 million years ago.
B Radioisotopic dating only reveals the order of fossil formation, not how many years ago the fossil was formed.
C Radioisotopic dating relies on the decomposition of unstable atoms into stable atoms.
D Carbon is primarily used when calculating the age of igneous rocks.

Adapted from VCAA 2017 Northern Hemisphere Section A Q34

Question 4 (1 MARK)

Scientists concluded two modern animals shared a vestigial structure. How can the presence of vestigial structures provide evidence that these animals have a recent common ancestor?

A Vestigial structures are the result of exposure to different selection pressures.
B It is unlikely that two animals would independently evolve the same vestigial structure. Therefore, they must share a recent common ancestor.
C Despite these structures serving different functions, their skeletal structures are similar. Therefore, they must share a recent common ancestor.
D Through exposure to similar selection pressures the two animals evolved structures with similar functions. Therefore, they must share a recent common ancestor.

Question 5 (1 MARK)

The fossil record provides evidence for evolution as it

A shows changes between fossils over time.

B tells us which species hold the record for most fossils.

C explains how fossils of the same species can be found across continents.

D allows scientists to calculate the age of fossils with relative dating techniques.

Question 6 (1 MARK)

Which statement about relative dating techniques is true?

A Relative dating techniques require the presence of index fossils.

B Carbon is the best element to use when determining the age of all fossils.

C Relative dating techniques are only useful for fossils formed less than 100 million years ago.

D Relative dating techniques indicate a fossil's age by determining if one fossil is older than another.

Question 7 (1 MARK)

Modern humans have wisdom teeth even though they are not necessary for us to chew food properly. They used to play an important role in grinding up the rough food in our ancestor's diet, which included hard seeds, nuts, and raw meat.

Wisdom teeth are

A modern structures.

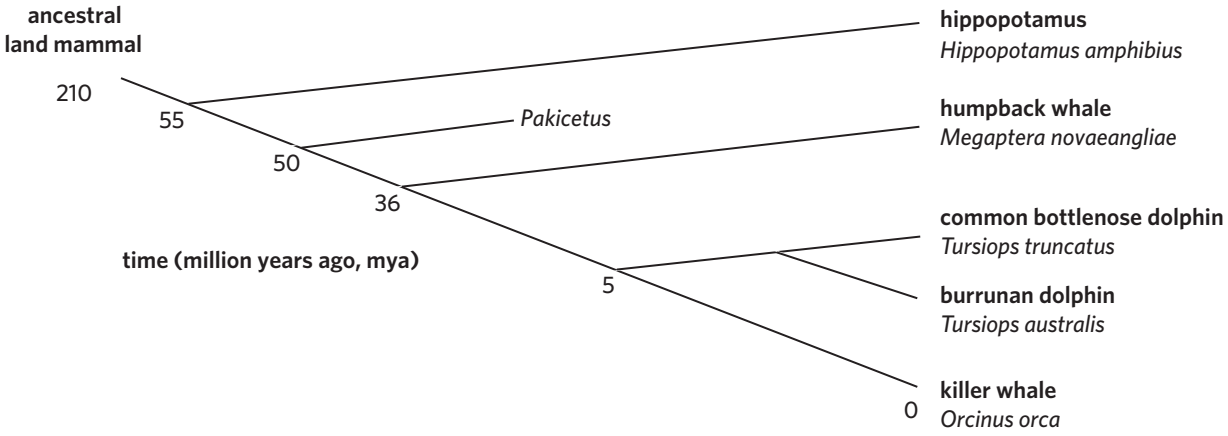
B vestigial structures.

C analogous structures.

D homologous structures.

Use the following information to answer Questions 8 and 9.

Cetaceans (whales, porpoises, and dolphins) are marine mammals belonging to the order Artiodactyla (mostly even-toed hoofed mammals). The closest living relative of cetaceans is *Hippopotamus amphibius*. The following phylogenetic tree summarises the evolutionary relationships of four present-day cetacean species and *Hippopotamus amphibius*.



Question 8 (1 MARK)

Which of the following statements is false?

A The length of the branch of *Pakicetus* suggests it is extinct.

B The *Hippopotamus amphibius* is most closely related to the ancestral land mammal.

C Burrunan dolphins are more closely related to common bottlenose dolphins than they are to killer whales.

D The killer whale diverged from the ancestor of the common bottlenose dolphin and the burrunan dolphin around 5 million years ago.

Adapted from VCAA 2018 Section B Q9a

Question 9 (1 MARK)

A fossil named *Ambulocetus* was found in 1992 and dated to 49 million years old. Some palaeontologists believe that it is a transitional fossil between the ancestral land mammal shown in the phylogenetic tree and present-day cetaceans.

Given that *Ambulocetus* is a transitional fossil, you would expect it to have

A eyes at the top of its head for surface vision.

B increased fur for better insulation.

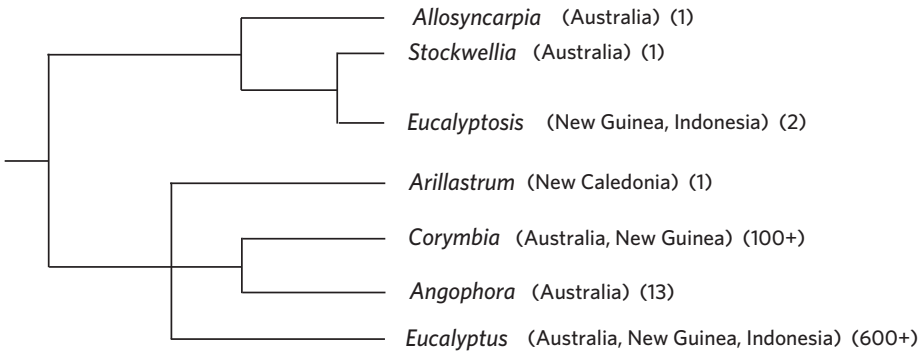
C gills for underwater breathing.

D hands with grasping ability.

Adapted from VCAA 2018 Section B Q9b

Question 10 (1 MARK)

Eucalypts, commonly known as gum trees, are found throughout Australia and other countries in Southeast Asia. They have been recently classified into seven different genera. A proposed phylogeny for the seven genera is shown in the phylogenetic tree, along with the countries in which they are found.



Key Numbers in brackets ( ) refer to the numbers of species in the genus.

It would be reasonable to conclude that the

A genus that evolved most recently was *Allosyncarpia*.

B fewer the number of species in a genus, the older the genus.

C DNA sequences of the species in *Corymbia* would be more similar to those in *Arillastrum* than to those in *Angophora*.

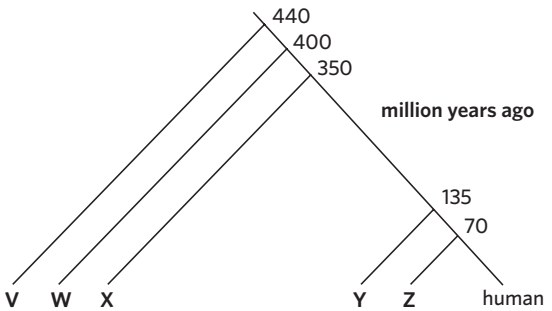
D amino acid sequences of the species in *Eucalyptosis* would be more similar to those in *Stockwellia* than to those in *Arillastrum*.

Adapted from VCAA 2012 Exam 2 Section A Q19

Question 11 (1 MARK)

Comparisons of the amino acid sequences of the α-globin polypeptide have been made between humans and a number of other vertebrates. The number of differences is shown in the table. A phylogenetic tree is also shown.

Organism	Stingray	Rabbit	Wombat	Frog	Clownfish
Amino acid differences in α-globin compared to humans	78	14	26	66	71



Based on the information provided, the correct placement of each animal on the phylogenetic tree to show the evolutionary relationship is

A V = stingray, W = wombat, X = frog, Y = clownfish, Z = rabbit.

B V = stingray, W = clownfish, X = frog, Y = wombat, Z = rabbit.

C V = clownfish, W = stingray, X = frog, Y = rabbit, Z = wombat.

D V = wombat, W = rabbit, X = frog, Y = stingray, Z = clownfish.

Adapted from VCAA 2006 Exam 2 Section A Q25

Question 12 (1 MARK)

The human, rabbit, mouse, and chimpanzee β-globin gene was compared in terms of the:

- DNA sequence of the β-globin gene
- amino acid sequence of the β-globin polypeptide.

The results are shown in the table.

	Sequence similarity (%)	
Organisms being compared	**DNA**	**Amino acid sequence**
human β-globin/chimpanzee β-globin	100	100
human β-globin/rabbit β-globin	89.3	90.4
human β-globin/mouse β-globin	82.1	80.1

It is possible to conclude from this data that

A humans are more closely related to mice than rabbits.

B the differences in DNA sequences always have an effect on the amino acid sequence.

C the variation between chimpanzees and humans occurs in a region of the β-globin gene which would code for amino acids.

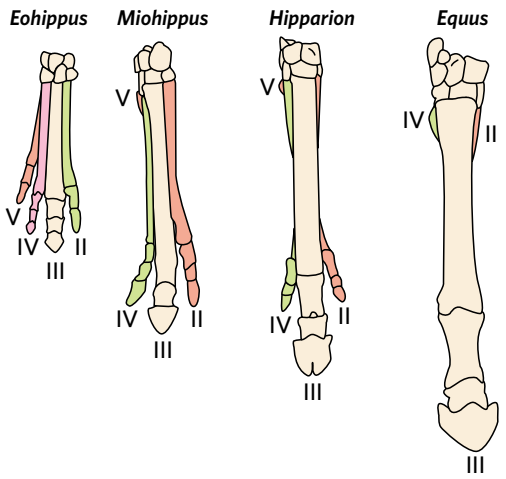
D it would be difficult to distinguish between humans and chimpanzees if only DNA and amino acid sequences of β-globin were being analysed.

Adapted from VCAA 2002 Exam 2 Section A Q18

Section B (28 MARKS)

Question 13 (6 MARKS)

Modern horses (*Equus* genus) are thought to have originated in North America approximately five million years ago. The leg fossils of different genera are shown.



a By observing the leg structures in the fossil record, scientists have concluded that horses in the *Equus* genus are descendants of the *Eohippus* genus, which lived approximately 33.9 – 56 mya.

- **i** Identify whether these foot structures are homologous or analogous. (1 MARK)
- **ii** Identify whether this is an example of convergent or divergent evolution. (1 MARK)

b Given that North and South America only recently joined together (approximately 3 mya), what is the oldest fossil of the *Equus* genus you would expect to find on the continent of South America? Why? (2 MARKS)

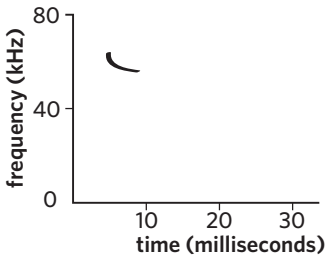
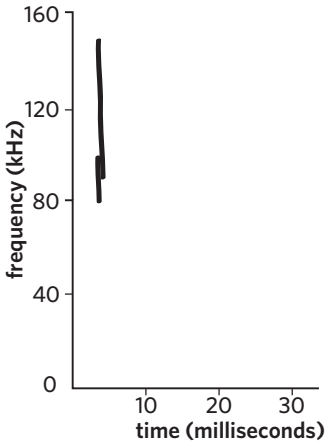
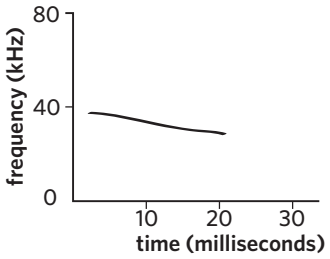
c Explain the difference between absolute and relative dating techniques. (2 MARKS)

Question 14 (3 MARKS)

Barro Colorado Island is a small island in Central America covered by a tropical forest. Seventy-four different bat species live in this forest.

Bats are nocturnal, flying mammals. To find their way around in the darkness, many bat species emit high-frequency pulses that bounce off obstacles and prey. These pulses enable bats to judge how far away an object is. A longer pulse allows bats to detect insects that are further away, whereas a shorter pulse allows bats to detect insects that are closer. This behaviour is called echolocation.

Three of the Barro Colorado Island bat species are described in the table.

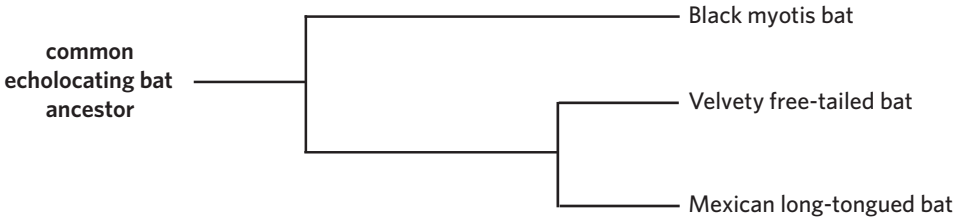
Species name	Facial appearance of bat	Diet	Feeding location	Echolocation signal
Black myotis bat (*Myotis nigricans*)		Insects	Around trees at forest's edge and in clearings	
Mexican long-tongued bat (*Choeronycteris mexicana*)		Nectar and pollen, flowers that open at night, for example cactus, agave	Narrow gaps and small spaces	
Velvety free-tailed bat (*Molossus molossus*)		Insects	Above trees, in open spaces	

a Analyse the data in the table.

i In terms of time, which of the three species emits the shortest echolocation signal? (1 MARK)

ii Explain how a short echolocation signal could be a selective advantage for this bat species. (1 MARK)

b A world-renowned biologist found that the three bat species share a recent common ancestor. To establish the order in which each species had evolved from this common ancestor, the biologist compared amino acid differences for several proteins between the various species. After analysing the results, the scientist drew the following phylogenetic tree.



Based on the diagram, which species would have the fewest amino acid differences when compared with the Mexican long-tongued bat? (1 MARK)

Question 15 (8 MARKS)

Over the past 20 years, a number of new mammalian fossils have been discovered. A fossilised *D. opatum* skull was found near the south-eastern Australian coast and a mummified *P. cinereus* skull was found within a cave in central Australia. A fossilised impression of *A. cignorum* was found in the same area as *D. opatum*.

a Consider the conditions that may have led to the fossilisation of members of these species. In the table, fill in one condition in the environment of each species that made fossilisation possible. The same answer cannot be used for multiple species. (3 MARKS)

Species	Environment	Condition
D. opatum	Near the coast of south-eastern Australia	
P. cinereus	Cave in western Queensland	
A. cignorum	Near the coast of south-eastern Australia	

Adapted from VCAA 2016 Section B Q10a

b What is the name given to the study of similar or different structures found between the bones and skeletal structures of animals, including fossilised remains? (1 MARK)

Adapted from VCAA 2015 Section B Q9b

c The skulls of *D. opatum* (left), *P. cinereus* (right) are given. After studying the skulls, scientists concluded that the two species were related.

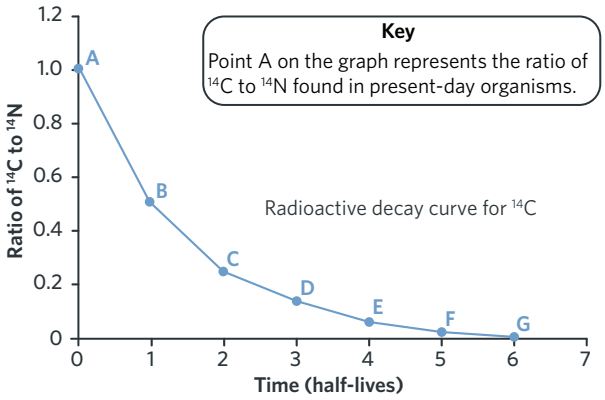
Discuss the evidence the scientists would have used to support their conclusion. Use an example from the skulls in your response. (2 MARKS)

d One method used to date fossils is radioactive carbon dating. The ratio of carbon-14 to nitrogen-14 (^{14}C:^{14}N) in the fossil is analysed and compared with the ratio of these elements in an organism living today.

The graph shows the rate of decay for carbon-14.

The *P. cinereus* skull ^{14}C:^{14}N ratio was analysed and found to contain three-quarters (¾) of the carbon-14 of a kangaroo that died recently.

Given the half-life of carbon is approximately 5 730 years, what is the absolute age of the *P. cinereus* skull? (1 MARK)



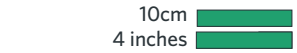
Adapted from VCAA 2012 Exam 1 Section B Q6a

e While walking back to their car, a scientist dropped the *P. cinereus* skull in a freshly dead kangaroo corpse. What type of error is this? (1 MARK)

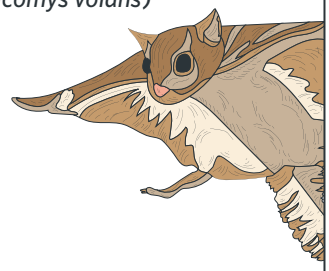
Question 16 (5 MARKS)

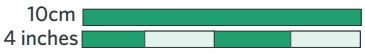
Australian sugar gliders and North American flying squirrels both have skin extensions connecting their front and hind legs. These extensions allow the animals to glide over short distances between trees. However, these animals do not share any recent common ancestors.

Short-headed glider, sugar glider, flying phalanger, flying possum (*Petaurus breviceps*)



Southern flying squirrel, North American flying squirrel (*Glaucomys volans*)





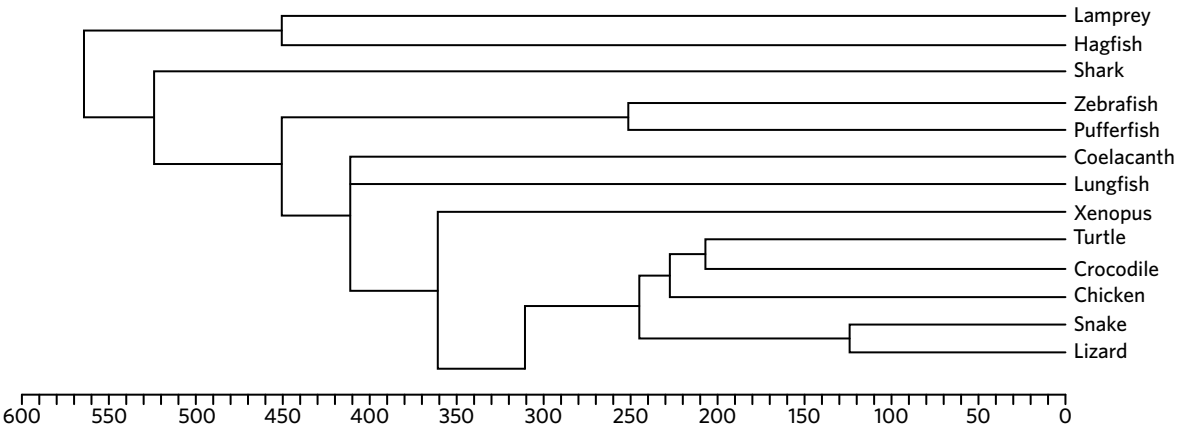
a Identify whether the skin extensions of these two animals are homologous or analogous structures. (1 MARK)

b Determine the process of evolution that Australian sugar gliders and North American flying squirrels have gone through. (1 MARK)

c Explain how these species both evolved the ability to glide. (3 MARKS)

Question 17 (6 MARKS)

The phylogenetic tree of several different animals is given.



a Consider the phylogenetic tree.

i Which species is most closely related to turtles? (1 MARK)

ii When did zebrafish and pufferfish diverge from their most recent common ancestor? (1 MARK)

iii Which species is more closely related to lungfish – the coelacanth or xenopus? (1 MARK)

b Determine whether scientists should compare amino acid sequences or DNA sequences to assess the relatedness between snakes and lizards. Explain your answer. (3 MARKS)

CHAPTER

11

Becoming human

Key knowledge

- the shared characteristics that define mammals, primates, hominoids, and hominins
- evidence for major trends in hominin evolution from the genus *Australopithecus* to the genus *Homo*: changes in brain size and limb structure
- the human fossil record as an example of a classification scheme that is open to differing interpretations that are contested, refined, or replaced when challenged by new evidence, including evidence for interbreeding between *Homo sapiens* and *Homo neanderthalensis* and evidence of new putative *Homo* species
- ways of using fossil and DNA evidence (mtDNA and whole genomes) to explain the migration of modern human populations around the world, including the migration of Aboriginal and Torres Strait Islander populations and their connection to Country and Place

11A DEFINING HUMAN

Imagine that the planet of the apes attack is actually happening, and that an army of gorillas is threatening to take over the entire world. After making quick work of South Australia, the hoards of angry beasts are barreling down on us here in Victoria. The Premier calls you up and asks you for a plan on how to stop them: one giant banana? A million smaller bananas?

You shake your head and suggest instead that we challenge them to a game of cricket... winner takes the Earth! 'The stupid monkeys won't even be able to hold the ball, let alone have a decent bowling action' – you explain. The Premier is very impressed with your idea and instantly makes you the Minister for Defence. But is this really such a great idea? Will the gorillas indeed bowl pies? Or worse yet – underarm?

Image: Jeff W. Jarrett/Shutterstock.com

Lesson 11A

In this lesson you will learn about the shared characteristics that define what it means to be a mammal, a primate, a hominoid, and a hominin.

◀ *Prerequisite knowledge*

Lesson 9B

You learned how species evolve over time in response to selection pressures and mutations.

Chapter 10

You learned about the fossil record and how we consider the relatedness between different species.

Future applications ▶

Chapter 11

Across the remainder of Chapter 11, you will build on the knowledge of this lesson when looking at hominin evolution in greater detail.

Study design dot point

- the shared characteristics that define mammals, primates, hominoids, and hominins

Key knowledge units

Categorising humans	4.2.9.1
Comparing mammals, primates, hominoids, and hominins	4.2.9.2

Categorising humans 4.2.9.1

OVERVIEW

Using the biological taxonomy we can categorise humans in relation to other species.

THEORY DETAILS

If you were to ask a philosopher 'what is a human being?', you might get a long-winded answer that leaves you more confused than you were to begin with. Asking a biologist the same question, however, will get you a more straightforward answer – modern humans are members of the species ***Homo sapiens***, which are the last remaining member of the tribe Hominini.

From a biological perspective, we can use a classification system to place a species into smaller and smaller categories, each of which shares a greater number of traits and morphological similarities with other members of that category (Figure 1). The further you categorise a species according to this classification system, the closer you move towards a working definition of that species. These categories are referred to as **taxa** and extend from the domain (which is the largest, and most inclusive level) at the top, to the species level at the bottom.

Homo sapiens the species name for modern humans

taxon (pl. taxa) a unit of biological classification into which related organisms are classified. Taxa are arranged in a hierarchical rank from kingdom down to species, where members of a specific taxon typically share certain morphological characteristics

You will notice that for each step we take down the taxonomic levels, certain groups are excluded from that category (e.g. plants do not fall into the Animalia kingdom, nor do rodents fit within the Primate order). Before long, you will be left with only the species you are examining, and will understand more clearly which characteristics separate that organism from even its closest relatives.

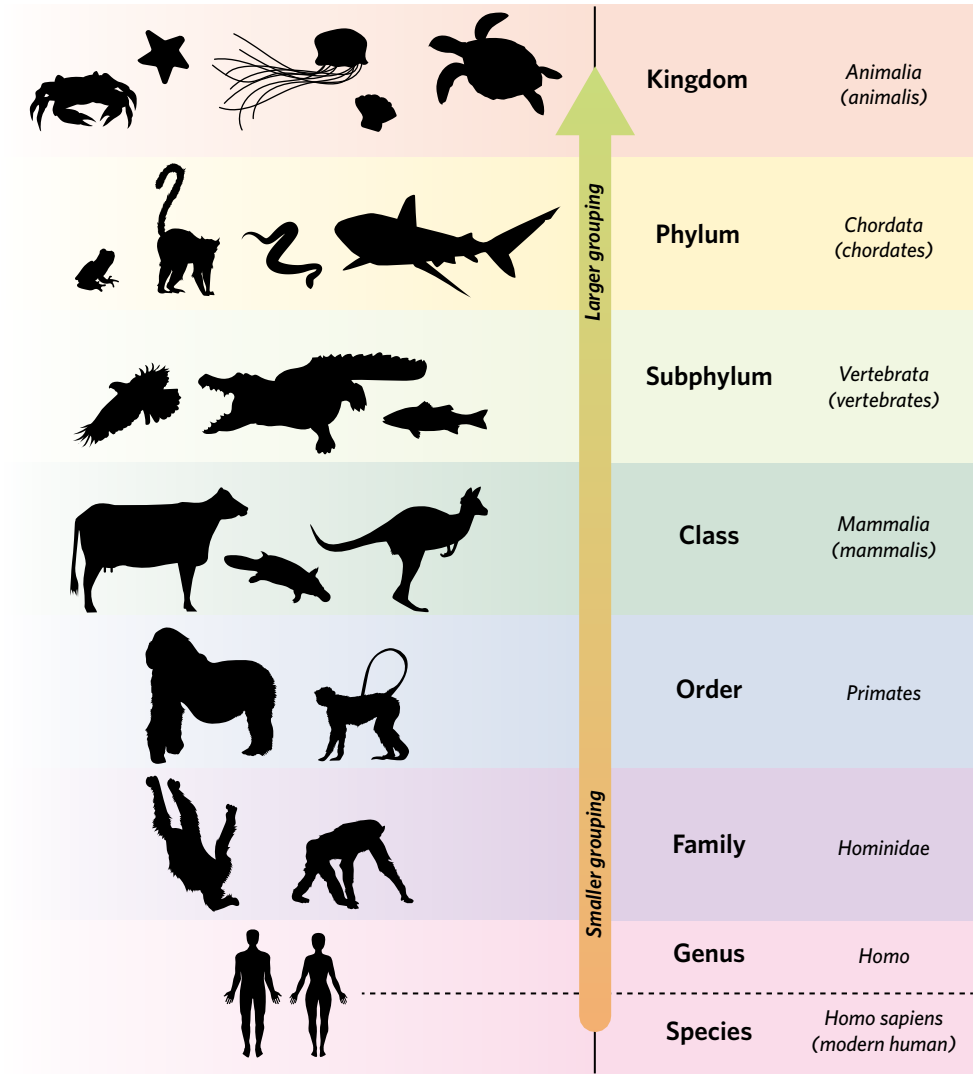


Figure 1 The taxonomic classification system goes from broader groupings at the top, to narrower groupings at the bottom, until you are left with a single species. The further down the list you move, the greater degree of traits are shared between members.

We can see that human beings (*Homo sapiens*) are primates belonging to the family Hominidae and genus *Homo*. This means that we are also mammals, as primates are an order under the mammalian class. You can think of this system like a babushka doll – where at each new level a new group of organisms are excluded until we are only left with humans (Figure 2).

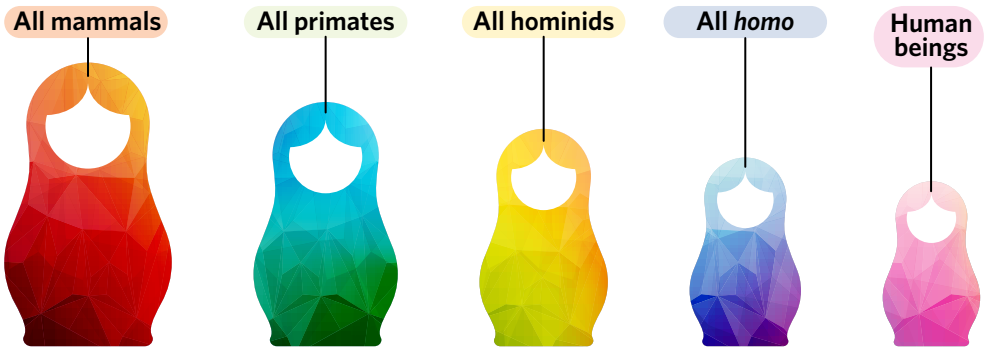


Image: Reamolko/Shutterstock.com

Figure 2 Defining humans is like playing with a babushka doll – we continue to specialise and move down different categories (or taxa) until we are left with a working understanding of a human being.

Comparing mammals, primates, hominoids, and hominins 4.2.9.2

OVERVIEW

Examining the shared characteristics of each of the taxonomic groupings, we can track our divergence away from our broader mammalian relatives and into our own tribe – Hominini.

THEORY DETAILS

Based on this classification system, we are able to classify humans into distinct categories, each of which become increasingly smaller and more exclusive (Figure 3). In this section, we will explore the shared characteristics of four of these categories to which humans belong: **mammals, primates**, **hominoids**, and **hominins**.

mammals warm-blooded vertebrates belonging to the taxonomic class Mammalia that have mammary glands, hair/fur, three middle ear bones, and one lower jawbone

primates the highest order of mammals, comprised of about 400 different living species who share a number of features including opposable digits and binocular vision

hominoids members of the superfamily Hominoidea that includes apes and humans

hominins members of the taxonomic tribe Hominini that includes modern humans and our upright-walking ancestors

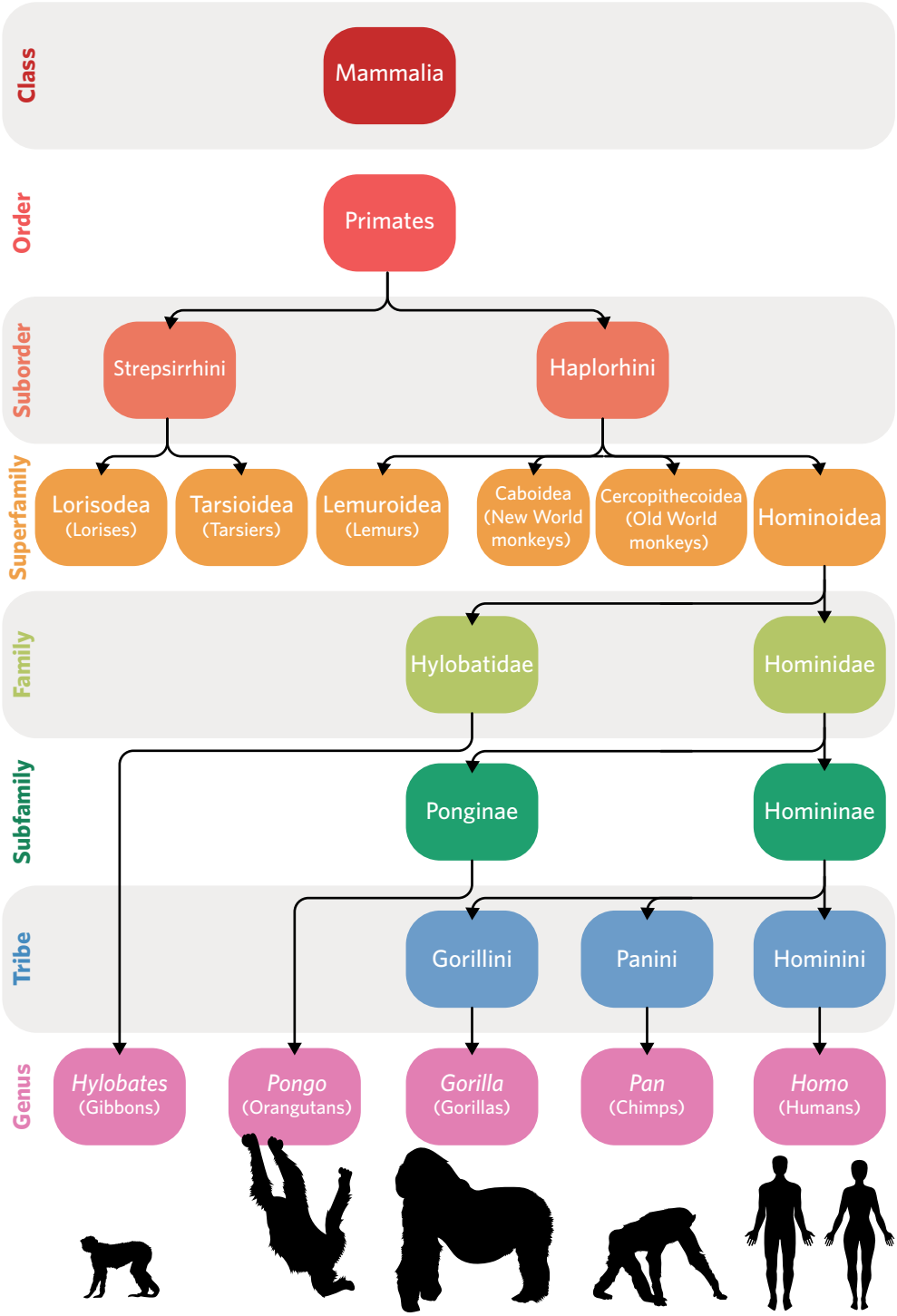


Figure 3 A detailed depiction of the classification of mammals. Notice that humans progress through the class Mammalia, the order Primates, the superfamily Hominoidea, and the tribe Hominini. We will examine what it means to belong to each of these categories in more detail throughout this section.

Mammals

Humans are mammals, meaning we are one of roughly 5 400 animal species belonging to the class Mammalia. This list includes other familiar species you know and love, such as cats and dogs, farm animals like sheep, pigs, and horses, as well as larger wild animals like whales and bears. The key characteristics shared by mammals are shown in Figure 4.

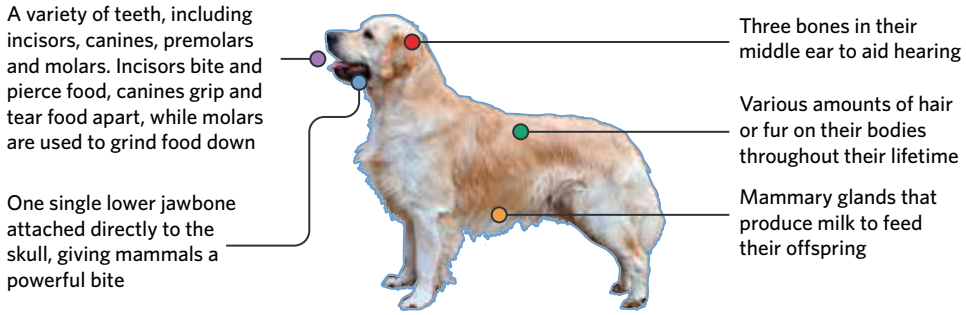


Figure 4 The key characteristics of mammals. Interestingly, only one of these five features needs to be present for an organism to be identified as a mammal.

Primates

Within the class Mammalia, humans are further classified into primates, which are a specific order of mammals that includes around 400 different species such as orangutans, lemurs, gorillas, and baboons. Primates are often referred to as 'the highest order of mammals', given their large brain size (in relation to body size) relative to other mammals and their increased reliance on visual acuity, which they use to help them find food and avoid predators. The key characteristics shared by primates are shown in Figure 5.

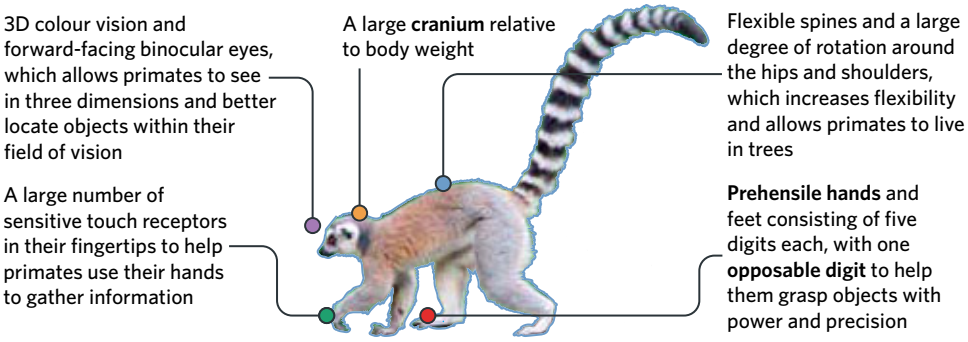


Image: ehtesham/Shutterstock.com

Figure 5 The key characteristics of primates

cranium the part of the skull that covers the brain

prehensile the ability to grasp objects

opposable digit a digit (either the thumb, big toe, or both) that is able to touch all the other digits on the same appendage

Theory in context

One of the defining features of primates includes the presence of an opposable digit (either the thumb or big toe), which allows primates to possess prehensile hands and feet. The opposable digit is fundamental in forming a precision grip, which is an ability that almost all primates possess, though to varying degrees. Humans have a highly developed **precision grip**, which allows us to finely manipulate objects and delicately grasp small items between our index finger and thumb. Other apes, such as chimpanzees, have relatively shorter thumbs and longer fingers, resulting in a weaker precision group. To counteract this, they are much more adept at using a **power grip**, which is particularly useful for tree climbing.

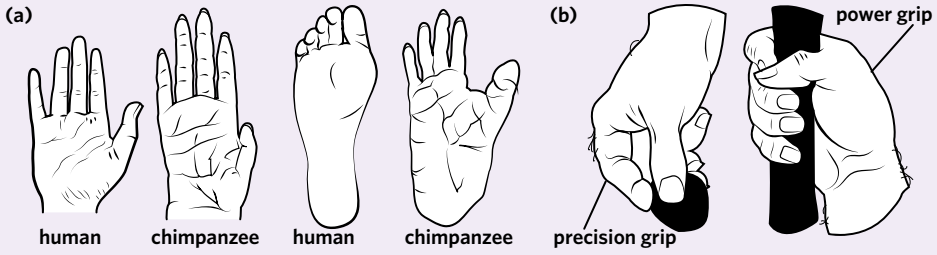


Figure 6 **(a)** When comparing the hands and feet of humans and chimpanzees, it is important to note that while both have an opposable thumb, humans do not possess an opposable toe. **(b)** The presence of an opposable thumb allows primates to possess prehensile hands, facilitating the use of a precision or power grip.

precision grip a type of grip involving the tips of the thumb and finger, used by primates (to varying extents) for precise manipulation of objects of various sizes

power grip a type of grip involving the palm and the fingers, used by primates (to varying extents) for moving and manipulating objects. The power grip generates more force due to the significant use of the palm

Hominoids

Within the order of primates, humans are further classified into the superfamily Hominoidea. Species belonging to this superfamily are called hominoids (or apes) and include:

- Great apes – orangutans, chimpanzees, gorillas, and humans
- Lesser apes – many different species of gibbons.

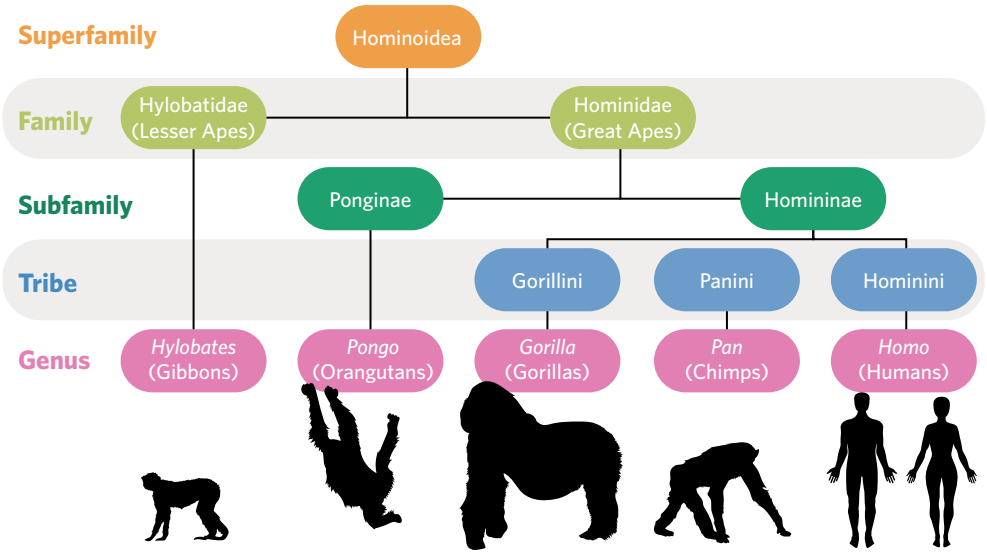


Figure 7 The classification of organisms within the Hominoidea superfamily.

The key characteristics shared by hominoids are shown in Figure 8. These are contrasted to a non-hominoid primate, the baboon, which is an Old World monkey.

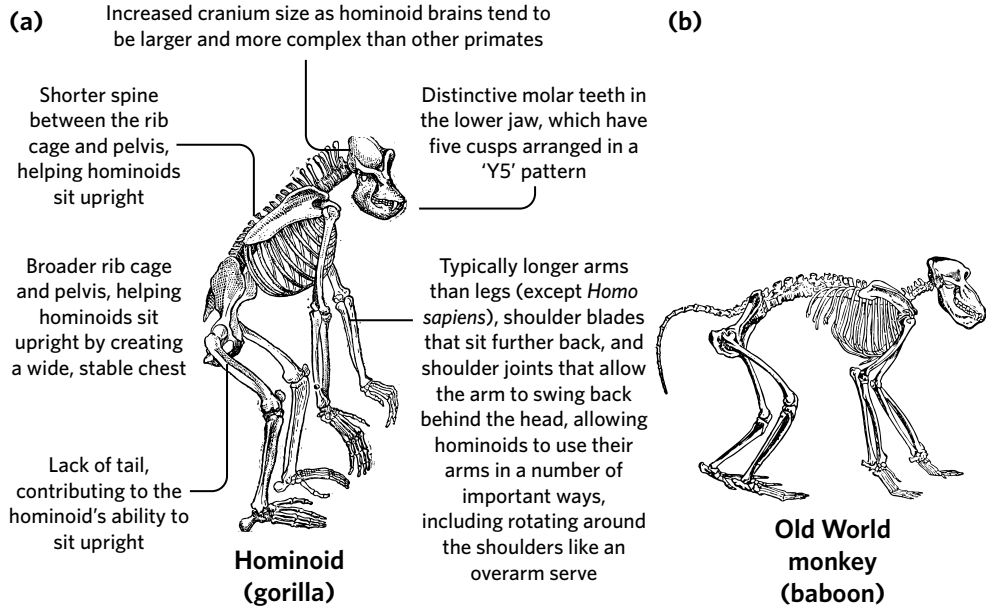


Image: Morphart Creation/Shutterstock.com

Figure 8 Defining characteristics of hominoids, comparing the skeleton of a **(a)** gorilla with a **(b)** baboon. Note the absence of a tail, shorter spine, broader rib cage, and longer arms than legs in hominoids.

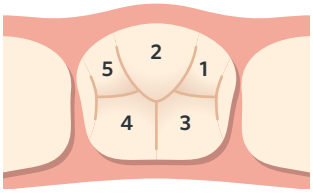


Figure 9 The distinctive Y5 molar teeth pattern of hominoids

Hominins

Finally, we reach the hominins, which is the taxonomic point where humans are separated from all other species in the animal kingdom! Humans are hominins, which places us in a tribe known as Hominini and includes all members of the genus *Homo* (of which we are the only living members), as well as some of our more distant ancestors.

The key characteristic of the hominins (us and our most recent ancestors), is our ability to walk erect on our hind legs over a sustained period of time. This is known as **bipedalism** and is considered to be the last distinguishing feature that separates us from our closest primate relatives such as the chimps and gorillas, who typically walk on four limbs with their knuckles on the ground.

bipedalism using two legs for walking upright

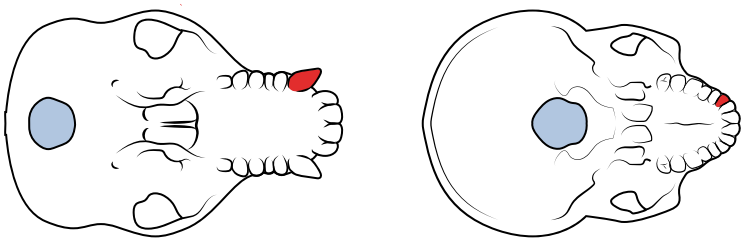
Theory in context

BECOMING BIPEDAL – HOW DID IT AFFECT US?

Becoming bipedal has resulted in a number of structural changes that have come to distinguish hominins from other primates. To illustrate these we'll compare a gorilla skeleton and a *Homo sapien* skeleton. These are shown in Figure 10 and the key differences are summarised.

Canine teeth – Humans have much smaller **canine teeth** than gorillas.

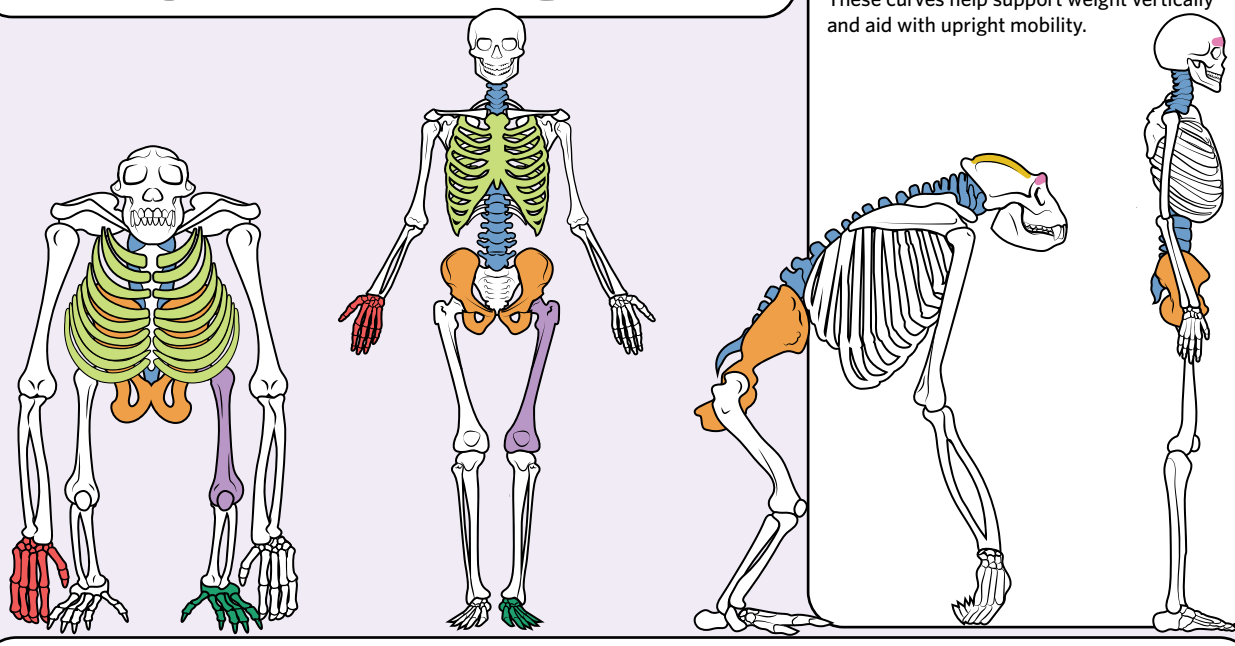
Foramen Magnum* – The **foramen magnum** is more central in human skulls, allowing the head to sit forwards while resting on top of the spinal column. In gorillas, however, the foramen magnum is closer to the back of the skull.



Brow ridge – Humans tend to have a much smaller **brow ridge** than gorillas.

Sagittal crest – The **sagittal crest** that is present on the top of the skull in gorillas is absent in humans. This crest is where jaw muscles attach. Having a big sagittal crest means large muscles can attach to it, increasing jaw strength.

Spine curve* – Gorillas have a "C-shaped" spine that curves forwards. Human spines, however, are "S-shaped" with a curve in the lower spine and another in the upper spine. These curves help support weight vertically and aid with upright mobility.



Rib Cage* – The rib cage in humans is more barrel-shaped than gorillas who have funnel-shaped rib cages instead. This helps humans to maintain an upright posture for a lengthy period of time.

Hand – Human hands have shorter, straighter fingers and longer thumbs compared to gorillas, making it possible for humans to have a further refined precision grip.

Pelvis* – Human pelvises are more shallow and bowl-shaped than other primates, whose pelvises tend to be vertically long and narrow. The bowl-shaped pelvis helps provide support for the upper body whilst standing and walking upright.

Femur angle* – Humans have a relatively large **femur angle** compared to gorillas. This helps to increase stability in humans while walking upright by ensuring the knee and foot are more centrally placed below the body.

Foot* – The human foot no longer has prehensile capabilities, and the big toe is in line with the other toes. Human feet also have two arches and a wide heel, making bipedalism more energy efficient and less impactful on the foot.

Figure 10 Structural features of gorillas (left) compared to hominins (right). *denotes a feature indicating bipedalism.

As you can see from Figure 10, many of the key structural differences between hominins and other primates arose due to bipedalism. Other differences, such as the reduction in canine teeth size, aren't directly related to bipedalism but are still important as evidence of other influences that drove our evolution – in this case, changes in hominin diet.

There are also many other non–structural differences between hominins and other taxa. Hominins have the ability to speak and communicate with one another and tend to form large, complex societies. They also have increased cognitive abilities, meaning they can think about abstract concepts and create language as well as tools in order to pass knowledge on from generation to generation. Many of these traits will be looked at in more detail in the next lesson as we trace the evolution of hominins more closely.

Theory summary

By understanding the taxonomic classification of organisms and the characteristics of mammals, primates, hominoids, and hominins, it is possible to see exactly what it is that defines us as human.

canine teeth a type of tooth in mammals that is relatively long and pointed

foramen magnum the hole in the base of the skull through which the spinal cord passes. A more centralised foramen magnum indicates bipedal locomotion

brow ridge a bony ridge above the eye sockets. It is found in all primates, but is greatly reduced in *Homo sapiens*

sagittal crest a ridge of hard bone running lengthwise (front to back) along the top of the skull. A pronounced sagittal crest indicates strong jaw muscles

femur angle the angle between the top and bottom of the femur when standing. It is greater in hominins when compared to other primates

Table 1 Summary of the defining characteristics of the taxonomic groups covered in this lesson

Classification	Characteristics
Mammals	• three middle ear bones • single lower jaw bone • mammary glands • variety of teeth • hair/fur
Primates	• flexible spines and hip rotation • prehensile hands and/or feet • binocular colour 3D vision • opposable thumb/big toe • sensitive touch receptors
Hominoids	• Y5-shaped molar teeth • broad rib cage • large cranium • long arms • no tail
Hominins	• bipedalism • structural consequences of bipedalism – centralised foramen magnum, S-shaped spine, broader rib cage, bowl-shaped pelvis, increased carrying angle of the femur • communication and formation of complex social groups

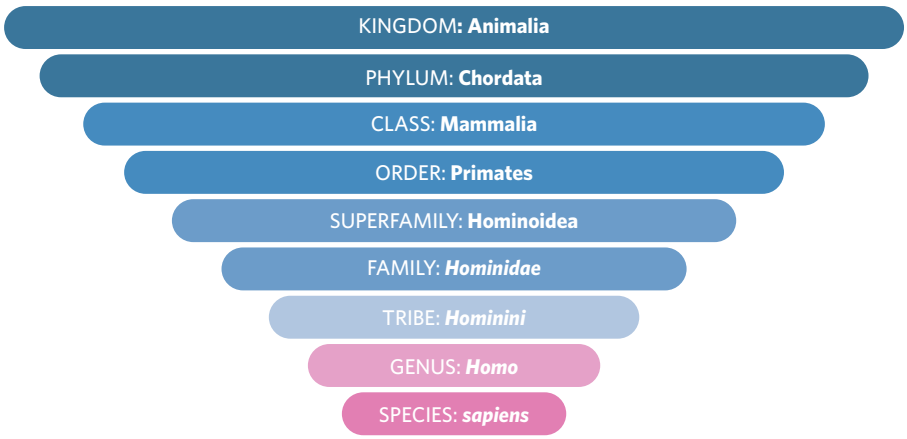


Figure 11 The taxonomic classification of modern humans

So the game of cricket goes ahead, and it's a complete disaster. Not only does the 200 kg opening bowler charge towards you at around 40 km/h, but you soon realise that his opposable thumbs make holding the ball as easy as holding a marble.

But perhaps your biggest mistake was underestimating their bowling action... it turns out that all apes have shoulder joints similar to ours which permit their arms to be swung back behind their heads with force. This ability comes from our primate ancestors and stems from their swinging between tree branches. You leave your stumps wide open and are literally devoured (along with the rest of Victoria) after less than two minutes at the crease. Within weeks of the invasion, the entire state is flattened and turned into a hyper-efficient banana farm. All hail our mighty ape overlords.

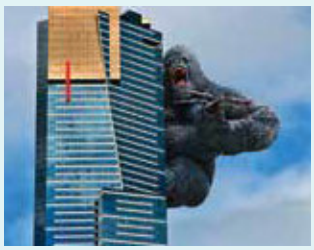


Image: rossco,meunierd/Shutterstock.com

11A QUESTIONS

Theory review questions

Question 1

Which of the following provides the most accurate definition of a human?

A a primate with forward-facing eyes, long arms, and no tail
B a bipedal primate mammal of the taxonomic tribe Hominini
C a mammal with prehensile feet and binocular 3D colour vision
D a carnivorous arthropod with a broader rib cage and a variety of teeth types

Question 2

Match the term to its definition.

Term	Description
• prehensile	**I** _____ the term used to describe individuals that walk upright
• mammals	**II** _____ animals that have mammary glands and fur
• hominins	**III** _____ the order of mammals to which humans belong
• primates	**IV** _____ capable of grabbing an object
• bipedal	**V** _____ members of the taxonomic tribe to which humans belong

Question 3

Which of the following options correctly shows the unique defining characteristic of each taxonomic group?

	Mammals	Primates	Hominoids	Hominins
A	Y5-shaped molar teeth	prehensile hands	one jaw bone	bipedalism
B	one jaw bone	prehensile hands	Y5-shaped molar teeth	bipedalism
C	prehensile hands	one jaw bone	Y5-shaped molar teeth	bipedalism
D	fur	Y5-shaped molar teeth	prehensile hands	bipedalism

Question 4

Which of the following is not a structural consequence of bipedalism?

A S-shaped spine
B centralised foramen magnum
C broader rib cage and bowl-shaped pelvis
D single lower jawbone and Y5-shaped molars

Question 5

Which of the following options is correct regarding the features of gorillas and hominins?

	Gorilla feature	Common to both	Hominin feature
A	tail	hair/fur	broad rib cage
B	mammary glands	tail	bipedalism
C	sagital crest	homininae	centralised foramen magnum
D	long arms	binocular vision	bipedalism

SAC skills questions

Case study analysis

Use the following information to answer Questions 6–10.

Evolutionary biologists deserve a pay rise. Despite the neat categories and structural characteristics described in this lesson, mapping evolutionary groupings is much messier and more complex than our taxonomic system would suggest.

One discovery back in 2012 illustrates this point perfectly. While working in northern Ethiopia at a site called Burtele, a team of researchers discovered a range of foot and toe bones that were thought to have belonged to an early human ancestor – a hominin that lived roughly 3.4 million years ago (mya). The discovery was important because it provided a rare glimpse into the form and function of the early hominin foot, but also called into question many of our assumptions about how bipedalism evolved in the taxonomic tribe Hominini.

The team were able to confirm that the bones belonged to a bipedal hominin due to the presence of many human-like features – the specimen had a slightly arched foot, which provides stability during walking, and also showed what biologists term 'toe-off', which is the little nuance of our walking cycle where our big toe gives extra lift and push just as we take our step.

However, the specimen also possessed similarities to both gorilla and monkey feet. For example, the big toe was small and opposable, suggesting grasping capabilities, while the fourth toe was unusually long for a typical hominin, resembling the long toes of monkeys. The researchers explained that these elements did not match the morphology of *Australopithecus afarensis*, who was thought to be the only living hominin species at that time, but were instead more similar to the earlier *Ardipithecus ramidus* – who had adaptations suited for both bipedalism and living in trees.

Question 6

What does the term 'bipedal hominin' refer to?

A any member of the taxonomic tribe Hominini who use two legs to walk upright

B any member of the superfamily Hominoidea who have feet with prehensile capabilities

Question 7

Which of the following can be inferred by the researchers' discovery?

A Early bipedalism was more varied than previously thought due to the persistence of *A. ramidus*-like locomotor adaptations.

B Bipedalism and the structural adaptations created by it emerged rapidly around 3-4 mya, where morphological characteristics largely resembled that of modern hominins.

Question 8

Based on the researcher's findings, what can be inferred about the lifestyle of the Burtele hominin?

A The Burtele hominin had a lifestyle very different to that of *A. afarensis*, which likely included tree-dwelling and moving on all fours.

B The Burtele hominin permanently stood upright, had a highly centralised foramen magnum and an increased reliance on hunted animal flesh compared to other primates.

Question 9

Which of the following assumptions is challenged by the information provided?

A The Burtele hominin was bipedal.

B An opposable digit is needed for precision grip.

C *A. afarensis* was the only living hominin species in Ethiopia 3.4 mya.

D Humans evolved from *A. afarensis*, which was a bipedal primate that lived during the period.

Question 10

Biologists believe this discovery has benefits for the bioethical concept of integrity. Which of the following options most accurately describes this potential benefit?

A The findings of the researchers cause us to rethink our evolutionary history, expanding on long-held assumptions about the development of bipedalism. Continued reevaluation of this kind adds to our working understanding of our own evolution and helps improve the accuracy of our fossil record.

B The findings of the researchers cause us to rethink what it means to be a hominin today, inferring additional rights and protections for gorillas and African monkeys. This ensures the fair and equitable treatment of other conscious beings and improves our relationships with our closest biological relatives.

C The findings prove that inferences are the best way to determine evolutionary histories, allowing us to make more accurate predictions when dealing with future fossil records. Future researchers ought to rely on these inferences to fill in the evolutionary 'picture' in places where existing explanations are lacking or without evidence.

Exam-style questions

Within lesson

Question 11 (1 MARK)

Consider the classification of hominins. Which one of the following statements about hominin classification is true?

A no primates have a tail

B all hominins are also primates

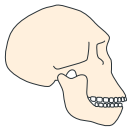
C all hominoids are also hominins

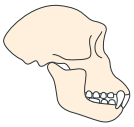
D *Homo sapiens* are the only present-day hominoid species

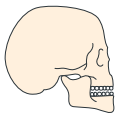
Adapted from VCAA 2018 Section A Q38

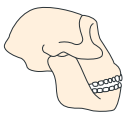
Question 12 (1 MARK)

Consider the following skulls. Which is likely the most distantly related to *Homo sapiens*?

A 

B 

C 

D 

Adapted from VCAA 2006 Exam 2 Section A Q22

Question 13 (1 MARK)

While transporting artefacts between museums, a young conservator mixes up the skulls from a primate exhibit with those from an Old World monkey exhibit. Which of the following would distinguish the chimpanzee skull from the skulls of the Old World monkey exhibit?

A Y5 molar teeth

B a small braincase

C a flat face and small teeth

D the presence of three middle ear bones

Adapted from VCAA 2002 Exam 2 Q23

Question 14 (1 MARK)

Members of the order Primates are mammals. Which combination of features is common to all primates and distinguishes them from other orders of mammals?

	Feature 1	Feature 2	Feature 3
A	binocular vision	fur or hair	opposable thumbs
B	fully rotating shoulder joints	opposable thumbs	large brains relative to body size
C	milk-producing mammary glands	fully rotating shoulder joints	nails instead of claws
D	large brains relative to body size	binocular vision	milk-producing mammary glands

Adapted from VCAA 2018 Section A Q37

Question 15 (4 MARKS)

Shown here are two photographs of a hominoid skull. Scientists compared this skull to that of a species belonging to a different primate superfamily.

Image: ivanpavlisko/Shutterstock.com

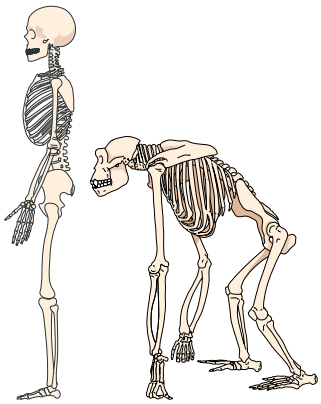
a Describe two features that could allow scientists to determine that this skull belonged to the hominoid superfamily. (2 MARKS)

b Describe two structural features (other than skull features) of hominoids that indicate they are more closely related to *Homo sapiens* than other primates. (2 MARKS)

Adapted from VCAA 2016 Section B Q10

Question 16 (4 MARKS)

The skeletons of two primates are shown. One is a hominoid skeleton, the other is a hominin skeleton.



Skeleton 1 **Skeleton 2**

Image: Emre Termin,Olga Bolbot/Shutterstock.com

a Identify the hominin skeleton. Justify your response. (2 MARKS)

b Redraw the table on a piece of paper.

	Skeletal structure	Differences	Significance
1			
2			

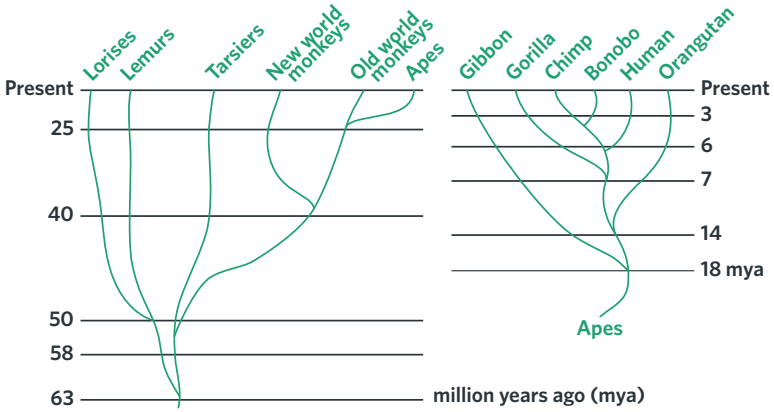
Identify two non-skull skeletal structures in the hominin, describe how they differ from the same structure in the hominoid, and state the functional significance ofthese differences. (2 MARKS)

Adapted from VCAA 2013 Section B Q11

Multiple lessons

Question 17 (1 MARK)

Examine the following primate evolutionary trees.



Which of the following statements is false?

A Lemurs and apes are unrelated.

B Chimps and humans are more closely related than gorillas and chimps.

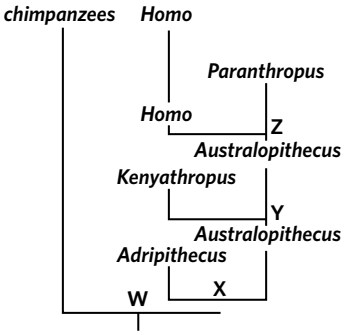
C Bonobos and chimps are more closely related than humans and chimps.

D Lorises and New World monkeys shared a common ancestor approximately 63 mya.

Adapted from VCAA 2009 Exam 2 Section A Q21

Question 18 (2 MARKS)

The figure shows the possible evolutionary relationships between chimpanzees and humans.



a From the diagram, which letter represents the common ancestor of Homo and chimpanzees? (1 MARK)

b *Ardipithecus* was said to be both free-standing and tree-dwelling meaning that it lived on both flat ground and in trees. Identify one possible morphological feature of *Ardipithecus* that allowed it to swing through trees. (1 MARK)

Key science skills and ethical understanding

Question 19 (6 MARKS)

The table shows the number of nucleotide differences between a region of DNA in humans, chimpanzees, and tarsiers.

	Human	Chimpanzee	Tarsier
Human		15	30
Chimpanzee			20

a Based on the data in the table, identify which species is most closely related to humans. Justify your response. (1 MARK)

b Explain how nucleotide differences can be used to determine relatedness between primate species. (2 MARKS)

c The DNA used in the tests was extracted from skeletons of the three species in the table.

What other type of information could be obtained from the skeletons to assist in determining the relationships between the three species? (1 MARK)

d In an effort to further explore the relatedness between the three species, scientists used the technique of DNA hybridisation. They used an uncalibrated thermometer, resulting in the DNA being overheated to 105 °C and their results being inaccurate.

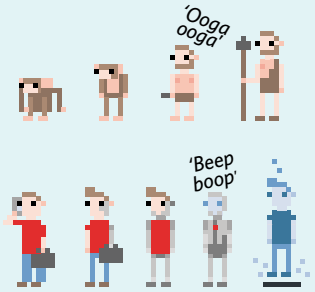
State the type of error which has taken place and explain your reasoning. (2 MARKS)

Adapted from VCAA 2003 Exam 2 Section B Q6

11B HOMININ EVOLUTION

Imagine if I took you out of class now and froze you in a cryogenic chamber for 1 000 years. Let's assume that you are wearing the same clothes that you have on now, you have your phone in your pocket, and I've left you a large cheeseburger meal to eat when you wake up. Not only would there be a lot of notifications to go through (or if you're anything like me... depressingly few notifications), but you would also probably be horrified by what you found in the world around you.

Yet 1 000 years is relatively quick, in evolutionary terms – when you woke up after your slumber, humans would probably still look pretty much the same. My question to you would be this: how would things be different if I had frozen you for say, one million years? Our human ancestry is riddled with changes and twists and turns... how well would you recognise your relatives from so many millions of years in the future – and more importantly, how well would they recognise you?



Tag you and your best friend!

Lesson 11B

In this lesson you will learn about the evidence of hominin evolution over time, including our increased brain size and changing limb structure.

◀ *Prerequisite knowledge* — *Future applications* ▶

Years 7–10
You learned about the theory of evolution by natural selection and how this theory is supported by a range of different scientific evidence.

Chapter 10
You learned about the fossil record and how we consider the relatedness between different species.

Lesson 11C
In this lesson, you will learn about the human fossil record and the difficulties we have with interpreting it.

Study design dot point

- evidence for major trends in hominin evolution from the genus *Australopithecus* to the genus *Homo*: changes in brain size and limb structure

Key knowledge units

Meet the hominins	4.2.10.1
Changes in brain size	4.2.10.2
Changes in limb structure	4.2.10.3

Meet the hominins 4.2.10.1

OVERVIEW

A hominin is any species, living or dead, that can be classified in the taxonomic tribe Hominini. This includes modern humans, as well as our upright-walking ancestors, such as those from the genera *Homo* and *Australopithecus*.

THEORY DETAILS

As you learned in the previous lesson, humans are hominins. The term is used to refer to any member of the tribe Hominini, which has been evolving and developing over the last 7 million years, and to which we modern humans are the last remaining member. As such, when we think of hominins, we are thinking of us, *Homo sapiens*, as well as all the extinct members of the human lineage – our ancestors. Many of these ancestors you may be familiar with, is in large part due to the extensive fossil records they left behind. Some of these ancestral species include *Homo neanderthalensis* (or, the Neanderthals), *Homo erectus*, and the australopithecines.

For the purposes of VCE Biology, it is only necessary to familiarise yourself with a working understanding of what it means to be a hominin, as well as some general trends in hominin evolution over time – which can be seen summarised in Figures 1 and 2. In short, you should familiarise yourself with the general trends in hominin evolution across time, starting with the **genus** *Australopithecus*, which existed around 4 million years ago (mya), through to the genus *Homo*, which first appeared between 2 and 3 mya and continues to exist to this day!

genus (pl. genera) a taxonomic rank above species and below family. Modern humans belong to the genus *Homo*

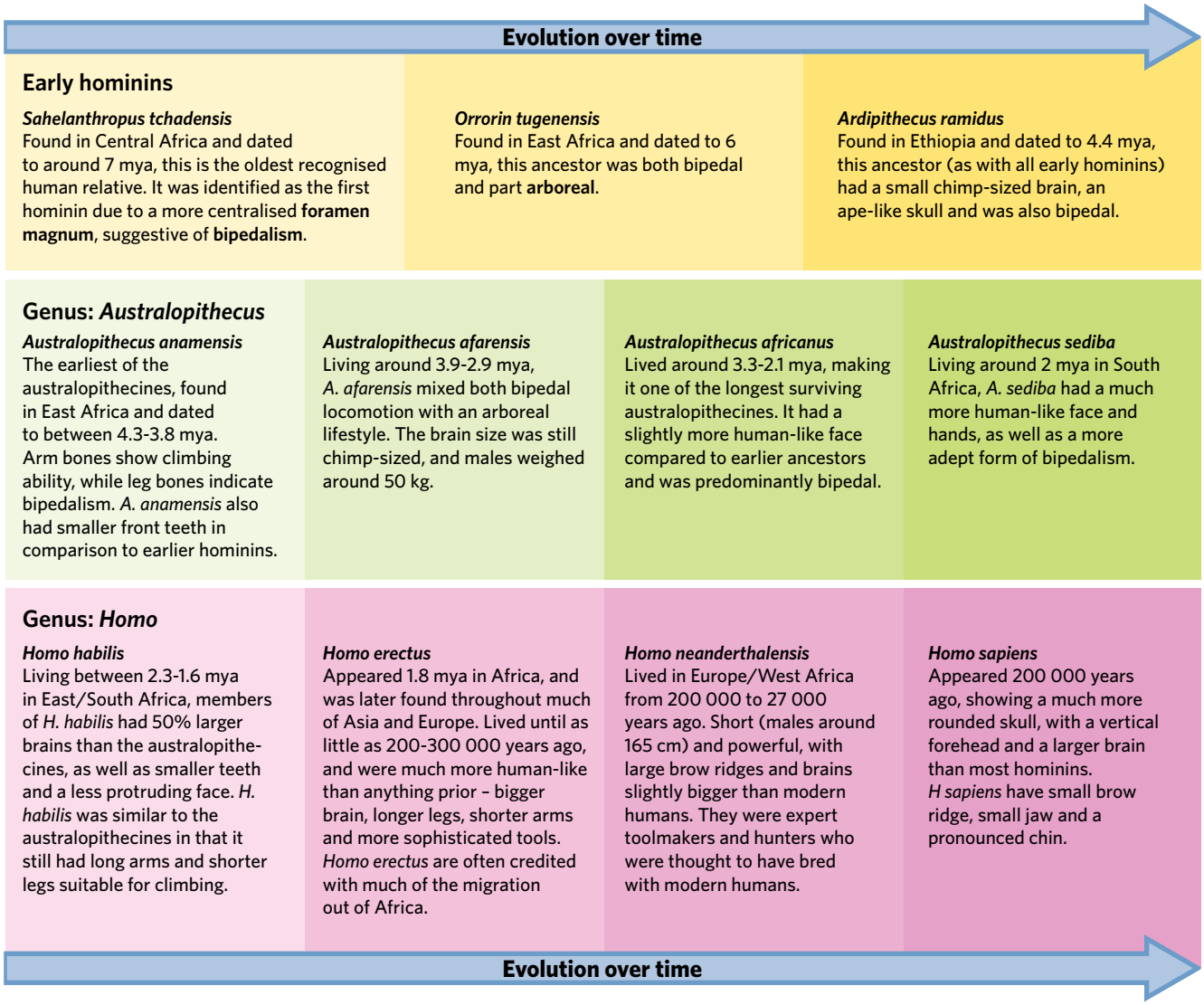


Figure 1 A general trajectory of hominin evolution across the past 7 million years. Note that modern day Homo sapiens have had many more hominin ancestors than those included in this diagram, and that this diagram is meant as a rudimentary overview of the general evolution of our species through time. Can you identify any general trends, especially in relation to brain size and limbs?

As you can see from Figure 1, the evolution to modern day *Homo sapiens* has been anything but simple. It has taken several millennia for different species to evolve to show the characteristics that we are familiar with today. While it is not necessary to memorise every detail of this trajectory, it is helpful to understand some general patterns, namely that our ancestors gradually moved from an arboreal lifestyle to one of complete bipedalism, and that this was facilitated by the shortening of our arms and the lengthening of our legs. What's more, our faces gradually became flatter, and our skulls rounder and smaller. Some of these changes will be explored in more detail across the remainder of the lesson.

foramen magnum the hole in the base of the skull through which the spinal cord passes. A more centralised foramen magnum indicates bipedal locomotion

biped an individual that moves on two legs (upright-walking)

arboreal living in or amongst trees

Note that while these changes are not perfectly linear, such that one descendant may have had a smaller braincase than their direct ancestor, they do represent overall directional trends in the 7 million years of hominin evolution. It is important to research some of the complexities within each of these trends, and look to understand how and why each structural change came about. Hint: our move to bipedalism had a lot to do with them!

Consider Figure 2, which shows a family tree of hominin species and the rough timing in which they existed. The progression of these trends can be seen in the successive species.

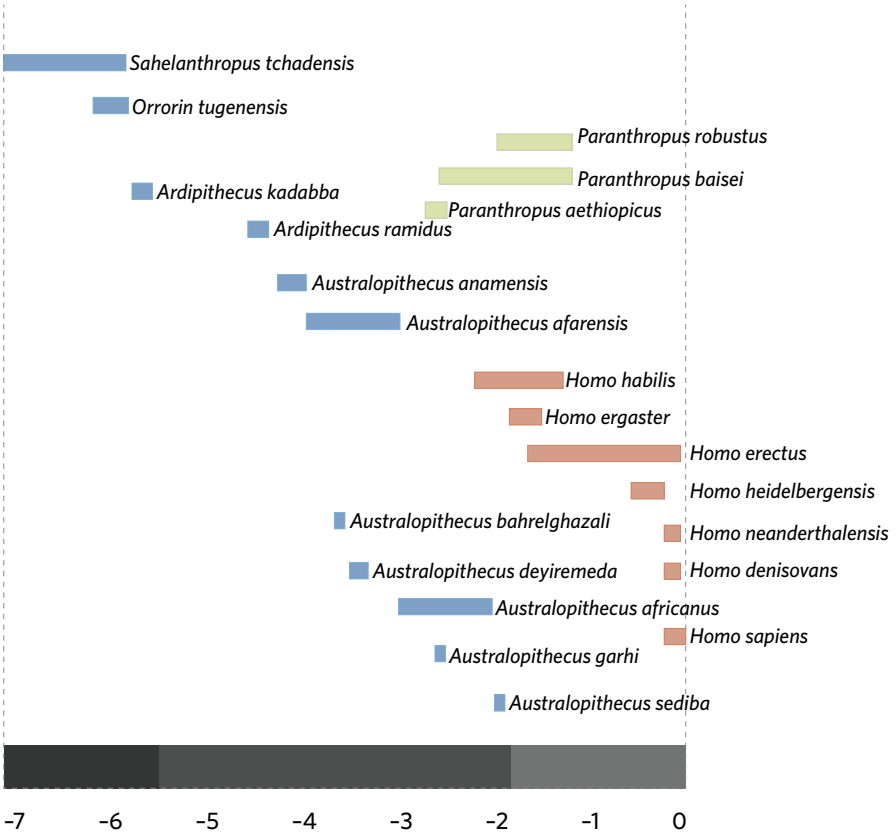


Figure 2 A family tree of hominin species showing when each species existed. The VCAA tests you on patterns of hominin evolution from *Australopithecus* to *Homo sapiens*, and while it is not necessary to commit each species and its timeframe to memory, it can be helpful to understand roughly how long different species were around and who they shared the earth with during their respective time periods.

Changes in brain size 4.2.10.2

OVERVIEW

Hominin brains increased in size over time, resulting in the evolution of higher cognitive processes such as planning, speech, and abstract thinking.

THEORY DETAILS

A defining feature of hominins is their large brains (when compared to their body size). This has been an evolutionary trend that has tended to increase gradually across time, with almost every subsequent hominin species having a larger brain than their recent ancestors. Why is this, and what has been the evolutionary significance of this trend for modern humans?

While there is no general consensus as to the exact rate at which hominin brains have evolved, scientists are able to determine the differences in brain size thanks to the fossils left behind by each species. While brains themselves are comprised of soft tissue and therefore do not fossilise, the volume of an extinct hominin's cranium (skull) can be estimated from recovered fossilised skulls. From studying this fossil record, for example, we know that brain size increased more than threefold from the earliest australopithecines to modern day human beings.

Research currently disagrees on whether the evolution in brain size happened gradually over time, or via brief episodes of rapid growth in response to different environmental challenges and stressors – such as periods of climate change or migration to new environments. One of the most commonly accepted drivers of the change, however, was the improved diet of hominin species over time, from earlier leaf-eating primates to more recent hominins who incorporated more fruit and animal products into their diets. This was further facilitated by the onset of the controlled use of fire around 800 000 years ago, which enabled our hominin ancestors to fuel brain growth through cooking and increased nutrition.

With an increase in hominin brain size comes an increase in the complexity of brain structure. Specifically, the **cerebrum** of hominin brains became more folded, which increased the total surface area of the brain, resulting in more neurons and an increase in the number of connections between brain cells, leading to enhanced cognitive ability (Figure 3). With this increase in folding and brain cell connections came the ability to do a number of unique activities, including speech, the feeling of complex emotions, higher-order decision making, enhanced self-control, abstract thinking, and planning.

cerebrum the largest part of the brain, which comprises two-thirds of the brain's entire weight and is responsible for a large range of vital functions including sensory processing, motor control, and visual and spatial learning

We can see an interesting dilemma here: larger brains with more complexity and folding require much more energy than smaller brains. They use more resources, and have much higher metabolic requirements than other organs and tissues in our body. Why then, did hominins evolve such large brains? This is part of what is known as the expensive brain hypothesis, which tries to understand advanced cognitive ability in terms of advantages like predation reduction and improved food production, compared to negative impacts such as increased energy requirements and higher childbirth complexity (Figure 4).

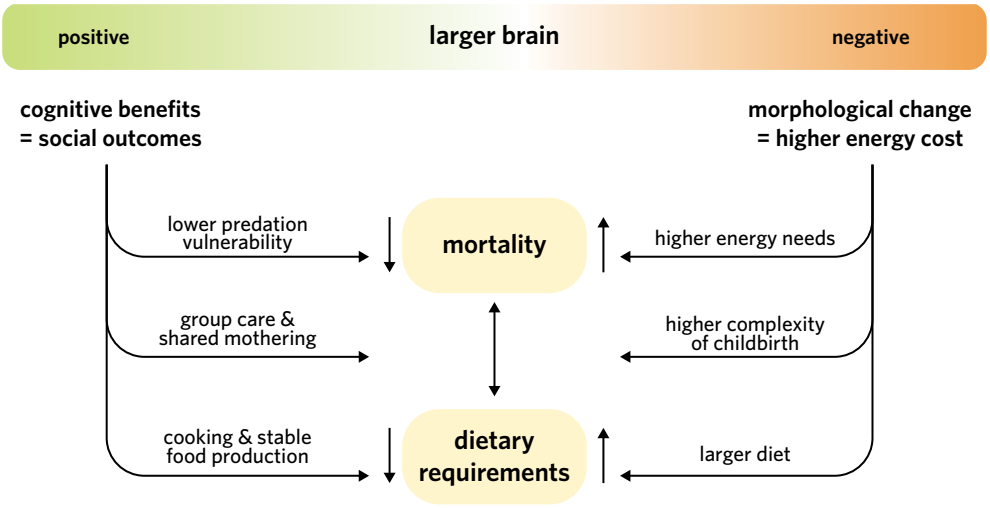


Figure 4 The expensive brain hypothesis . Source: Adapted from Maslin et al., 2015

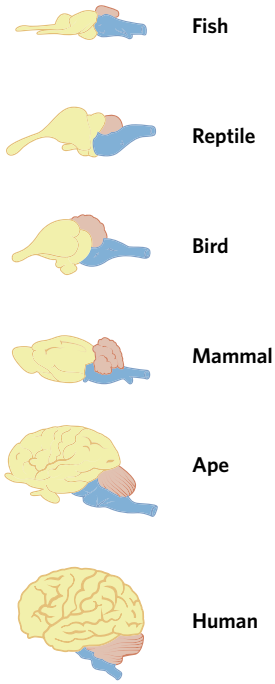


Figure 3 The brain of *Homo sapiens* has many more folds than those of other species.

As well as increasing in size, the structure of the hominin skull has also changed in a number of other ways since the early australopithecines. Some of these general trends are shown in Figure 5. Note that this is not all of the morphological changes across our evolutionary history, but show some of the more obvious structural differences between the skulls of us and our earliest ancestors.

- a more centralised foramen magnum (1)
- a shrinking of the **sagittal crest** (2)
- a lessening of the brow ridge (3)
- a flattening of the face (4)
- a less protruding chin (5)
- a more domed skull (6)
- smaller teeth (7)

sagittal crest a ridge of hard bone running lengthwise (front to back) along the top of the skull. A pronounced sagittal crest indicates strong jaw muscles

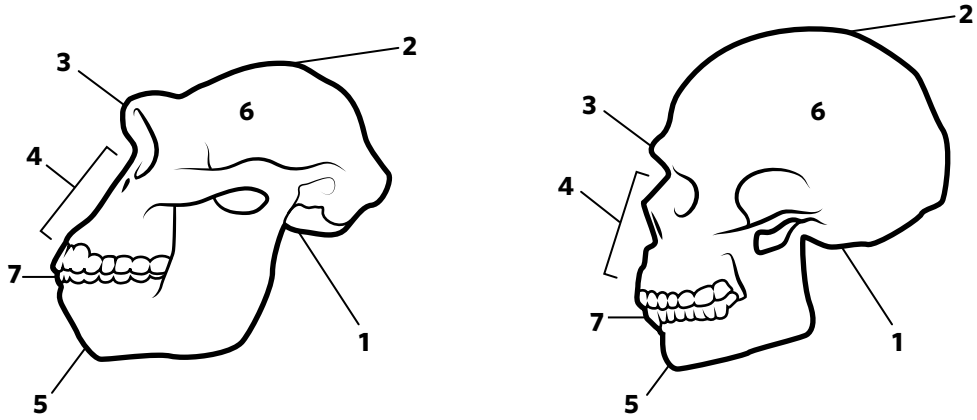


Figure 5 Some of the morphological changes to skull shape over time

Changes in limb structure 4.2.10.3

OVERVIEW

The arm to leg ratio of hominins decreased over time in response to an increased reliance on bipedal locomotion. The pelvis shape of hominins also changed in response to an increasingly upright-walking lifestyle.

THEORY DETAILS

Another major evolutionary change that has occurred to the hominins over time is a changing in limb structure, namely a decreasing **arm to leg ratio**. Three related changes that we will look at here are: shortening of the arms, lengthening of the legs, and changing of the pelvis shape. While some evolutionary biologists argue that these evolutionary changes occurred independently of one another, the consensus is that all three were driven by the evolution of bipedalism from our earlier arboreal ancestors. Let's take a brief look at possible explanations for these changes:

arm to leg ratio the ratio of arm length to leg length. Tree-dwelling hominids have longer arms and shorter legs, or a larger arm to leg ratio

Shorter arms

This change makes sense when you think about it: movement in trees requires all four limbs to be in contact with the external environment, grabbing and pushing off branches to move the body along. When our ancestors changed from arboreal living and began moving primarily on the ground, their forelimbs were no longer in contact with the external environment during locomotion. This placed less of a need for available contact points on the forelimbs, 'freeing' the arms up for other tasks, such as carrying children, preparing food, and building tools.

Longer legs

At the same time, longer legs were selected for in bipeds due to the fact that longer legs positively affect stride length and make upright walking more energy efficient. Longer legs also lessen the intensity of the rising and falling motion that the body's centre of mass experiences each time you take a step, reducing the volume of muscles activated as we walk.

Change to pelvis shape

The pelvis shape of hominins also became shorter and more bowl-shaped over time (Figure 6). This shape provided hominins with more support for the upper body whilst standing and walking upright. In later hominin species the leg attaches to the pelvis at an angle, allowing them to walk upright more easily. The legs of the earlier hominin species, by contrast, attached to the pelvis in more of a straight line, meaning they had to swing their legs wide when walking. This method of walking made them slower and less energy efficient. Further changes to pelvis shape occurred over time, largely due to different demands of childbirth as the cranial capacity of hominin babies increased. This mostly entailed a narrowing of the pelvis with a more circular birth canal, which reflected the overall narrowing of the body shape and more barrel-like chest of the offspring.

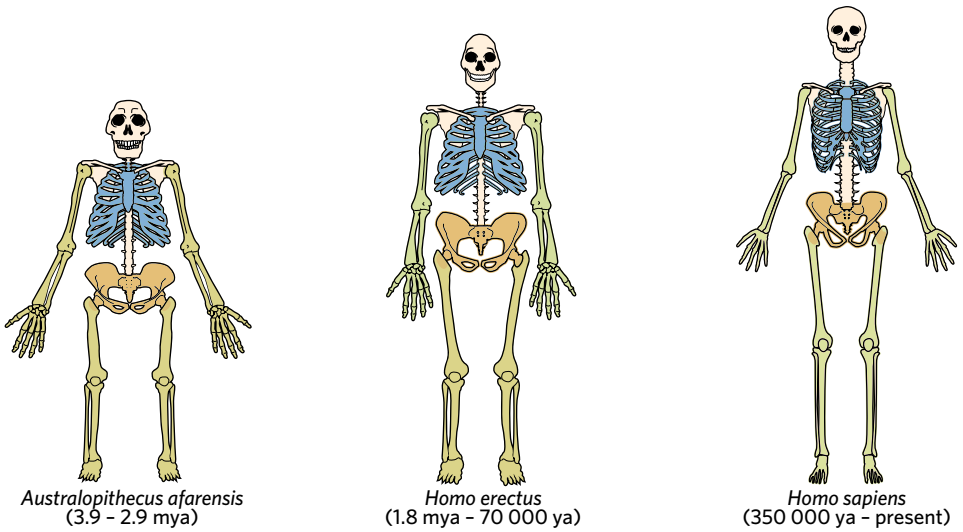


Figure 6 Tracing some of the structural changes in hominins across time. Notice the decrease in arm to leg ratio, and the changing shape of the pelvis.

Theory summary

For the purposes of VCE Biology, it is necessary to be familiar with the general trends of hominin evolution, including the approximate periods and patterns of development of our taxonomic tribe from the earlier australopithecines to modern day *H. sapiens*. Three main overarching trends over time have been:

1. Larger brain size – over time, the hominin braincase has tended to increase in size. This included a range of other morphological changes, including a flatter face and a more domed skull.
2. Smaller arm to leg ratio – modern humans have a smaller arm to leg ratio than our earlier ancestors. This was due to a variety of reasons, including the fact that longer legs were more energy efficient for bipedalism.
3. Changing pelvis shape – hominins evolved a narrower and more bowl shaped pelvis over time, which developed in relation to our bipedal lifestyle and larger brain cases.

Chances are that after a million years humans would be quite different. Our ancestors from one million years ago, likely Homo erectus, were very very different from us today. Another million years in the future and little old you might be fraternizing with an entirely separate species – terrifying!

11B QUESTIONS

Theory review questions

Question 1

Which of the following statements is true?

A *Homo sapiens* have coexisted with other hominin species.

B *Australopithecus afarensis* was a direct descendant of *Homo ergaster*.

C All members of the genus *Homo* have evolved from *Australopithecus africanus*.

D At one stage of our evolution, *Homo heidelbergensis* was the only hominin species on earth.

Question 2

Categorise the following statements as either **true** or **false**.

I The longest surviving hominin species is the *Homo sapiens.* _____

II A more centralised foramen magnum helps facilitate bipedal locomotion. _____

III The earliest australopithecines existed at the same time as the first members of the genus *Homo*. _____

IV The term hominin refers to our upright walking ancestors who are members of the same taxonomic tribe as us. _____

V The size of the braincase between modern humans and the earliest australopithecines is thought to have increased by as much as 300%. _____

Question 3

Given that all previous hominin species are extinct, how are scientists able to determine the increase in brain size between modern humans and our hominin ancestors?

A using fossilised remains of hominin skulls

B using fossilised remains of hominin brains

C investigating the high degree of folds in modern day human brains

D by measuring the length of the arms and legs of previous hominins and estimating brain size

Question 4

Match the change in limb structure to its correct explanation.

Limb structure	Explanation for change
• shorter arms	**I** _____ provided more support to the upper body while standing and walking upright
• longer legs	**II** _____ reduced contact points needed for bipedal locomotion
• change in pelvis shape	**III** _____ lessened the rising and falling motion of the body's centre of mass and reduces the volume of activated muscles while walking upright

SAC skills questions

Case study analysis

Use the following information to answer Questions 5–9.

Fossils provide a record of the size and structure of early hominin skulls. Using these fossils, researchers are able to infer information about the size of the brains housed within them. However, these inferences regarding size and structure do little to explain the actual functionality of the brains themselves.

However, researchers have recently begun analysing the genetic material taken from those fossils in an attempt to better explain the cognitive abilities of earlier hominins. One particularly interesting piece of research, for example, has succeeded in growing 'mini brains' that combine human tissue with important Neanderthal DNA responsible for neurodevelopment. These mini brains have been shown to not only change shape in response to the Neanderthal DNA, but also appear to mature more quickly than modern human brains. The neurons of the Neanderthal mini brains have been shown to be far more active at earlier stages of development when compared to regularly developing human brains.

In addition to earlier developing neural networks, Neanderthal brains appear to favour the creation of brain pathways devoted to visual and spatial abilities, rather than social interactions. This leads to stronger processing of spatial localities, possibly allowing Neanderthals to better identify objects in their field of vision. Modern humans, by contrast, have brains that promote the formation of social networks and the ability to foster social interactions.

Question 5

Which of the following statements best summarises the overall methodology of this study?

A Researchers have extracted ancient Neanderthal DNA from the fossilised skulls of dead specimens, and through DNA sequencing have been able to reconstruct particular Neanderthal genes responsible for neurodevelopment.

B Researchers have combined particular Neanderthal genes with human brain tissue and have grown an artificial mini organ that resembles a working brain. This was done to mimic and better analyse the neurodevelopment of an actual Neanderthal brain.

Question 6

The researchers extracted Neanderthal DNA from a hominin fossil they had discovered. Based on this information, which of the following statements can be inferred?

A The fossil was taken from the skull of *Homo sapiens*.

B The fossil was found somewhere in Eurasia or Africa.

C The fossil was created more than 1 million years ago.

Question 7

The term 'neurodevelopment' refers to

A the rate at which neurological pathways form in the brain as it grows, and how that development affects performance or functioning.

B the field of biological research concerned exclusively with the comparison of brain development across hominin species, in this case between Neanderthals and modern humans.

Question 8

Which of the following hypotheses do the findings support?

A Neanderthal brains made them more capable at birth, while human brains continue to develop as we age and encounter a greater need for social interaction.

B Neanderthal brains are more devoted to spatial abilities so as to better allow them to define their territory and identify when they are encroaching on a rival's space.

Question 9

Assume that a research team interested in these findings has designed a further experiment where the Neanderthal genes are to be implanted into the developing brain of a human foetus. The head of the research team had this to say to the press: 'Neanderthal DNA has tremendous potential to improve the rate of cognitive development in young children. As researchers, we have the obligation to investigate this potential further, and should gather as much data as possible to determine if this is a viable option for humanity moving forward'.

Assume that you have been called in to debate this researcher and argue that their proposed experiment is unethical. Using the bioethical concept of respect, which of the following arguments would be most appropriate?

A 'We ought to consider the value of others when planning further research. Risking the health and viability of a young child in this way ignores the personal welfare of that child, and removes their ability to make their own decisions'.

B 'We ought to prioritise the freedom of others to make their own decisions. This research team should not be persecuted for deciding to conduct this research, provided they have the authority of the parents to do so'.

C 'We ought to act in a way that maximises positive outcomes for all of humanity. If it turns out that this child flourishes with the neanderthal DNA, then we may unlock an incredible opportunity for other humans'.

Exam-style questions

Within lesson

Question 10 (1 MARK)

Which general trend is shown by hominin fossils?

A The older the fossil, the smaller the braincase that surrounds the cerebral cortex.

B The older the fossil, the smaller the sagittal crest on the top of the skull.

C The older the fossil, the less bowl-shaped the pelvis.

D The older the fossil, the smaller the arm-to-leg ratio.

Adapted from VCAA 2018 Section A Q39

Question 11 (1 MARK)

Which feature would indicate fossil bones belong to the genus *Australopithecus* rather than *Homo*?

A a flatter face

B a long forearm

C a larger braincase

D a less protruding chin

Adapted from VCAA 2017 Section B Q7a

Use the following image to answer Questions 12 and 13.

The following image shows a hominin skull.

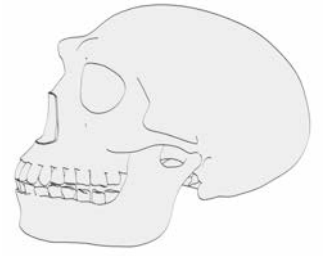


Image: 3drenderings/Shutterstock.com

Question 12 (1 MARK)

Which features of the skull shown in the diagram allow scientists to determine that this is a much earlier species of the genus *Homo* than modern humans (*H. sapiens*)?

A brow ridge and small jaw

B brow ridge and a flat face

C large canines and no brow ridge

D brow ridge and a less domed skull

Adapted from VCAA 2016 Section B Q10b

Question 13 (1 MARK)

Which feature of the skull shown in the diagram allows scientists to determine that this is a more modern hominin species than members of the genus *Australopithecus*?

A flatter face

B smaller braincase

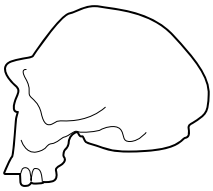
C more sloping forehead

D less central foramen magnum

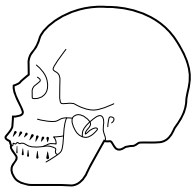
Adapted from VCAA 2016 Section B Q10c

Question 14 (1 MARK)

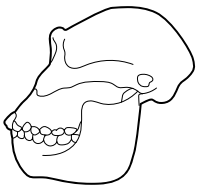
Here are three images of fossilised hominin skulls.



Skull 1



Skull 2



Skull 3

Which sequence gives the most likely order from the most modern fossil skull to the most ancient fossil skull?

A Skull 1, Skull 2, Skull 3

B Skull 3, Skull 1, Skull 2

C Skull 2, Skull 1, Skull 3

D Skull 3, Skull 2, Skull 1

Adapted from VCAA 2015 Section A Q37

Question 15 (1 MARK)

Fossil remains of a number of individuals from a hominin species were found at various sites in the eastern half of Africa and have been dated to between 3–4 million years old.

Compared to *Homo sapiens*, these fossil remains would have a

A more bowl-shaped pelvis.

B a smaller sagittal crest.

C longer arm to leg ratio.

D larger brain.

Adapted from VCAA 2015 Section A Q38

Question 16 (3 MARKS)

In 2013, about 1 500 fossil bones of a hominin species were found in a cave in South Africa. The fossil bones have some features in common with those of the genus *Australopithecus;* however, they have enough similarities to the genus *Homo* that scientists have classified the fossil skeleton as belonging to a new species, *Homo naledi*.

a Scientists estimate that the fossil is approximately 2 million years old. Given this information, would you expect this species to be bipedal? Explain. (1 MARK)

b The scientists also found that the arm to leg ratio of the skeleton was much less than some of the earlier *Australopithecus* skeletons they had been comparing it to. Explain how this finding is an indication of *H. naledi's* bipedalism. (2 MARKS)

Multiple lessons

Question 17 (1 MARK)

Members of the genus *Homo* are hominins. Which combination of features is common to all hominins and distinguishes them from other primates?

	Feature 1	Feature 2	Feature 3
A	forward-facing eyes	less fur and hair	opposable thumbs
B	arboreal lifestyle	large braincase	narrower, more bowl-shaped pelvis
C	no brow ridge	bipedal	large braincase
D	shorter arm to leg ratio	large braincase	bipedal

Question 18 (6 MARKS)

A hominin species, *Homo floresiensis,* was identified from fossils found on an isolated Indonesian island. These fossils were dated to be 18 000 years old.

The adult skull of this upright, bipedal hominin had a cranial volume less than one-third the average cranial volume of a modern adult human. It had harder, thicker eyebrow ridges than *Homo sapiens*, a sharply sloping forehead, and no chin.

H. floresiensis was just over one metre tall and their arm to leg ratio was slightly larger than modern humans. They are estimated to have weighed between 25–30 kg.

The fossils were found in sediment that also contained stone tools and fireplaces for cooking. The fireplaces contained the burnt bones of animals, each animal weighing more than 350 kg. The stone tools included blades, spearheads, and cutting and chopping tools.

a *H. floresiensis* are unusually small for such a recent hominin species. Using the theory of natural selection, describe how this species evolved to be so small compared to *Homo sapiens* and *Homo neanderthalensis.* (4 MARKS)

b The fossils were found in sediment. Assuming that this adult *H. floresiensis* died in a river, briefly describe the fossilisation process of permineralisation as it relates to the likely formation of this fossil. (2 MARKS)

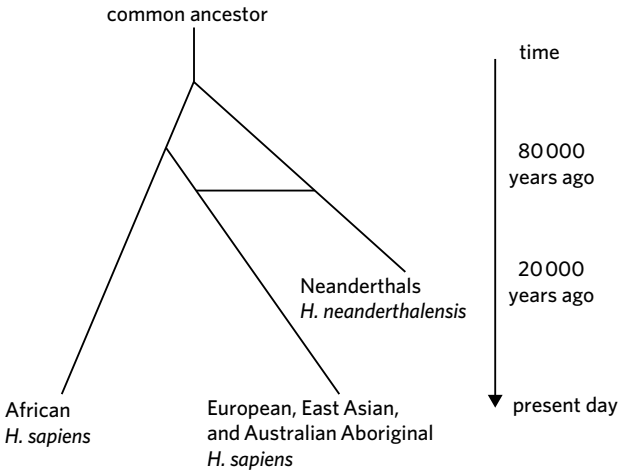
Adapted from VCAA 2014 Section A Q39

Key science skills and ethical understanding

Question 19 (6 MARKS)

Fossil evidence indicates that between 30 000–80 000 years ago, populations of modern humans (*Homo sapiens*) and the extinct Neanderthals (*Homo neanderthalensis*) lived close to one another in parts of the Middle East, Asia, and Europe.

Using molecular and structural homology analyses, researchers have constructed a theory about the relationships between ancient populations. This is represented in the diagram.



a Assume that the common ancestor depicted in the phylogenetic tree is not from the genus *Homo*. With reference to your understanding of the general trends in hominin evolution, suggest which other genus this common ancestor is likely to have belonged to. Justify your response. (2 MARKS)

b Given the phylogenetic tree above, would you expect *Homo sapiens* of African descent to have Neanderthal DNA in their genome? Justify your response. (2 MARKS)

c In collecting evidence for this molecular analysis, a team of researchers from Australia uncovered an extensive collection of ancient Neanderthal fossils found deep within a cave in the Middle East.

Outline one bioethical issue associated with the uncovering of fossils in foreign countries. (2 MARKS)

Adapted from VCAA 2015 Section B Q11ai

11C THE HUMAN FOSSIL RECORD

We humans love to contest and debate things. However, there is perhaps no argument more difficult than the one we have with our Biology teacher when she gives us our SAC back. I deserved that one mark for Question 10, I know I did, but it's recess and I don't want to be back of the line at the tuck shop. What I really needed was evidence. I'm sure if I had been using my Edrolo textbook at the time, then I'd be able to show Ms. Franklin exactly where she was wrong and secure that precious extra mark.

Building and mounting hard evidence for your position is central to the scientific dogma, and often takes years and years of hard work. Now imagine a scenario where your only available evidence for a hypothesis was a small toe bone hidden somewhere deep in a cave on a faraway island in the southern Pacific ocean. Sounds shaky? How would you feel if you then needed to convince the world that the little piece of bone actually belonged to an entirely new species of ancient human that lived in small colonies tens of thousands of years ago? Providing irrefutable evidence of your position is hard, especially when it comes to interpreting the fossil record.

Image: Sensvector/Shutterstock.com

Lesson 11C

In this lesson you will consider the difficulties in interpreting the human fossil record as well as consider recent discoveries of new ancestors.

◀ *Prerequisite knowledge* *Future applications* ▶

Years 7–10
You learned how to interpret evidence for evolution, including the fossil record, anatomical similarities, and the geographical distribution of species.

Lesson 10A
You learned about changes in species over time as evidenced by the fossil record, including the law of fossil succession and transitional fossils.

Chapter 11
So far in this chapter, you have considered hominin evolution across time, including general trends of our evolutionary past.

Lesson 11D
In this lesson, you will consider ways of using this fossil and DNA evidence to explain the migration of hominin populations around the world.

Study design dot point

- the human fossil record as an example of a classification scheme that is open to differing interpretations that are contested, refined, or replaced when challenged by new evidence, including evidence for interbreeding between *Homo sapiens* and *Homo neanderthalensis* and evidence of new putative *Homo* species

Key knowledge units

Why is interpreting the human fossil record so difficult?	4.2.11.1
Did we breed with Neanderthals?	4.2.11.2
New hominin species	4.2.11.3

Why is interpreting the human fossil record so difficult? 4.2.11.1

OVERVIEW

The human fossil record is like a puzzle that is slowly being filled in as new fossils are discovered. However, it is ultimately still incomplete and different interpretations can be made from the few pieces of evidence we have.

THEORY DETAILS

Over the first two lessons of this chapter, we explored what it means to be human. We learned that scientists study the fossils and genomes of our ancestors to make **inferences** about when they lived, how they moved around, and even how big they were. These inferences are assumptions and are only as good as the evidence from which they stem.

inference conclusions or assumptions reached by analysing and extrapolating from evidence

This lesson will explore some of the difficulties we face in trying to accurately interpret our evolutionary history. The human fossil record is far from a complete depiction of our evolution as a species as it only shows very limited, and often imperfect, fossilised fragments of a small collection of our ancestors.

On the most basic level, interpreting the human fossil record is so difficult simply because the record itself is not complete. This could be due to an almost innumerable set of reasons, some of which include the fact that:

- Not all individuals die in conditions that promote fossilisation. For example, organisms might decompose completely or be eaten by scavengers when they die.
- Rock layers and the fossils they contain might erode and disappear over time.
- Many rock layers are still inaccessible to paleontologists, so not all fossils have been found.

Lesson link

If you need to familiarise yourself with the fossil record and how it operates, please revisit ***lesson 10A*** before continuing with the lesson.

Because the fossil record itself is incomplete, the inferences we are able to make are also often incomplete and can be challenged or refined in light of new and/or competing evidence. For example, there are times when scientists disagree on the identity of a hominin fossil, typically in situations where the fossil shares features with previous fossils of other known species. One possible articulation of this dilemma can be represented as follows. Imagine scientists found a new fossil – Fossil A – and are trying to determine what species of hominin this fossil represents. The following facts are known about Fossil A:

- Fossil A was found in a cave and dated to around the same period as two other species of hominin that lived in the same area at the same time – Species X and Species Y.
- Fossil A also shares characteristics of both Species X and Species Y, therefore making it difficult for scientists to determine which species they are looking at by reference to the fossil alone.

What should these scientists do? Was this new fossil taken from an individual of Species X or Species Y, or is this new fossil an example of a completely new and previously undiscovered species – Species Z?

Theory in context

HOMO NALEDI

The case of *Homo naledi* is a terrific example of this difficulty. In 2013, fossils of an unknown hominin species were discovered in a cave in South Africa (Figure 1). The fossils showed features resembling those of both modern humans (*Homo*) and older hominins (*Australopithecus*) (Table 1) and some scientists suggested that it may be a **transitional fossil** between *Australopithecus* and *Homo*.

Figure 1 Fossils of *Homo naledi*

transitional fossil a fossil that shows traits that are common to both its ancestral group and its descendant group. They are particularly important when the descendant species is physically very distinct from the ancestral species, such that the transitional fossil can help demonstrate evolutionary changes between the two

cont'd

Theory in context

HOMO NALEDI – CONTINUED

Table 1 Features of *H. naledi* grouped by genus resemblance

Australopithecus	*Homo*
• more primitive shoulders • fingers are long and curved, suggesting tree-climbing lifestyle • wider, flatter pelvis • skull size is small • wide rib cage	• rounded skull shape • hands well suited for object manipulation • arched feet and smaller ankles

The fossils were not dated upon their initial discovery, so a crucial piece of the puzzle was missing. Interestingly, they were arranged in a peculiar pattern, suggesting some sort of burial rite. This finding could be groundbreaking if *H. naledi* really is older than the genus *Homo*, since it would mean that complex behaviour such as burial rituals would have evolved much earlier than previously thought. Figure 2 shows each of the possible ages for *H. naledi* and their implications for hominin evolution.

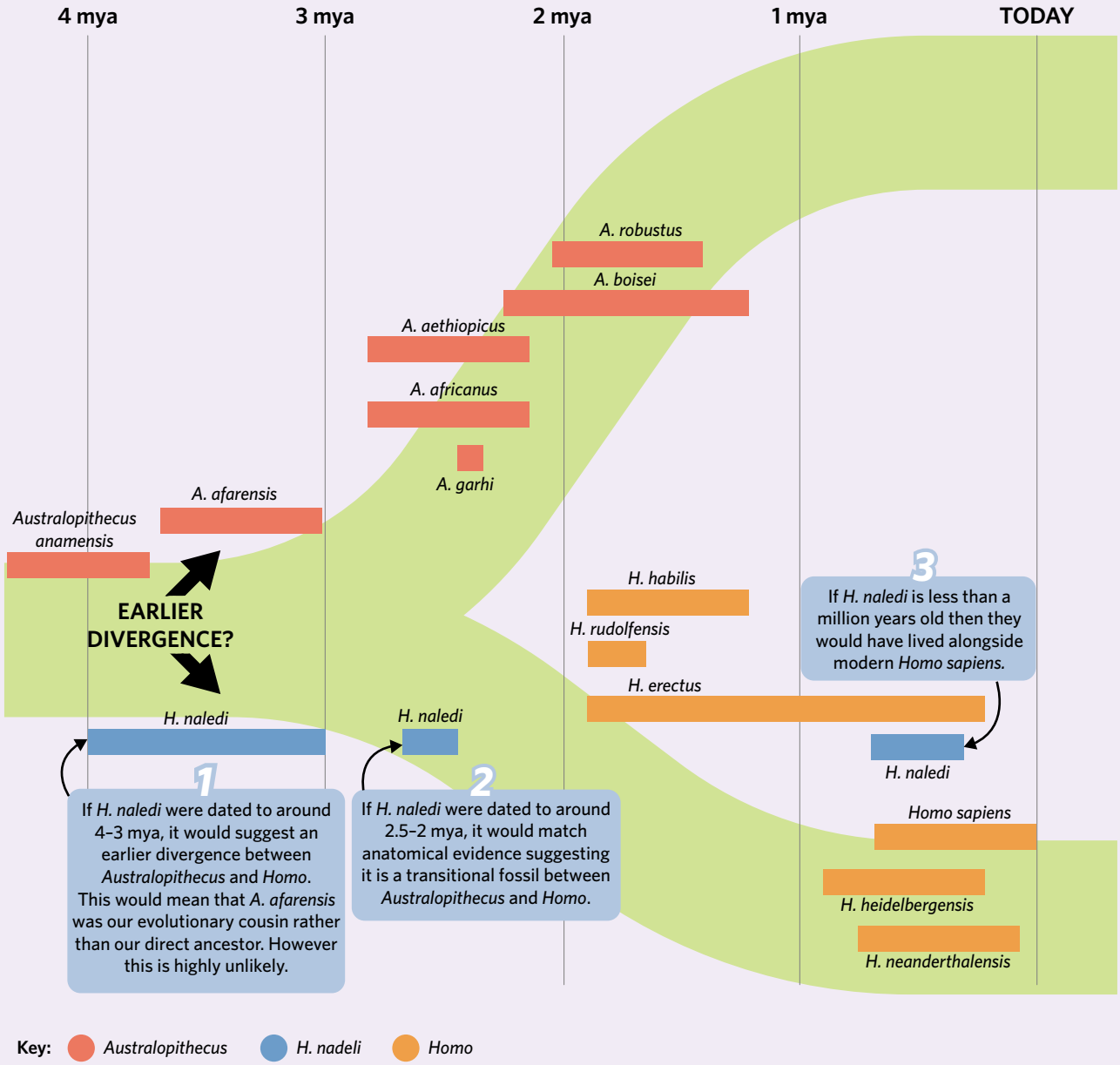


Figure 2 The implications for hominin evolution of three potential outcomes of *H. naledi* dating. Each outcome is dependent on the age of *H. naledi* fossils, as based on dating data.

Later in 2017, the fossils were dated to around 250 000 years ago, matching up with outcome 3 in Figure 2. This indicates *H. naledi* isn't as old as earlier scientists had suspected and actually lived alongside modern humans, adding another piece to the puzzle of hominin evolution. This is a prime example of the uncertainty shrouding our evolutionary history.

Examiners' tip

Extrapolated out on a much larger scale, it's easy to see how this type of ambiguity could really make things difficult when trying to properly understand hominin evolution. We don't know how many more ancestors we will uncover in the future, nor whether the classification we've arrived at today is even accurate. For example, consider Question 9 of the 2020 exam, which included the following figure:

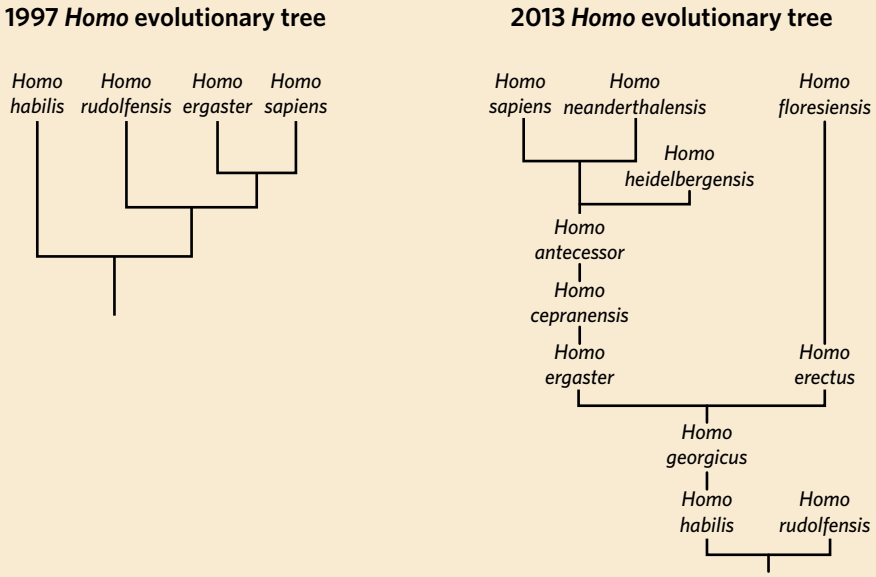


Figure 3 A figure included in Question 9 of the 2020 VCAA Biology exam, showing two *Homo* evolutionary trees, one of which was created in 1997 and the other in 2013.

We can clearly see that a lot changed between the evolutionary tree in 1997 and the corresponding tree in 2013. Not only have many more species of *Homo* been uncovered in that time, but the relationships between different members have also been updated. Take *Homo ergaster* and *Homo sapien*s as an example:

- In 1997, the two species were said to share a common ancestor, but did not share one in 2013.
- In 1997, *H. ergaster* was not a direct ancestor to *H. sapiens*, but is considered one in 2013.
- In 1997, the two species shared a more recent common ancestor and were therefore thought to be more closely related than they are in 2013.

We understand that scientists do not always agree on the positioning of a particular *Homo* species in the evolutionary tree. This is because there are significant gaps in the fossil record, and when new discoveries of fossils are made, this can significantly alter previous views held by scientists.

Lesson link

When engaging with evolutionary trees such as the one included in Figure 3, it is important to be clear on the difference between ancestors and descendants, regardless of how the tree is drawn (i.e. left to right, bottom-up, with arrows etc.). It is necessary to be able to understand evolutionary relationships when represented visually in different ways, as this is something that the VCAA have examined closely in the past. To refamiliarise yourself, please revisit ***lesson 10C***.

Did we breed with Neanderthals? 4.2.11.2

OVERVIEW

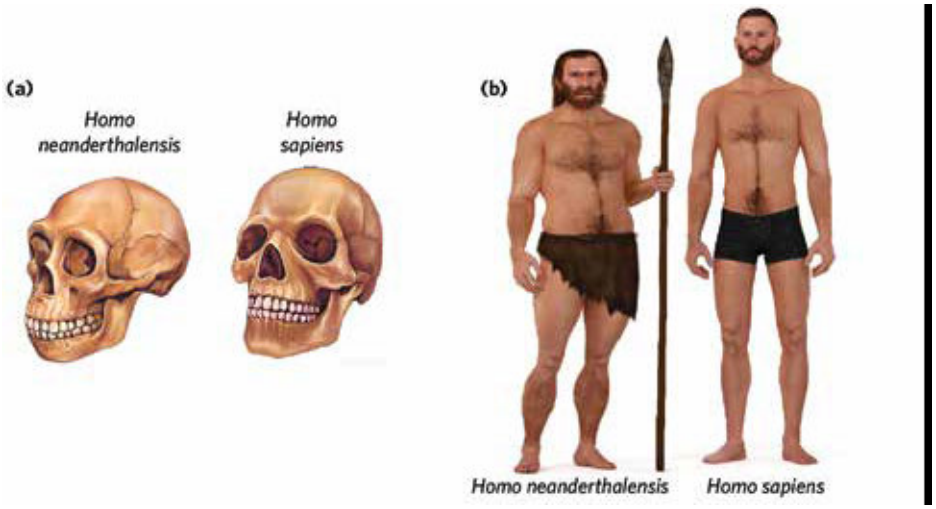
How do we know that Neanderthals and humans interbred 50 000 years ago? Here we will look at the evidence supporting this hypothesis.

THEORY DETAILS

Despite the ambiguities of the human fossil record, scientists are able to make compelling inferences and hypotheses about our evolutionary history. One particularly interesting example of this is the idea that early humans actually interbred with the Neanderthals – a completely different species – 50 000 years ago. How could we possibly know this? Firstly, let's revisit who the Neanderthals were, and then look at the evidence to suggest that **crossbreeding** occurred between our two species.

interbreeding refers to the mating between different species (e.g. between *Homo sapiens* and other closely related species such as Neanderthals and Denisovans). Also known as **crossbreeding**

Homo neanderthalensis, commonly known as the Neanderthals, were our close evolutionary cousins that existed in Europe and Asia between 40 000 and 400 000 years ago. They are known from fossil evidence that has accumulated over time since the first Neanderthal fossils were discovered in the Neander Valley in Germany in 1856. The mtDNA taken from Neanderthal fossils and compared with *H. sapiens* suggests that we are separate species that shared a recent common ancestor around 400 000 years ago.



Images (left to right): Liliya Butenko, Nicolas Primola/Shutterstock.com

Figure 4 **(a)** A side by side comparison of a Neanderthal skull (left) and a human skull (right), and **(b)** an artist's interpretation of a Neanderthal man (left) and human man (right).

Being closely related to humans, Neanderthals share a lot of features with us but differ in a few important ways. We can see a lot of these differences in Figure 4, and Neantherthals can be summarised as having:

- a wider nose
- shorter limbs
- a stockier build
- a flared rib cage
- a sloping forehead
- an enlarged brow ridge
- a larger cranial capacity
- better resistance to colder climates.

Modern humans and Neanderthals lived in the same regions of Eurasia between 40 000 and 100 000 years ago, and there is evidence to suggest that crossbreeding occurred between our two species (Table 2).

Table 2 Recent evidence supporting Human-Neanderthal interbreeding

Evidence for interbreeding	Inference
Nuclear DNA studies in 2010 show around 1–4% of the human genome is identical to DNA found in Neanderthals (Figure 5a). This 1–4% similarity was only found in the genomes of non-African populations and not in sub-Saharan African genomes.	Neanderthals may have interbred with humans as they left Africa somewhere in the Middle East around 65 000 years ago and did not interbreed with African humans.
100 000 year old DNA from Neanderthal fossils found in Siberia in 2016 contained a significant amount of ancient human DNA not found in other Neanderthal populations.	A population of Neanderthals in Siberia may have interbred with an early form of humans around 100 000 years ago. This suggests a second interbreeding event with humans.

Although around 1–4% of some human genomes are made up of Neanderthal DNA, the part of the Neanderthal genome found in each person differs quite a lot. In fact, a team of researchers at Princeton University were able to recover around 41% of the total Neanderthal genome from a sample of 2500 individuals. Here, rather than directly sequencing the DNA of a Neanderthal, the researchers were able to string together bits and pieces of the Neanderthal genome from the small pieces of shared DNA found in the individual genomes of modern humans in their sample.

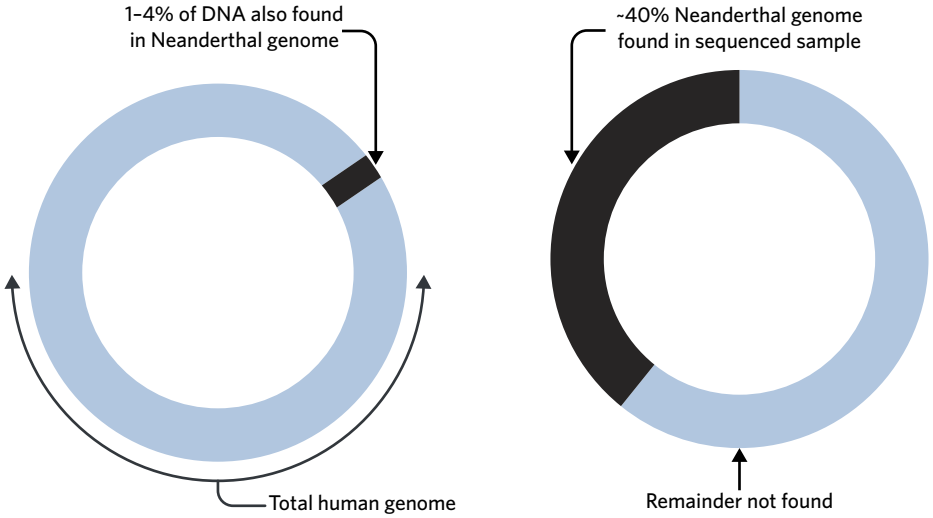


Figure 5 **(a)** Modern day humans (of non-African descent) share around 1–4% of their genome with that of ancient Neanderthals. **(b)** This shared DNA is different from person to person, as shown by Princeton University researchers who recovered around 40% of the total Neanderthal genome from different sections of the genomes of their sequenced sample (N=2500). Adapted from the University of California Television (UCTV, 2020).

New hominin species 4.2.11.3

OVERVIEW

Because of our constant effort to improve our understanding of the human fossil record, there are times when we discover completely new hominin species. Two of the most recent hominin species to have been discovered and added to our evolutionary tree are *Homo denisova* and *Homo luzonensis*.

THEORY DETAILS

Homo denisova

In 2010, scientists reported the discovery of bone fragments of a new hominin species in Denisova Cave in Siberia. The bones were dated to around 40 000 years ago. Upon analysis, nuclear DNA from the bone was found to be very closely related to Neanderthals, but different enough to be a new distinct species, termed *Homo denisova*, or Denisovans. Due to a lack of discovered fossil evidence, most of the inferences about the Denisovans have been made purely from DNA evidence. For example, only a few small teeth were uncovered in the original Denisova Cave (Figure 6), as well as a partial jawbone discovered in the Tibetan Plateau in 2019.

One particularly interesting point about the Denisovans is that they are thought to have interbred with a particular group of ancient humans from Melanesia, a subregion of Oceania that includes the modern-day countries of Fiji, Vanuatu, the Solomon Islands, and Papua New Guinea. We can infer this thanks to DNA taken from the genomes of Melanesian *Homo sapiens* which revealed that they share 4–6% of their DNA with Denisovans, but that other human populations do not. This interbreeding event likely occurred between 15 000 and 44 000 years ago as the ancestors of Melanesians migrated south through Southeast Asia.

Figure 6 Denisovan molar tooth fossil found in Denisova Cave in Siberia. Some teeth and a few bone fragments are the only fossil evidence we have of Denisovans.

Theory in context

While we are particularly looking at early interbreeding with *H. sapiens*, it can be interesting to consider interbreeding events between other hominin species and the possible implications stemming from this. For example, evidence suggests significant interbreeding between the Denisovans and the Neanderthal population of the time – about 17% of the Denisovan genome derived from the fossils in Denisova Cave was identified as Neanderthal DNA. What's more, a first-generation hybrid was discovered with a Denisovan father and a Neanderthal mother. The hybrid was nicknamed 'Denny'.

Homo luzonensis

In April 2019, researchers in the Philippines announced that they had discovered a new species of hominin, originally stemming from a single metatarsal (long foot bone) that had been uncovered back in 2007 in Callao Cave in the northern parts of Luzon, the largest island of the Philippines. The original bone was dated to 67 000 years old. Since that discovery, two more toe bones, along with seven teeth, two finger bones, and part of a femur have been uncovered on return trips to the cave in 2011 and 2015. Thanks to these further discoveries, we now estimate that *H. luzonensis* was a relatively small-bodied hominin ancestor that lived on the Philippine island of Luzon between 50 000 and 67 000 years ago.

Image: Soltchy/Shutterstock.com

Figure 7 Callao Cave, Philippines, the site of *Homo luzonesis*' discovery

What was interesting was that the fossils showed an unexpected mix of both ancient and more modern hominin traits, making it difficult to identify the bones as belonging to an existing species. For example, the teeth were small in size and simple in shape, which is indicative of a more modern hominin species, while the foot bones resembled those of the most ancient *Australopithecus* ancestors. In all, the specimens showed a new combination of features that were different from the combination of features found in other species in the genus *Homo*. Therefore, the specimens were believed to warrant their classification as belonging to a new species – which was named *Homo luzonensis*.

Theory summary

In this lesson, you have learned about how biologists interpret the fossil record differently and have considered how a range of different *Homo* species demonstrate these difficulties.

You have also looked closely at two species in particular, Neanderthals and Denisovans, both of which are thought to have interbred with early *Homo sapiens* at different points in our evolutionary history. These crossbreeding events are summarised in Figure 8.

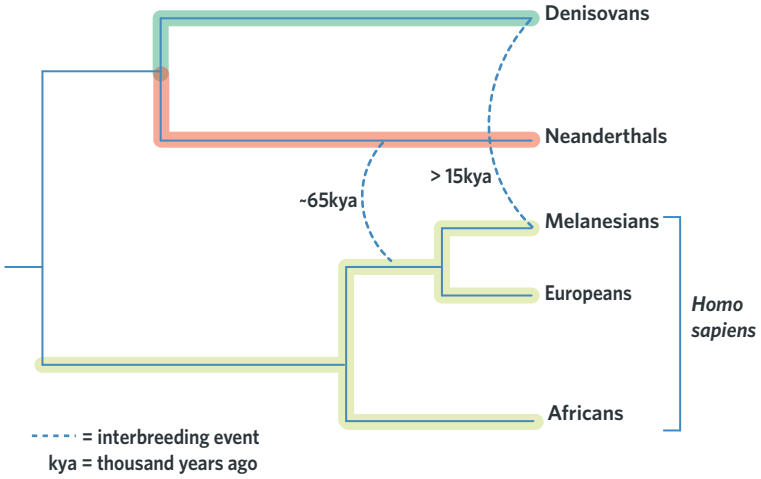


Figure 8 Phylogenetic tree depicting interbreeding events among modern humans, Denisovans, and Neanderthals.

This type of sleuth-work is exactly what evolutionary biologists and paleontologists do for a living, and was perfectly displayed by the team of researchers who discovered traces of Homo luzonensis *in Callao Cave back in 2007. Sure, it took another decade of work, several years of intense study and expertise, and a small fortune in grant money – but it was all worth it to add another branch to our ever-growing evolutionary tree.*

Worth it, for sure – perhaps even more worthwhile than me spending my lunchtimes arguing over the answer to Question 10 and ending up with a cold tuck shop focaccia...can you tell I'm still bitter?

11C QUESTIONS

Theory review questions

Question 1

Fill in the blanks in the following sentences.

Mating between humans and other closely related hominin species over time is known as _____. One example of this was the hominin species _____, who mated with non-African *Homo sapiens* and share 1–4% of their DNA. Another example would be the hominin species _____, who share 4–6% of their DNA with modern-day Melanesian humans. As well as interbreeding events, researchers are able to make other inferences based on the fossil record. A useful measure is identifying _____, which exhibit a combination of traits that are common to both an ancestral group and a derived descendant group.

Question 2

Using the human fossil record to determine the human evolutionary history is a difficult task. Which one of the following statements about the human fossil record is false?

A Fossilisation is difficult and only likely to have occurred amongst small numbers of our ancestral populations.

B Ancient *H. sapiens* interbred with the Neanderthals and Denisovans, raising questions about how we define a species.

C Rather than a single leap from ape-like ancestors to more human-like individuals, our evolutionary history involves numerous intermediaries and iterative changes over time.

D The fossil record shows us that hominin evolution takes 'big leaps' across time, where one species serves as a direct bridge to the next and shows linear morphological changes. This is like the bridge between *Australopithecus* and our own genus *Homo*.

Question 3

Categorise the following structures based on whether they would more likely to be found in an ***Australopithecus*** or ***Homo***. (*Select all that apply*)

I small skull _____

II legs similar to modern humans _____

III primitive rib cage shape _____

IV dated to around 250 000 years old _____

V fingers well adapted to climbing trees _____

Question 4

Which pieces of evidence support the hypothesis that *Homo neanderthalensis* interbred with *Homo sapiens*? (*Select all that apply*)

I Neanderthals have a larger cranial capacity than modern humans.

II Both modern humans and Neanderthals have evolved bipedalism.

III Modern non-African humans have 1–4% Neanderthal DNA in their genome.

IV Modern humans and Neanderthals both lived in Eurasia around the same time.

V mtDNA from humans was found to be different enough from mtDNA from Neanderthals to classify them as separate species.

SAC skills questions

Bioethical deep dive

Use the following information to answer Questions 5–8.

Each year, tens of millions of dollars are spent by universities and governments on paleoanthropological research. This work greatly contributes to filling in gaps in the knowledge of our evolutionary history. However, wherever there is a large amount of money, funding, and international interest, it is important to regularly revisit ways in which power may have been abused in the past, and where it might still be abused today.

Paleoanthropology's colonial history and identity is one of those ways. In the past, colonialism has been used to gain access to important research sites and collect and distribute fossils and artefacts. Many of these colonial powers, traditionally Europeans and researchers from the global north, have exercised undue control over the heritage of other cultures, especially in Africa, where they may have failed to consider the social context and importance of local research sites. This has often resulted in many culturally important fossils and artifacts being taken to institutions outside of Africa. For example, when Napoleon Bonaparte of France invaded Egypt in 1798, his army took home hundreds of tonnes of important Egyptian artifacts (which today sit in the Louvre Museum in Paris).

Unfortunately, many indigenous communities have lost control and access to significant cultural materials as a consequence. While many European nations have begun returning items taken from Africa in recent years, it is imperative that future research acknowledges this potential to damage and ignore indigenous connection to research sites. These days, many African countries now have formal laws and procedures regulating access to paleontological sites. For example, Ethiopian law protects the conservation of its cultural heritage and outlines ownership rights for uncovered paleontology discoveries in the country.

Question 5

Colonialism refers to

A the exertion of power and/or control over a country by foreign peoples, often for economic reasons.

B the donation of important cultural artefacts to local communities without their informed consent.

Question 6

Paleoanthropology refers to

A the study of our evolutionary history via the recovery and analysis of important historical material, such as fossils and artefacts.

B the settling of foreign countries by Europeans and invading armies of the global north for the sole purpose of gaining economic control of important cultural sites.

Question 7

Based on the key issues raised in the text, which of the following is not likely to be a concern for the research ethics of an international expedition?

A funding the expedition and paying the scholars involved

B incorporating the recommendations of local communities and indigenous peoples

C correctly handling uncovered material and determining how best to make it accessible

D protecting sacred sites and understanding the cultural and spiritual significance of the objects and landscapes of interest

Question 8

Assume you are an ethics advisor for a university that is about to approve a large grant for a research expedition to an isolated island overseas. Which of the following questions best corresponds to the bioethical concept of respect?

A How will my team excavate the site in a way that does not unnecessarily disturb local fauna and flora or disrupt the cultural significance of the landscape?

B How will I encourage my team to include the information and opinions of local scholars in a way that improves the fullness and multicultural accuracy of our final report?

C How will my team share our findings with the local community, making our research accessible in a culturally appropriate way and allowing access to the economic benefits that result?

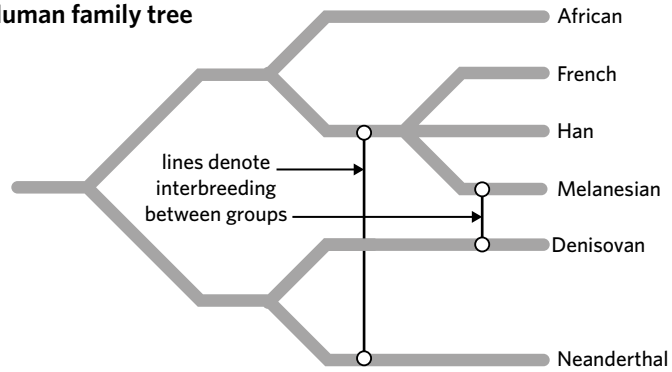
Exam-style questions

Within lesson

Use the following information to answer Questions 9 and 10.

This human family tree shows the relationships between different human groups.

Human family tree



Source: Ghosh (2010), adapted by VCAA 2017 Sample Exam Section A Q35

Question 9 (1 MARK)

Which group would most likely contain Denisovan DNA?

A Han group
B African group
C Melanesian group
D Neanderthal group

Adapted from VCAA 2017 Sample Exam Section A Q35

Question 10 (1 MARK)

The group most closely related to the Neanderthal group is the

A Han group.
B French group.
C Denisovan group.
D Melanesian group.

Adapted from VCAA 2017 Sample Exam Section A Q36

Use the following information to answer Questions 11–13.

Scientists have been studying the genomes of groups of present-day *Homo sapiens*. Most of the DNA in these present-day groups is the same as the DNA found in *Homo heidelbergensis*, suggesting that *H. heidelbergensis* is the direct ancestor of *Homo sapiens*. The scientists found that there were traces of DNA in these present-day groups from three separate *Homo* ancestor species, indicating earlier crossbreeding. The table provides information about eight species of *Homo*.

Species	When this species lived	Where this species lived
Homo rudolfensis	2.1–1.8 million years ago	Eastern Africa
Homo habilis	1.9–1.4 million years ago	Eastern and Southern Africa
Homo erectus	1.9 million – 100 000 years ago	Africa and Asia
Homo antecessor	approximately 1.2 million years ago	Spain
Homo heidelbergensis	700 000–200 000 years ago	Africa, Europe and possibly Asia
Homo neanderthalensis	500 000–40 000 years ago	Western Eurasia (as far east as Siberia)
Homo denisova	100 000 years ago	Siberia
Homo sapiens	approximately 180 000 years ago to present	worldwide

Source: Lewis (2016), as adapted by VCAA 2018 Northern Hemisphere Exam Section A Q21

Question 11 (1 MARK)

Two of the species that crossbred were identified as *Homo erectus* and *Homo neanderthalensis*. Which species of *Homo* shown in the table above could have been the third species to crossbreed?

A *H. rudolfensis*

B *H. antecessor*

C *H. denisova*

D *H. habilis*

Question 12 (1 MARK)

Based on the information in the table, which species would be least likely to interbreed with *Homo neanderthalensis*?

A *H. heidelbergensis*

B *H. sapiens*

C *H. erectus*

D *H. habilis*

Question 13 (1 MARK)

From the information in the table above it can be concluded that

A species diversity within the *Homo* genus was much greater around 400 000 years ago.

B *Homo neanderthalensis* was much more widespread than *Homo sapiens*.

C *Homo rudolfensis* was the longest living and most successful species.

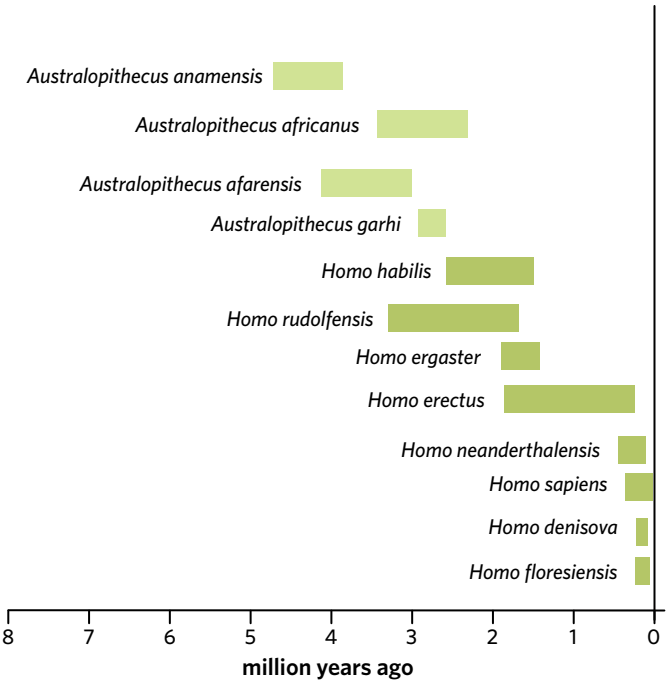
D the ancestor of the *Homo* genus likely lived in Siberia.

Question 14 (3 MARKS)

In 2013, about 1500 fossil bones of the recently discovered *Homo naledi* were found in a cave in South Africa. Finding out the age of *H. naledi* fossils is difficult and hotly contested. Assume that two different groups have the following contrasting opinions regarding the age of the fossils:

- Group 1 believes that the fossils are more than 2 million years old. They suggest that *H. naledi* was a 'link' species that represented a transition between the ancient genus *Australopithecus* and the more modern genus *Homo*.
- Group 2 believes that the fossils are less than 1 million years old. They disagree with Group 1's assessment, arguing that *H. naledi* is not a 'link' between *Australopithecus* and *Homo*.

The diagram indicates the time periods for different *Australopithecus* and *Homo* species.



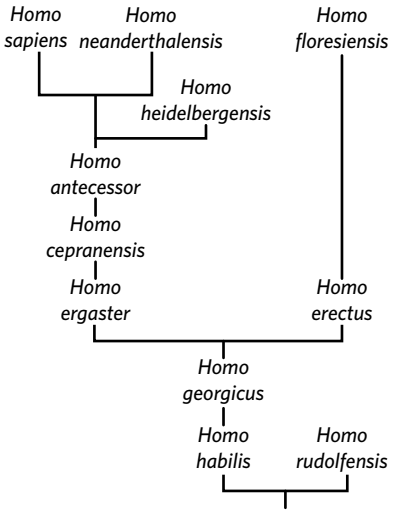
a Briefly describe what is meant by a transitional fossil. In your answer, describe why a transitional fossil is useful for researchers. (2 MARKS)

b Assume that Group 2 has accurately dated the fossils to less than 1 million years old. Using the diagram provided, suggest one reason as to why *H. naledi* cannot be the 'link' between the australopithecines and *Homo*. (1 MARK)

Multiple lessons

Question 15 (6 MARKS)

The diagram shows the evolutionary relationships between different *Homo* species, and is up to date as of 2013.



a Natasha has been given a collection of fossils and is asked to identify whether they belong to *Homo sapiens* or the earlier ancestor *Homo habilis*. Identify two key structural features that would suggest that the fossils belong to *H. habilis* rather than *H. sapiens*. (2 MARKS)

b Research into the nuclear DNA of modern humans shows that between 1–4% of our genome is identical to the genome of *Homo neanderthalensis*. However, this similarity is only shared between *H. neanderthalensis* and individuals of non-African descent. What does this suggest about the interbreeding events between *Homo sapiens* and *Homo neanderthalensis*? (2 MARKS)

c This evolutionary tree is up to date as of 2013. Nonetheless, evolutionary relationships are constantly being revised and updated, and are not always agreed upon by all scientists. Explain why this is the case. (2 MARKS)

Adapted from VCAA 2020 Exam Q9

Key science skills and ethical understanding

Question 16 (7 MARKS)

Denisovans are a species of the genus *Homo* that existed around 40 000 years ago. They are known from fossils found in Siberia that contained DNA, allowing researchers to sequence the Denisovan genome.

Researchers have surveyed the genomes of the modern *Homo sapiens* population and have concluded that interbreeding must have occurred between Denisovans and *H. sapiens* between 15 000 and 50 000 years ago. Modern African *H. sapiens* do not contain Denisovan DNA whilst modern Asian and Australian *H. sapiens* contain a small percentage of Denisovan DNA.

a A common definition for a species is any group of individuals who are able to exchange DNA and interbreed with one another to produce viable offspring. Given this definition, what implication does the DNA evidence have for the classification of the two hominin species, *H. sapiens* and Denisovans? (1 MARK)

b Explain why Denisovan DNA is not present in modern African *H. sapiens* but is present in other *H. sapiens* populations. (2 MARKS)

c Further research into the *H. sapiens* population has led to two new findings:

- The DNA from the Denisovan fossil in Siberia is more similar to Denisovan DNA in the East Asian genome than the Papua New Guinean genome.
- Modern individuals in Papua New Guinea contain 5% Denisovan DNA whilst East Asian populations contain far less Denisovan DNA.

Suggest a hypothesis that explains the origin of Denisovan DNA in both the East Asian and Papua New Guinean genomes. Justify your response by referring to both of the findings. (3 MARKS)

d Some groups of modern day humans have been found to contain sequences of hominin DNA that are not found in other humans, Neanderthals, or Denisovans.

Provide an explanation to account for these findings. (1 MARK)

11D HUMAN MIGRATION

In 1974, the remains of an ancient human were found buried in Lake Mungo, New South Wales. Named 'Mungo Man', he had been carefully buried on his back with his hands crossed neatly in his lap, with red ochre sprinkled across his chest. A peculiarly dignified and emotional burial – so who was Mungo Man?

Lesson 11D

In this lesson you will learn how the fossil record is used to demonstrate the migration patterns of our early *Homo sapiens* ancestors, Aboriginal Australians.

◀ *Prerequisite knowledge* *Future applications* ▶

Lesson 11A
You learned about the characteristics that define *Homo sapiens* as hominins, including the development of bipedalism. These characteristics helped ancient humans spread across the globe.

Lesson 11B
You learned about major trends in hominin evolution from the genus *Australopithecus* to the genus *Homo*. Evidence for these trends includes an increased brain size and changing limb structure over time. We can model these changes thanks to fossils we uncover around the globe.

Lesson 11C
You learned about particular intricacies in the interpretation of the human fossil record, including possible interbreeding events and periods of shared habitancy between ancient *Homo* species. We can better understand human migration from these periods of overlap.

Study design dot point

- ways of using fossil and DNA evidence (mtDNA and whole genomes) to explain the migration of modern human populations around the world, including the migration of Aboriginal and Torres Strait Islander populations and their connection to Country and Place

Key knowledge units

Tracking migrations	4.2.12.1
Aboriginal and Torres Strait Islander people's connection to Country and Place	4.2.12.2

Tracking migrations 4.2.12.1

OVERVIEW

Researchers are able to examine the fossil record and analyse DNA evidence to help understand the ancient migratory patterns of our species. The prevailing understanding is that *Homo sapiens* evolved in Africa around 200 000 years ago before migrating to different parts of Eurasia, where they replaced existing populations of other *Homo* species.

THEORY DETAILS

Evidence for examining our evolutionary past

In Chapter 10, we learned about the different methods which can be used to demonstrate the relatedness between different species over time. Two of those methods are the fossil record (lesson 10A) and molecular homology (lesson 10B). Let us quickly familiarise ourselves with how each of these measures can be used to demonstrate relatedness:

1. the fossil record – we can use fossils as evidence for relatedness by pointing to comparative anatomy, particularly the presence of homologous structures.
2. molecular homology – we can use similarities between nucleotides in DNA sequences or amino acid sequences in proteins as evidence for relatedness.

Using these two measures, we have been able to better explore major trends in our own evolutionary history over the course of this chapter. Now, in this lesson, we will be considering how these two methods – fossils and molecular homology – can be used as evidence for different theories of human migration over the course of our expansion around the globe.

How *Homo sapiens* spread: human migration

According to the fossil record, the earliest known hominins first evolved in Africa approximately 4 million years ago. These early hominins, namely the *Australopithecines*, remained solely in separate regions of Africa and continued to evolve, resulting in the emergence of new species over time, including the earliest members of the *Homo* species (such as *Homo habilis*). However, it wasn't until around 2–2.25 million years later before any of these early hominin species began to migrate out of Africa and into the nearby regions of Europe and Asia. This feat is currently believed to have been accomplished by a small population of *Homo erectus*, whose fossils were the first to be found outside of the African continent (in China and Indonesia).

Fast forward another 2 million years and we *Homo sapiens* are the last remaining lineage of the *Homo* genus. What's more, our population totals close to 8 billion and spans across almost all the habitable land on the planet. In light of this, we are left to ponder two questions:

1. How did this extraordinary geographical expansion of *Homo sapiens* occur?
2. How can we leverage the information contained in DNA and the fossil record to help us better understand these ancient migratory patterns?

In this lesson, we will be considering how *Homo sapiens* made their way out of Africa and into other parts of the globe. To do this, we will consider the two main hypotheses – (1) the **'multiregional' hypothesis** (Figure 1a) and (2) the **'Out of Africa' hypothesis** (Figure 1b). Both hypotheses agree that the *Homo* lineage originated in Africa and expanded to Eurasia about 1.8 million years ago with the departure of *Homo erectus* – but they differ in explaining the origin of modern humans. The central question separating both models can be summarised as follows: did *Homo sapiens* evolve as a collection of separate, individual populations from the existing hominin populations that had left Africa before us, or did we evolve independently in Africa before migrating outwards?

multiregional hypothesis
a model for the geographical spread of *Homo sapiens* which suggests that separate human populations evolved independently from earlier hominins that had spread throughout Eurasia and experienced gene flow. Also known as the **regional continuity model**

Out of Africa hypothesis
a model for the geographical spread of *Homo sapiens* which suggests that humans first developed and evolved in Africa before migrating outwards and expanding their colonies, replacing the earlier hominins that had spread prior. Also known as the **African replacement model**

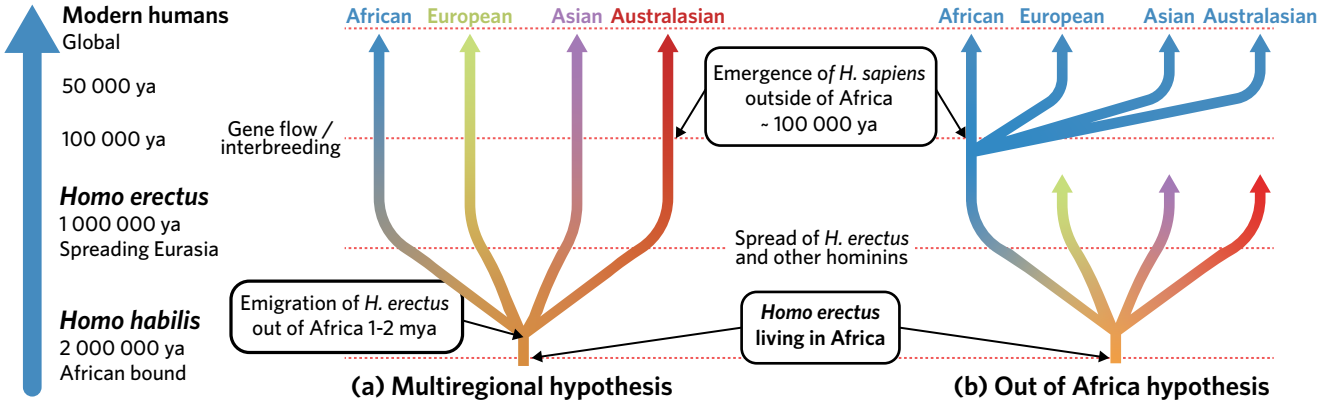


Figure 1 A summary of the alternative theories of *Homo sapiens* development. **(a)** The multiregional hypothesis posits that separate *Homo sapiens* populations evolved from earlier hominins that had spread throughout Eurasia over the millenia prior. **(b)** The Out of Africa hypothesis posits that *Homo sapiens* first evolved in Africa before migrating outwards, replacing the earlier hominins that had spread prior.

It is worth noting that almost all existing evidence supports the Out of Africa hypothesis and that this is generally taken as the accepted model of human migration. In this lesson, the Out of Africa hypothesis has been compared to the multiregional hypothesis to highlight the uncertainty and complexities present in our understanding of the human fossil record, an exercise we began in the previous lesson.

Out of Africa hypothesis

This hypothesis suggests that *Homo sapiens* evolved in Africa around 200 000 years ago, long after the departure of *Homo erectus* into Eurasia, and remained there for an extended period of time (up to 100 000 years) before **emigrating** in waves and replacing existing hominin species such as *Homo erectus* and *Homo neanderthalensis* in different parts of Europe and Asia. This would mean that all modern human beings are of African descent.

The evidence for the Out of Africa hypothesis is compelling:

- Repeated large-scale analysis of the mitochondrial DNA (mtDNA) of modern humans demonstrates that our mitochondrial lineages can all be traced back to a common ancestor that lived in Africa between 140 000 and 290 000 years ago.
- Modern-day humans show very little genetic diversity compared to other species. One reason for this is due to our relatively short existence (~200 000 years), as well as the suggestion that we originated from a small, centralised population. What's more, the greatest genetic diversity is thought to exist in African populations, where *Homo sapiens* first appeared, suggesting that there has been more time for spontaneous mutations to accumulate in mtDNA.
- While fossil evidence of *Homo sapiens* is limited due to the short time frame of our existence, scientists are still able to model what is thought to be our first migratory wave as a species from early fossilised remains found along the east coastline of Africa. For example, some of the oldest *Homo sapiens* fossils found were uncovered in East Africa and dated to around 160 000 years ago, as well as later fossils uncovered in the Middle East and dated to 100 000 years. This suggests migration into and out of Northern Africa (Figure 2a).
- As well as DNA evidence and fossilised remains, scientists have also uncovered other artefacts along the far north-west into deeper parts of Europe (and south-east into Asia). These include stone tools, carvings, and cave paintings, which indicate an increased complexity and cultural evolution. These can be dated to further map our migratory pattern throughout Eurasia. For example, stone tools were found in the United Arab Emirates and dated to 80 000 years old, as well as 74 000 years old in India. Cave paintings and carvings have also been found in western European regions and dated to around 40 000 years.

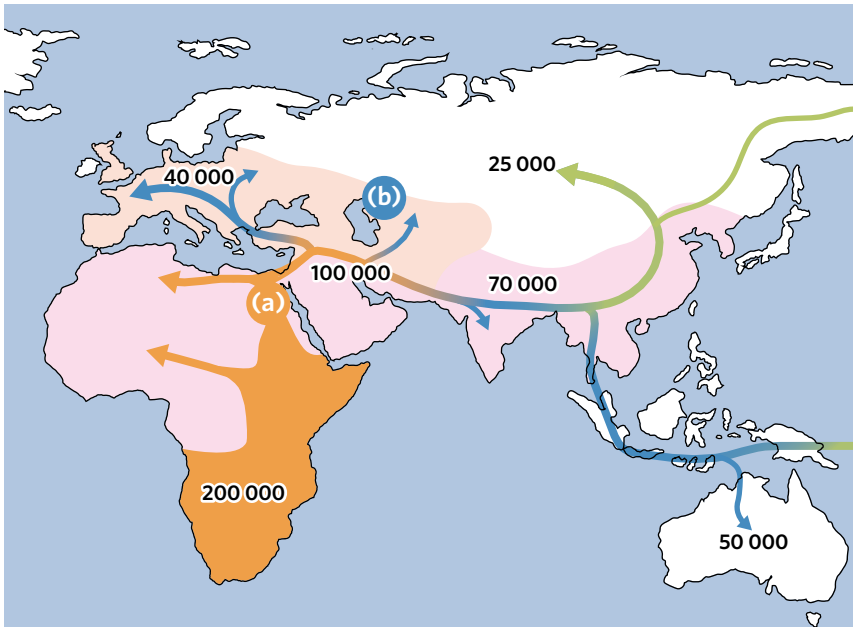


Figure 2 **(a)** Proposed spread of *Homo sapiens* during their first migratory wave out of Africa. Fossils found in East Africa show that *Homo sapiens* emerged as early as 200 000 years ago before travelling along the eastern coastline and into the Middle East (via the northern tip) around 100 000 years ago. **(b)** Proposed second migratory wave of *Homo sapiens* further into Eurasia between 40 000 and 80 000 years ago.

Theory in action

Check out scientific investigation 11.1 to put this into action!

emigration leaving one place to settle permanently in another

Lesson link

In ***lesson 10B***, we considered the use of mtDNA as a tool for understanding human evolution and relatedness. Ancient mtDNA can be retrieved from museum specimens and fossil remains, and is extremely useful in providing direct evidence for population origins and migratory processes.

This usefulness is largely owing to the fact that mtDNA has an apparent lack of recombination, meaning that any variation seen is likely due to mutations. This mutation rate is also high and tends to be geographically restricted, meaning that mutations will accumulate over time and can be used to highlight genetically distinct populations.

Multiregional hypothesis

This hypothesis suggests that the evolution of modern humans, from *Homo erectus* to *Homo sapiens*, was actually an ongoing process across all regions of the world with gene flow between different continental populations. In other words, the multiregional model posits that *Homo sapiens* evolved from several different geographically separate groups of *Homo erectus* who had migrated across much of Africa and Eurasia in the million years prior to the emergence of modern humans. This would mean that all modern human beings are not of African descent, but rather descendants of smaller, localised populations of early hominins. For instance, modern Africans originated from early Africans that evolved from African hominins, whereas modern Asians came from archaic Asians that evolved from Asian hominins.

There is limited evidence to support the multiregional hypothesis today. Some proponents argue that the ancient fossil record demonstrates what are known as **morphological clades**. For example, some researchers point to a morphological clade in the Chinese region characterised by a combination of ten features commonly seen in fossils uncovered in this locality, including facial flatness and a non-depressed nasal root. However, many critics of this theory argue that the fossil record is too incomplete to rely on morphological clades, or that different studies do not include enough fossil specimens to be relied on.

morphological clades combinations of various physical characteristics that are unique to particular geographical regions across a wide timespan

Aboriginal and Torres Strait Islander people's connection to Country and Place 4.2.12.2

OVERVIEW

Aboriginal Australians are the longest continuous population on earth. They share a strong Connection to Country that can be difficult for non-Aboriginal peoples to fully appreciate.

THEORY DETAILS

The second wave of migration proposed in Figure 2b is believed to have led to the emergence of Indigenous populations in the Oceanic region, including the arrival of Aboriginal Australians between 50 000 and 65 000 years ago. Research that sequenced the genomes of a group of Aboriginal Australians suggests that most of the genomes of modern-day Aboriginals can be traced back to the original 'Out of Africa' event in which the first ancient humans spread throughout the globe.

It is thought that around 50 000–65 000 years ago, the wave of migrants reached a now prehistoric supercontinent called Sahul, which was composed of present-day Australia, Tasmania, and New Guinea, before the supercontinent separated due to rising sea levels around 10 000 years ago (Figure 3a). This separation made Aboriginal and Torres Strait Islanders (also known as First Nations people) geographically and genetically isolated, which is thought to make them the world's oldest surviving civilisation. In other words, Indigenous Australian people are the longest surviving population of modern humans to have lived in a given location, and are thought to have one of the strongest **Connection to Country** of any living population on earth.

Connection to Country a reciprocal relationship between First Nations people and their ancestral lands and seas

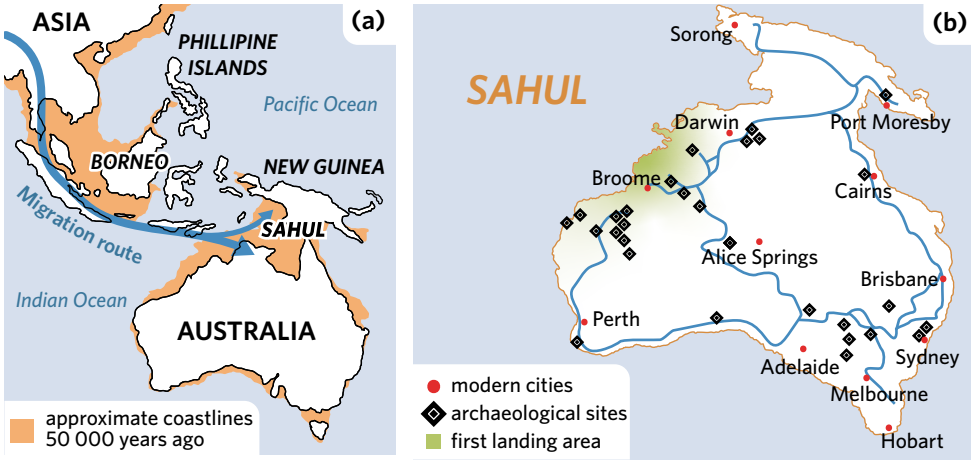


Figure 3 **(a)** The proposed route of Indigenous Australians into our country roughly 50 000 years ago. The supercontinent Sahul would later separate, leaving the populations geographically isolated and making them the longest unbroken example of an indigenous population. **(b)** The assumed migration routes of Indigenous Australians once arriving in Sahul. Current understanding hypothesises that the first Australians landed on the shores of Western Australia, around the Kimberley region, and spread from the far north of the tropics to the deep south of Tasmania in as little as 6 000 years.

Connection to Country

For many First Nations people, the land on which they live goes beyond simply a physical environment, and is instead seen as fundamental to identity. The term '**Country**' is used to refer to a special kindred relationship between individuals and their ancestral lands and seas. It is a reciprocal relationship that goes two ways: the land provides for the people, while the people manage and sustain the land through their culture, ceremonies, and care. The words and descriptions that many Aboriginal language groups use when talking about Country express this living relationship. For example, Country may be mother or grandfather. Kinship terms like these impose a mutual responsibility of caring, knowledge transfer, and shared growth. Land sustains the lives of Indigenous Australians in every aspect – spiritually, physically, socially, and culturally. This can be a hard concept for non-Aboriginal people to understand, but it is central to the lives of Aboriginal Australians.

It is therefore important to acknowledge the existence of two parallel scientific theories of early human migration. Up to this point in the chapter, we have considered a Westernised view of the migration of early humans out of Africa, and while the observational framework of the Indigenous scientific process is similar to the Western process, the way of explaining and communicating knowledge can be very different. For example, it is the understanding of some Aboriginal Australian language groups that their people have been in Australia since the beginning of time. Another example is the different meaning of **Dreaming** beings for Aboriginal language groups, who sometimes believe that children's spirits are present in the landscape and enter women's bodies when the child quickens, or first moves. Neither understanding should be privileged and both should be respected and communicated without bias.

Country an area that is traditionally owned and looked after by an Aboriginal language group or community, or by certain people within that group. The term may indicate more than simply a geographical area – it is also a concept that can encompass the spiritual meaning and feelings of deep connection and attachment associated with that area

Dreaming an Aboriginal philosophy that describes the time when Ancestral Spirits (Dreaming Beings) moved over the land and created life and important geographical sites. It explains the origins of the universe, as well as the relationships between humans, animals, and the land on which they live. The Dreaming is passed down through generations and governs familial, relational, communal and spiritual obligations for Aboriginal Australians. Also known as **The Dreamtime**

totem emblems or symbols that represent the spiritual connection (Dreaming) between Aboriginal people and Country. Totems can take a range of different forms, such as animals, plants, and landscapes

Theory in context

DREAMING BEINGS AND THE WALPIRI

The Dreaming is a complex term that may encompass a range of different beliefs depending on the individual language group one is referring to. Whenever we discuss terms related to the Dreaming or give examples of Dreaming beings, it is important to mention the language group wherever possible. For example, sometimes, rather than the Dreaming being physically housed within a person, the Dreaming of a specific cultural site becomes what defines the person and therefore can relate to their personal **totem** and cultural responsibilities. Indigenous Australians believe that Dreaming can define and shape character and personality.

One example is the Walpiri language group, who are one of the central Australian peoples who collaborated with Western anthropologists to create an understanding of the terms we have used throughout this lesson, such as Dreaming. The Walpiri talk about 'Yiwirinngi', which is a person's 'Conception Dreaming'. It is defined in the Walpiri dictionary as an individual's 'life-force or spirit which is localised in some natural formation and which may determine the spiritual nature of a person from conception and the relation of that person to the life-force'. For the Walpiri, this natural place and a child's Conception Dreaming coincides with where the mother believes the child was conceived.

Theory in context

ABORIGINAL APPROACH TO HEREDITY AND INBREEDING

One example of the overlap between the scientific understandings of Western and Aboriginal traditions is the biological notion of heredity, and the importance of protecting the delicacy of genetic and familial lines. The fact that various characteristics are passed down through these family lines has been strictly observed and understood in Aboriginal communities for countless generations, as evidenced by the importance placed on avoiding inbreeding, particularly within quite small and geographically isolated communities throughout central Australia.

Complex systems of avoidance and regulations of marriages exist in Aboriginal societies from all over Australia, including skin naming systems, **totemic relationships**, **moieties**, and marriage responsibilities. These various classifications are all associated with places, animals, and Dreaming figures and are part of systems of avoidance, responsibility, and regulation of both human and natural systems. It is the understanding of Aboriginal people that these rules are part of the Dreaming and have been passed down since before time to help guard against the possibility of inbreeding by carefully regulating who is married to whom.

totemic relationships shared kinship between specific totems and the family, clan, individual and/or language group

moieties a two way division of society into maternal and paternal groups

It is also important to remember that Aboriginal people's understandings, laws, and cultures are not consistent around Australia. Different people have very different beliefs and systems of social organisation. Where possible the specific language group is used, or where generalisations are made it is important to note that they do not apply to all Aboriginal cultural groups.

The Out of Africa model, which places the advent of Aboriginal Australians at around 50 000–65 000 years, diminishes the traditional view of many First Nations communities who believe that their people have been in Australia since the time of creation. Genomic research carried out by non-Aboriginals into the origins of Aboriginal people in this country can therefore create a degree of angst in these Indigenous communities for a number of reasons, including a diminishment of their cultural connection to their creation story, a challenge to their identity and status as First Nations people, or even risks regarding land rights and status within certain communities.

Theory in context

JUUKAN GORGE

One way to understand First Nations' Connection to Country is to examine the importance of key cultural sites. One example is the famous Juukan Gorge, which is located in the Pilbara region of Western Australia. The site holds particular importance for the Puutu Kunti Kurrama and Pinikura peoples, and to Aboriginal Australians more generally, owing to the fact that a 3 000 year old lock of hair was found and tested to show that it belonged to a direct ancestor of this community. The Juukan Gorge was continually occupied by Indigenous Australians for over 50 000 years and had been used as rock shelters during the last ice age. This made it the only such site discovered in inland Australia to which continual occupation through the last glacial period was known.

The cave was permanently destroyed by the multinational mining company Rio Tinto in May 2020 as part of their expansion plans for one of their mines. This gravely damaged the relationship between Rio Tinto and the Puutu Kunti Kurrama and Pinikura peoples. Any damage to Country is incredibly damaging to Indigenous peoples, especially significant sites such as Juukan Gorge, which was thought to be the only site of its age with fossilised remains and stone tools – a direct example of Aboriginal lineage and a symbol of their Connection to Country.

Theory in context

LAKE MUNGO

Lake Mungo, in the south-west of New South Wales, was the site of the discovery of the oldest human remains in Australia. Three bodies were uncovered between 1969–1974 and dated at around 42 000 years old! To put this into perspective, when we think about the ancient Egyptians we are really talking around 5 000 years ago. King Tut, for example, reigned over ancient Egypt around 3.5 thousand years ago. Lineages like those of Aboriginal Australians are incredibly rare and important. Two of these skeletons – named Mungo Man and Mungo Lady – were determined to be ritualistically buried in a complex burial process, which places this site as one of the earliest examples of ritualistic burial anywhere in the world.

Later, in 2003, 460 footprints were uncovered around the lake and dated to around 20 000 years old. This represented the largest collection of fossilised footprints ever found in a single discovery anywhere in the world. The lakes area is now World Heritage listed and holds incredible significance for the Paakantji, Mutthi Mutthi and Ngyimpaa people. The skeletons have since been returned to the Aboriginal communities to which they belong.

Image: Leah-Anne Thompson/Shutterstock.com

Figure 4 Lake Mungo

Theory summary

Through the use of fossil and DNA evidence, scientists have come to a working hypothesis for human migration out of Africa. According to this hypothesis, early humans migrated out of Africa around 100 000 years ago, spreading in waves throughout much of Eurasia and replacing existing hominin populations that had lived in those areas prior.

Modern Aboriginal Australians can trace their lineage back to this first migration out of Africa, where it is believed that around 50 000 to 65 000 years ago the first humans arrived on the land. This makes Aboriginal Australians one of the oldest continuous populations on earth, and has tremendous impacts on Aboriginal peoples Connection to Country.

Mungo Man was a 42 000 year old Aboriginal man and holds tremendous importance to Aboriginal people nationwide, especially the Paakantji, Mutthi Mutthi, and Ngyimpaa peoples. Mungo Man lived around the shores of Lake Mungo with his family, and died around the age of 50. He died with significant arthritis after a hard life as a hunter. He cared for his Country and kept the special men's knowledge of his people safe.

11D QUESTIONS

Theory review questions

Question 1

Based on our current knowledge of the human fossil record and mtDNA data, which of the following options best describes the prevailing view of *Homo sapiens* early migratory patterns?

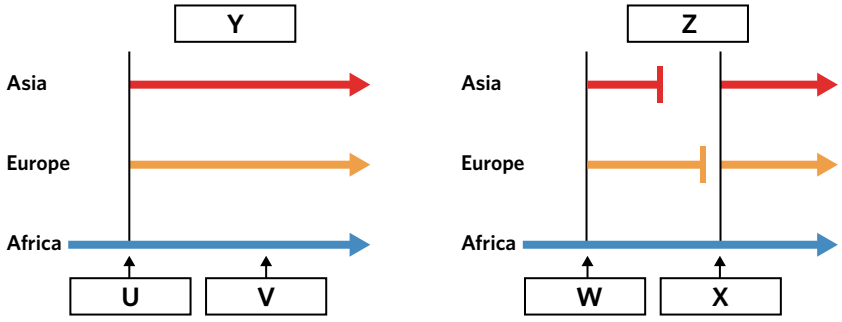
A A transition from earlier hominin species to *Homo sapiens* occurred within Africa, before a later migration throughout parts of Eurasia and the rest of the world led to the replacement of remaining hominin populations by *Homo sapiens*.

B A slower transition from subpopulations of *Homo erectus* to *Homo sapiens* occurred throughout much of Eurasia, where significant gene flow between these subpopulations led to genetic similarities between geographically distinct populations of ancient humans.

Question 2

Label the parts of the diagram from the list of terms provided. Terms may be used multiple times or not at all.

- interbreeding
- *Homo erectus*
- *Homo sapiens*
- *Homo neanderthalensis*
- Multiregional hypothesis
- Out of Africa hypothesis



Question 3

Fill in the blanks with the following terms.

- dated
- fossil record
- *Homo sapiens*
- DNA evidence
- migratory patterns
- high mutation rate
- genome-wide analysis

One measure that researchers use to help explain the migration of modern human populations around the world is the _____. When new fossils are discovered, they are _____ in order to provide rough time frames for the appearance of early _____ populations in different regions outside of Africa. These dates then help researchers map the _____ of our earliest ancestors, which can also be supported by _____, such as mtDNA and _____. Studies of mtDNA in modern humans is especially useful given its _____.

Question 4

Connection to Country refers to the

A attachment many First Nations people feel to a specific physical location, and the strict rules that govern their travel outside of that location.

B reciprocal relationship between many First Nations people and their ancestral lands and seas, and the strict culture around caring for and honoring these lands.

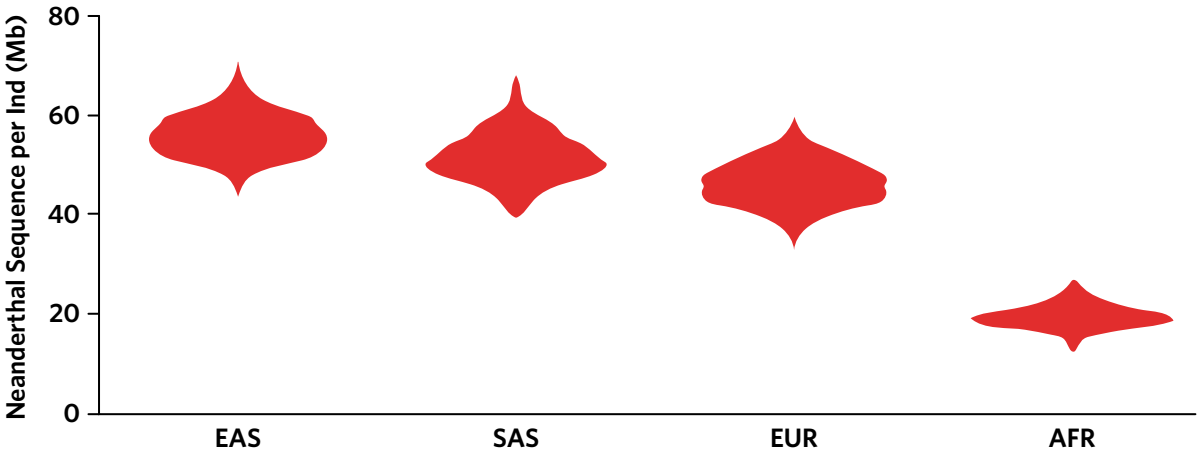
SAC skills questions

Data analysis

Use the following information to answer Questions 5–8.

Homo neanderthalensis arose roughly 430 000 years ago and lived predominantly in Europe and Central Asia until as recently as 40 000 years ago. Thanks to modern genetic sequencing technologies, we know that *Homo sapiens* interbred with the Neanderthals at various points in our migration across the globe. This interbreeding is assumed to have occurred almost entirely in Europe and central Asia as *Homo sapiens* populations spread outwards and encountered existing Neanderthal communities in these Eurasian regions. What is interesting, however, is that despite never living in Africa, recent research demonstrates Neanderthal ancestry in not only modern-day Eurasian populations but also in modern-day African populations.

Given that the Neanderthals are assumed to have never physically lived in Africa, how were researchers able to identify Neanderthal ancestry in modern-day African populations? This research provided two revelations into the migratory past of our species. It was determined that the Neanderthal ancestry in Africans was not due to an independent interbreeding event between Neanderthals and African populations, but instead due to migrations from ancient Europeans back into Africa at some point after the first emigration wave.



The amount in megabases (Mb) of Neanderthal DNA found in the genomes of separate populations of modern humans: East Asians (EAS), South Asians (SAS), Europeans (EUR), and Africans (AFR). Data was sourced from the 1 000 genomes project (internationalgenome.org) and collated by Chen et al. (2020).

Question 5

The number of megabases of Neanderthal sequence found per individual of African descent is closest to

A 0.
B 10.
C 20.
D 60.

Question 6

From the data provided, which population likely experienced the highest number of interbreeding events with ancient Neanderthal populations?

A African
B European
C East Asian
D South Asian

Question 7

Based on the information provided, which of the following options best describes the two primary explanations for Neanderthal ancestry in modern African populations?

	Explanation 1	Explanation 2
A	Established *Homo neanderthalensis* populations from Eurasia emigrated into parts of Africa and interbred with the earliest *Homo sapiens* populations prior to their own emigration.	An earlier Out of Africa (OOA) dispersal and subsequent gene flow from Neanderthals to humans. In other words, humans have far more admixing events with Eurasian Neanderthals than previously thought.
B	*Homo sapiens* populations emigrated out of Africa in the major OOA dispersal before admixing with Neanderthals and returning back to Africa at later dates, carrying the Neanderthal sequence with them.	An earlier OOA dispersal and subsequent gene flow from humans to Neanderthals. In other words, Neanderthal genomes possessed modern human DNA sequences.
C	Modern humans in Asia, Europe, and America inherited approximately 2% of their DNA from Neanderthals – proving that humans and Neanderthals had interbred after *Homo sapiens* first OOA emigration.	The hybridisation between humans and closely related species was a recurrent part of our evolutionary history, especially due to a number of independent interbreeding events between Neanderthals and early African populations.

Question 8

Which of the following is not a conclusion that can be drawn from the information provided?

A There have been multiple human dispersals both into and out of Africa.
B Human and Neanderthal interbreeding is unlikely to have ever occurred in Africa.
C Human and Neanderthal DNA has mixed multiple times and in multiple locations.
D The fitness consequences of human and Neanderthal admixture has always been positive.

Exam-style questions

Within lesson

Question 9 (1 MARK)

Based on our current knowledge of the human fossil record and mtDNA data, which of the following options best describes the prevailing view of modern human ancestry?

A The human fossil record only allows for migratory hypotheses and is far too incomplete to be able to make inferences about modern human ancestry.
B Modern humans can trace their ancestry to multiple hominin groups living in separate regions, with significant gene flow explaining much of the genetic diversity.
C Most of the genetic diversity in modern populations descends from a small group of early *Homo sapiens* who spread out of Africa sometime in the last 55–200 000 years.
D Current knowledge suggests that most modern humans can trace their ancestry to a group of early *Homo sapiens* who migrated from Africa around 2–2.5 million years ago.

Question 10 (1 MARK)

According to the Out of Africa model

A now-extinct hominins spread out of Africa sometime in the last 20 000 years.

B there were no interbreeding events between now-extinct hominins and modern humans.

C most of the genetic diversity in contemporary populations descends from a single or multiple groups of ancient humans.

D significant gene flow occurred among subpopulations of *Homo erectus* living in different parts of the globe throughout the Pleistocene.

Question 11 (4 MARKS)

Juukan Gorge, which is located in the Pilbara region of Western Australia, holds particular importance for the Puutu Kunti Kurrama and Pinikura peoples.

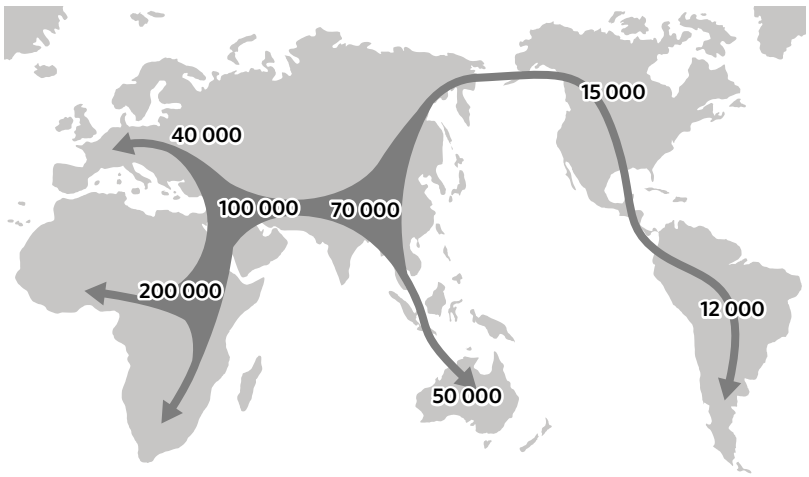
a With reference to the ancient landmass of Sahul, describe the geographical migration that led to Aboriginal Australians being named the world's oldest continuous population. (2 MARKS)

b Explain what is meant by Aboriginal Australians' Connection to Country. (2 MARKS)

Multiple lessons

Question 12 (9 MARKS)

The diagram shown depicts the presumed geographical spread of *Homo sapiens*.



a Which model of human migration is the diagram showing? Justify your response. (1 MARK)

b The genomes of living humans of European, East Asian, and Aboriginal Australian descent all contain small amounts of Neanderthal DNA (1–4%).

i Explain whether *Homo neanderthalensis* was hominin. In your answer, define what it means to be hominin. (2 MARKS)

ii Suggest how DNA from *Homo neanderthalensis* entered the genome of present-day European, East Asian, and Aboriginal Australian *Homo sapiens*, but is not found in present-day African populations. (2 MARKS)

c The morphology of modern humans is very different to that of our earliest ancestors. One model suggests that as early humans evolved and spread into different environments, they picked up new genes and alleles via a process known as adaptive introgression.

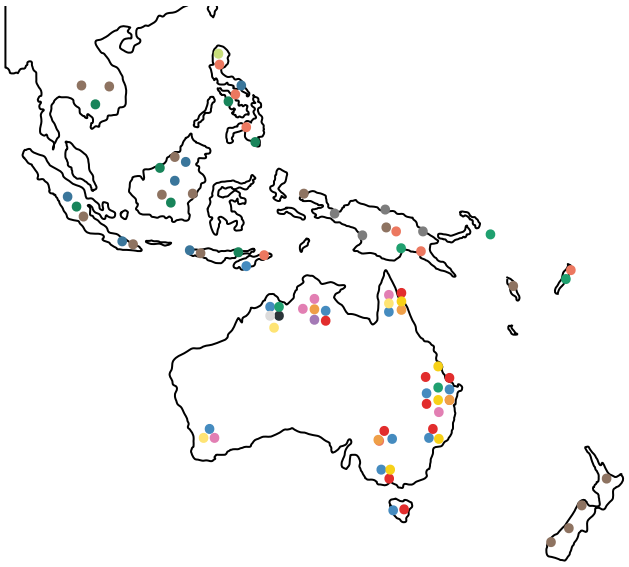
i Identify one structural difference between modern humans and their earliest hominin ancestors. (1 MARK)

ii Based on your understanding of the conditions of natural selection, explain how the structural difference you identified entered into the morphology of modern humans. (3 MARKS)

Key science skills and ethical understanding

Question 13 (5 MARKS)

A recent study into the mitochondrial genomes of Aboriginal Australians has demonstrated greater genetic diversity and variation between Aboriginal populations. By sequencing the mitochondrial genomes of 127 contemporary Aboriginal Australians, researchers were able to uncover novel Indigenous lineages across different geographical localities. The diagram models this updated geographical distribution of Aboriginal Australian mtDNA lineages and those of the surrounding regions.



Source: (Nagle et al., 2017)

a Based on your knowledge of mitochondrial DNA, explain why Aboriginal populations might show greater genetic variation in their mitochondrial lineages than non-Aboriginal populations. (1 MARK)

b The diagram shows a model of this updated geographical distribution of mtDNA lineages. Distinguish between a model and a simulation. (2 MARKS)

c Assume that the findings of the research uncovered new information regarding the lineages of certain Aboriginal communities that contradicted their traditional understandings of Connection to Country. How might this finding challenge the bioethical concept of respect? (2 MARKS)

CHAPTER 11 SUMMARY

11A: Can you classify modern humans?

General

- Domain
- Kingdom
- Phylum
- Class
- Order
- Family
- Genus
- Species

Specific

Humans are in the mammalian class: We are warm-blooded with fur and/or hair. Females give birth to live young and produce milk. We have a range of specialised teeth including incisors, canines, and molars.

Humans are in the primate order: flexible shoulders and hips to swing our arms and legs, plus forward-facing eyes and relatively large regions of the brain that relate to vision and hand-eye coordination. We have dexterous hands and feet with five digits and an opposable thumb.

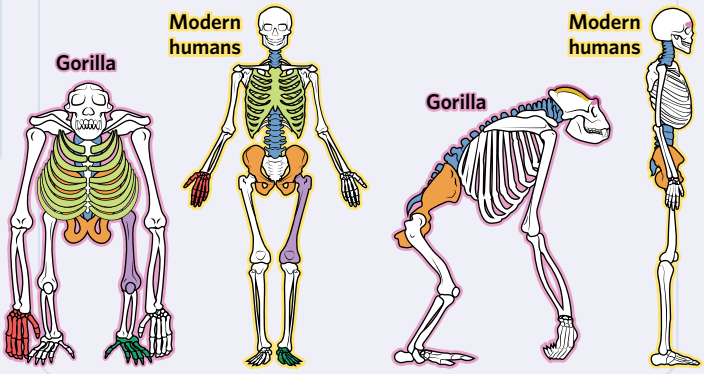
Humans are hominoids in the Hominoidea superfamily: a broad, flat rib cage and molars with five cusps (rather than four). No tail.

Within the hominoids, humans are also hominins (subfamily): includes only humans and our ancestral species (*Australopithecus* and *Homo*). Hominins are marked by their bipedalism.

11B: Can you describe general trends?

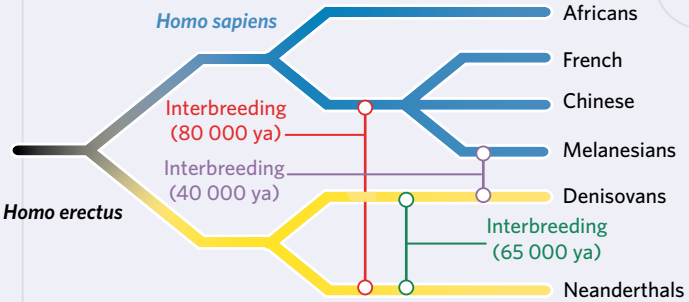
Brain size + skull shape: the general size of the brain has increased (although not always linearly – Neanderthals had a much larger brain volume than us). Certain areas of the brain have also developed (areas to do with memory, problem solving, and language).
Other parts of the skull have also changed over time (less prominent brow ridge, more rounded skull, central foramen magnum, flatter face, and a more parabolic jaw).

Limb structure: the development of bipedalism in humans came with a number of crucial structural changes to our limbs. For example, arm-to-leg ratio became shorter, our pelvis became more broad and bowl-like to support the upright torso, our spine became more curved (s-shaped), and our feet became more arched.



11C: The human fossil record is a contested classification scheme

The human fossil record is incomplete and is therefore open to differing interpretations, and may be refined and updated as new evidence is discovered.



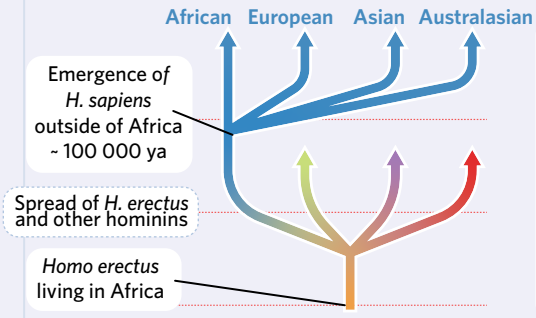
For example, recent discoveries of hominin fossils and analysis of their DNA sequences have confirmed that there was interbreeding between ancient hominin species - including early humans!

Fossil evidence suggests interbreeding events between early humans and the Neanderthals. We believe this interbreeding occurred with human populations outside of Africa most likely during the second wave of migration between 80 000 and 40 000 years ago.

Fossil evidence also suggests interbreeding between early humans and the Denisovans. We believe this interbreeding occurred slightly later in our migration as a species, around 40 000 years ago, particularly through southern parts of Asia and into island locations of Oceania.

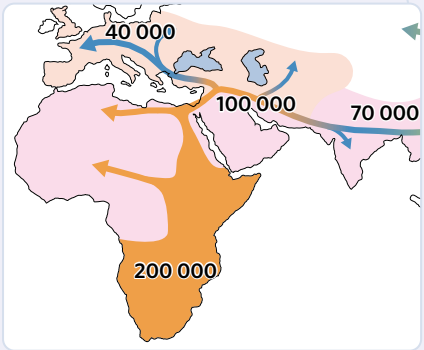
As new fossil evidence is uncovered, so too are new 'putative' species of the *Homo* genus. These species are those who are assumed to have existed, but for which more evidence is needed to gain a full understanding of when and where. One example of this is *Homo naledi*.

11D: Human migration



Evidence for the 'Out of Africa' model of early human migration includes:

- greater genetic diversity in African populations
- mtDNA traced back to common ancestor in Africa
- existing fossil record, especially along the east coast of Africa and into the Middle East
- cultural artefacts, such as stone tools and cave paintings.



Aboriginal and Torres Strait Islander populations and their Connection to Country and Place

Indigenous populations in Australia are thought to have arrived around 65 000 – 50 000 years ago and can be traced back to the first *Homo sapiens* populations to have left Africa. This makes Aboriginal Australians one of the longest surviving populations of modern humans to have lived in a given location, and is one of the reasons why their Connection to Country and Place is so strong and important.

The Connection to Country refers to the relationship experienced by an Aboriginal language group or community with an area that they have traditionally owned and looked after for generations. The term indicates more than simply a geographical area – it is also a concept that can encompass the spiritual meaning and feelings of deep connection and attachment associated with that area. Kinship names and familial spirits are used to demonstrate the mutual responsibility for care and growth of the land.

CHAPTER 11 SAC PRACTICE

SAC skills covered in this section:

✓ Case study analysis ✓ Data analysis

BRAIN SIZE AS A BARRIER FOR ACCURATE CATEGORISATION (21 MARKS)

The hominin fossil record is increasingly well documented, especially across the Pleistocene epoch (2.58–0.012 mya). In particular, the record for *Homo erectus*, the Neanderthals, and modern humans is becoming more reliable. However, we know that there were many other hominins to exist during this period, though the taxonomic status of these populations is more controversial and difficult to assess. One of the most critical issues impacting accurate categorisation is an incomplete understanding of the variation and evolution in skull form between separate species. For example, the average brain volume of *H. erectus* is 950 cm^3, while in a series of Middle Pleistocene crania from Africa and Europe, the average brain volume is 1 230 cm^3. It is not known what facilitated this increase, and whether other structural changes to the skull are linked to an increase in brain volume.

1 Describe what is meant by the term 'hominin'. In your response, name two hominin genera. (2 MARKS)

2 Other than changes in skull form over time, the evolution of hominins also involved a range of structural changes to the limbs alongside the development of bipedalism. Name two of these structural changes. (2 MARKS)

3 The evolution of brain size in hominins can be described as non-linear. Explain what this means with reference to your knowledge of Neanderthals. (2 MARKS)

Using brain size and skull formation to help categorise ancient hominins

A group of researchers conducted a correlation study to better understand the relationship between brain size, skull form, and species recognition. A simplified version of their method is described.

Method

1 Collect specimens of intact and well-preserved *H. erectus* crania (N = 30).

2 Collect specimens of intact and well-preserved Middle Pleistocene (MP) hominin crania (N = 15).

3 Identify the following measurements for the crania in each group of specimens.

- Cranial volume in cm^3 (VOL)
- Cranial length in cm (GOL)
- Cranial breadth in cm (XCB)
- Frontal sagittal arc in cm (GBR)
- Occipital sagittal arc in cm (LOR)

4 Collate all measurements into a table in preparation for Step 5. An example of a possible table for each group of specimens is provided.

Table 1 *H. erectus* cranial measurements

	VOL	GOL	XCB	GBR	LOR
Specimen 1					
Specimen 2					
Specimen 3					

Table 2 MP hominin cranial measurements

	VOL	GOL	XCB	GBR	LOR
Specimen 1					
Specimen 2					
Specimen 3					

cont'd

5 Conduct a correlation study to find the association between measurements for both the *H. erectus* crania and the MP hominin crania.

6 Use Pearson's correlation coefficient (*r*) to measure the strength of association between measurements. The strength of *r* will demonstrate patterns of covariation and assess the extent to which the components comprising the skull are related.

Results

The correlation techniques used by the researchers showed that *H. erectus* crania have relatively small capacities, a large basicranium, protruding occipitals and brow ridges, and highly projecting (slanted) faces. For the MP hominins, on the other hand, the average cranial volume is about 280 cm^3 greater than the average for *H. erectus*. Additionally, there is an increase in the vertical height of the braincase, a broader frontal region, and slight differences in the shape of the brow ridge. Based on the strength of the correlations, the MP hominin specimens were allocated to one or more species that are distinct from *H. erectus*, the Neanderthals, and modern humans.

4 Define a correlation study. In your response, suggest one difference between a correlation study and a controlled experiment. (2 MARKS)

5 Assuming the *Homo erectus* crania were dated to the early Pleistocene, state a possible hypothesis for this experiment in relation to the average brain volume of the specimens. (1 MARK)

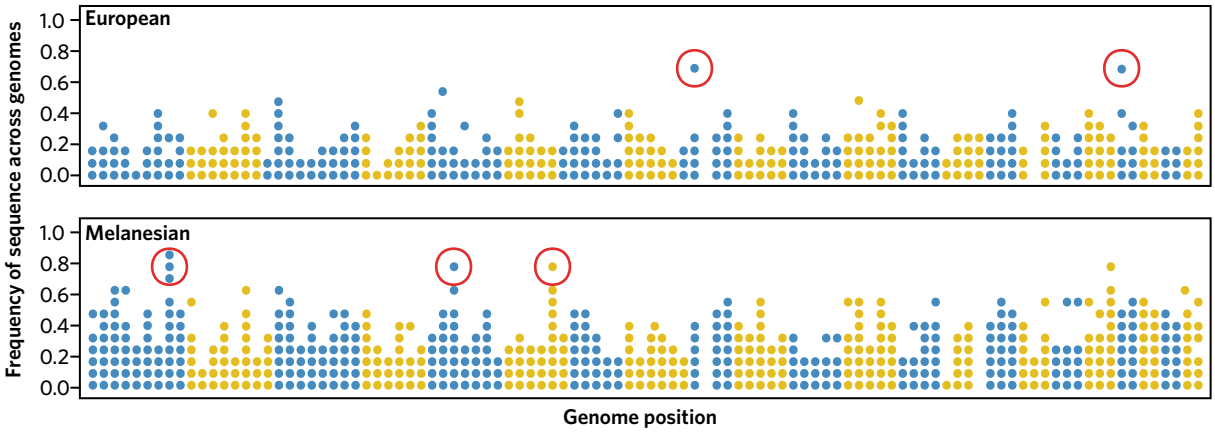
6 Assume a team of researchers discover a fossilised cranium with a large frontal region, a tall braincase, and a relatively flat face. Based on the results of this study, would the specimen likely be thought of as distinct from *H. erectus*? (2 MARKS)

7 Suggest a potential source of error in Step 3 of the researchers' method. In your answer, state one way in which this error might be avoided. (2 MARKS)

Adaptive introgression in hominin evolution

Genomic analysis of DNA sequences from archaic hominins demonstrates significant gene flow between the genomes of archaic hominins and modern humans. While genome-scale maps allow researchers to identify mixed sequences, there is still a lack of clarity regarding the functional and evolutionary significance of archaic hominin DNA that persists in present day humans. Nonetheless, recent genomic analysis demonstrates roughly 50–100 locations along the genome which show high rates of adaptive introgression – Neanderthal and Denisovan haplotypes which were beneficial and became much more frequent in the *H. sapiens* population as they interbred. Some examples of such sequences are highlighted in the graph provided (red circles).

Examples of this across regional populations suggests that as modern humans dispersed into new environments with new selection pressures, they collected beneficial genes from earlier hominin populations that had been there for generations before. More than 100 high-frequency archaic sequences have been identified as potential examples of adaptive introgression. These candidate genes tend to fall into specific categories, including those related to the immune system (OAS/1/2), adaptations to high-altitude, as well as skin and hair biology (OCA2).



Source: (University of California Television (UCTV, 2020)

8 In your own words, describe what is meant by the term 'adaptive introgression'. Justify your response with reference to migration into new environments. (2 MARKS)

9 'Haplotype' refers to a group of alleles found on a single chromosome which are often linked and typically inherited together. Based on the graph, which population demonstrates the highest amount of adaptive introgression? (1 MARK)

10 According to the text, candidate genes that show evidence for adaptive introgression tend to fall into particular categories. One such category is skin and hair biology, particularly genes relating to skin pigmentation. Describe one selection pressure that may have facilitated the adaptive introgression of genes responsible for skin pigmentation. (2 MARKS)

Genomic research and Aboriginal Australians

Some of the data used in genomic studies are taken from publicly available human genome databases, which sequence and store thousands of human genomes from different populations and geographical locations around the world. This includes indigenous populations globally, who provide blood samples to help scientists investigate various genetic disorders. Some of this research may be translated into extremely profitable medicines and genetic treatments. However, questions about where these profits flow and whether they help benefit indigenous communities is controversial. There is a high level of distrust for the motivations and methods of scientific researchers in indigenous communities globally. This is because the results of genomic studies have often been used to discredit indigenous peoples' claims regarding their ownership of land and heritage.

11 Suggest one bioethical issue in relation to the use of indigenous samples in genomic research. (1 MARK)

12 Using the bioethical concept of justice, suggest one way in which future genomic researchers might ensure indigenous participants receive equitable access to the benefits arising from their participation. (2 MARKS)

CHAPTER 11 EXAM PRACTICE

Section A (15 MARKS)

Question 1 (1 MARK)

With respect to the classification of modern humans and their ancestors, it is true to say that

A all primates are hominins.
B all primates are both arboreal and bipedal.
C the genus *Homo* includes all hominoids and hominins.
D the term hominin includes all members of the genera *Homo* and *Australopithecus*.

Adapted from VCAA 2018 Northern Hemisphere Exam Section A Q20

Question 2 (1 MARK)

Which of the following statements regarding the classification of modern humans is true?

A All mammals must give birth to live young.
B Modern humans belong to the genus *Hominoidea*.
C The common ancestor of all primates evolved an opposable digit.
D *Homo sapiens* and *Homo neanderthalensis* are the only two surviving hominins.

Question 3 (1 MARK)

Compared to their non-hominin relatives, human beings show

A a sagittal crest.
B a more centralised foramen magnum.
C a more c-shaped spine that curves forwards.
D a vertically longer and more narrow pelvis, lacking in bowl-shape.

Adapted from VCAA 2018 Biology Exam Section A Q39

Question 4 (1 MARK)

Which one of the following statements about hominin evolution is correct?

A Members of the *Australopithecus* genus are not considered hominins.
B The Australopithecines overlapped, but members of the *Homo* genus did not.
C Different members of *Australopithecus* and *Homo* coexisted in time with one another.
D The *Homo* genus first emerged around 2 million years ago when *H. erectus* first left Africa.

Adapted from VCAA 2018 Biology Exam Section A Q38

Question 5 (1 MARK)

Fossil remains of an ancient hominin species were discovered at various sites along the eastern coast of Africa and dated to between 3–4 million years old. These fossil remains are most likely

A ancestors of *Homo erectus*.
B from the genus *Australopithecus*.
C members of some putative hominin species.
D the earliest example of the hominin subfamily.

Question 6 (1 MARK)

Which of the following statements about the evolution of brain size in hominins is most correct?

A Brain size decreased over time, while the skull became more domed.

B Changes to brain size happened gradually over time and occurred linearly.

C Energy requirements increased as the brain became larger and more complex.

D Other than increasing in size, the overall structure and folding of the brain has remained the same.

Question 7 (1 MARK)

With respect to the structural evolution of hominin limbs over time, it is true to say that

A longer legs developed primarily due to a need to better locate food sources.

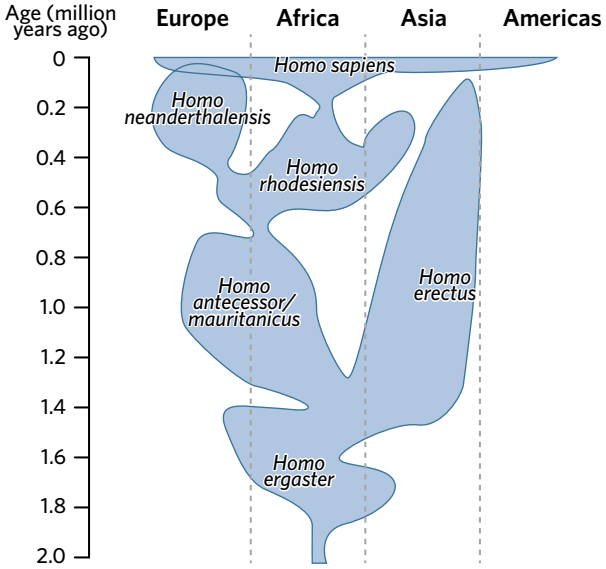
B hominin hands and feet evolved in response to an increasingly arboreal lifestyle.

C hominin species developed a less centralised foramen magnum to assist in the swinging of their legs.

D hominin species developed shorter arms and longer legs to increase the efficiency of bipedal locomotion.

Use the following information to answer Questions 8 and 9.

The diagram depicts one model for the evolution of hominin species over the last 2 million years. Note that not all species are shown and that this interpretation is contested.



Question 8 (1 MARK)

From the information in the diagram it can be concluded that

A *Homo sapiens* is the longest living hominin species.

B species diversity within the *Homo* genus is greatest today.

C *Homo erectus* was much more geographically widespread than *Homo sapiens*.

D there was overlap between two separate hominin species in Europe in the last 200 000 years.

Question 9 (1 MARK)

Which of the following assumptions is supported by the diagram?

A *Homo neanderthalensis* was the longest surviving and most successful of the hominins.

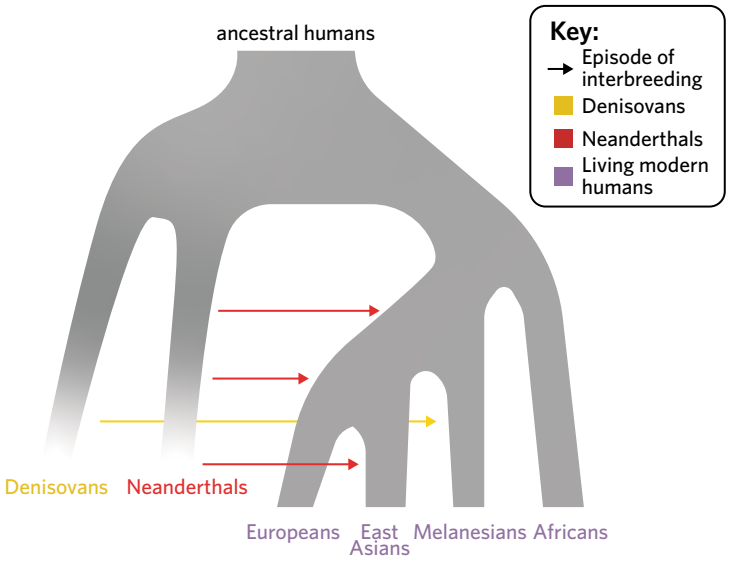
B *Homo ergaster* is the direct ancestor of *Homo sapiens* and interbred with both modern humans and the Neanderthals.

C *Homo sapiens* evolved in Africa 200 000 years ago and began spreading quickly throughout the world around 100 000 years ago.

D *Homo erectus* was the first real 'migrator' of the *Homo* genus, and spread throughout Europe and Asia around 1.8 million years ago.

Use the following information to answer Questions 10 and 11.

The diagram depicts different interbreeding events between three hominin species. Note that each living modern human population is geographically distinct.



Question 10 (1 MARK)

Which of the following assumptions can be concluded from the information provided in the diagram?

A Interbreeding events are likely to have only occurred in Europe.

B *Homo neanderthalensis* lived and interbred in both Europe and Asia.

C Modern-day East Asians experienced several separate interbreeding events over a period of 2 million years.

D Modern-day African populations are the only living population with both Neanderthal and Denisovan DNA in their genome.

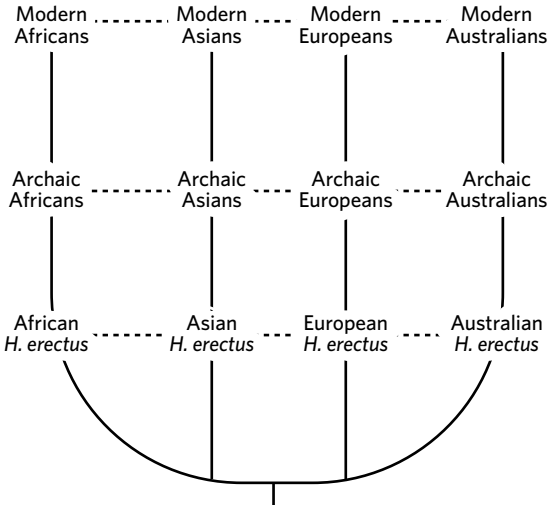
Question 11 (1 MARK)

Assuming the information in the diagram is correct, which of the following pieces of evidence is unlikely to have been used to support the corresponding assumptions?

	Assumption	Evidence
A	*Homo sapiens* interbred with the Neanderthals.	Nuclear DNA studies show around 1–4% of the human genome is identical to DNA found in Neanderthals.
B	The Neanderthals did not breed with African *Homo sapiens*.	The 1–4% DNA similarity is only found in the genomes of non-African populations and not in sub-Saharan African genomes.
C	*Homo sapiens* interbred with the Denisovans.	The mtDNA taken from Denisovan fossils and compared with *H. sapiens* suggests that we are separate species that shared a recent common ancestor around 400 000 years ago.
D	The Denisovans only interbed with Melanesian humans.	DNA taken from the genomes of Melanesian *Homo sapiens* revealed that they share 4–6% of their DNA with Denisovans. This similarity is not shared with other human populations.

Question 12 (1 MARK)

The diagram shown represents one view for the migration of modern humans across the globe.



Which of the following pieces of evidence would support this model?

A mtDNA analysis of modern humans demonstrates that our mitochondrial lineages can all be traced back to a common ancestor that lived in Africa between 140 000 and 290 000 years ago.

B Stone tools, carvings, and cave paintings, indicative of an increased complexity and cultural evolution, are found sequentially along the far north-west into deeper parts of Europe and south-east Asia.

C The greatest degree of genetic diversity in modern humans exists in African populations, where there has been more time for spontaneous mutations to accumulate, suggesting that we originated in a small, centralised African population.

D The existence of morphological clades in the ancient fossil record shows that there are combinations of various physical characteristics that are unique to particular geographic regions. These clades appear across a wide timespan and indicate potential localised evolution of separate populations.

Question 13 (1 MARK)

Researchers have dated some of the oldest *H. sapiens* fossils in East Africa to around 160 000 years old. They also uncovered some fossils in the Middle East and dated those to 100 000 years old. Additionally, stone tools were found in the United Arab Emirates and dated to 80 000 years old. From this data, we can assume that

A no *H. sapiens* fossils exceed 160 000 years in age.

B no stone tools have been uncovered in East Africa or the Middle East.

C migratory waves occurred back and forth between Africa and the Middle East, including into southern parts of the African continent around 80 000 years ago.

D an early migratory wave of *Homo sapiens* occurred along the east coastline of Africa, into and then out of Northern Africa via the Middle East around 100 000 years ago.

Question 14 (1 MARK)

Which of the following best summarises the migration of early humans into Australia?

A Modern Aboriginal populations in Australia arrived on the west coastline around 6 000 years ago, replacing existing hominin populations before spreading to other parts of the country.

B Early Aboriginal Australians migrated from Africa into Oceania around 180 000 years ago, as *Homo erectus* moved throughout Eurasia. These original populations died out, making way for modern-day Aboriginal groups.

C Early Aboriginal Australians evolved independently from existing *Homo erectus* populations in the Australian region around 50 000 years ago, making Aboriginal Australian groups some of the oldest continuous populations on Earth.

D Early Aboriginal Australians arrived in Oceania between 50 000 and 65 000 years ago. Their lineage can be traced back to the original populations of *Homo sapiens* in Africa, making Aboriginal Australian groups some of the oldest continuous populations on Earth.

Question 15 (1 MARK)

The notion that Aboriginal Australians first appeared in Australia between 50 000 and 65 000 years ago diminishes some Aboriginal groups' Connection to Country because it

A is unfounded, and is denied by all Aboriginal groups.

B contrasts with the traditional view that Aboriginal communities existed in the land since the beginning of creation.

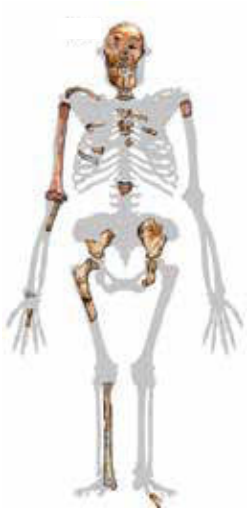
C necessarily removes Aboriginal land rights, given that important cultural sites can no longer be attributed to continued occupation.

D contrasts with the traditional view held by all Aboriginal communities that their culture has existed in the land for at least 180 000 years.

Section B (25 MARKS)

Question 16 (7 MARKS)

The skeletal structure of *Australopithecus sediba* is shown. *A. sediba* utilised a mixture of arboreal and terrestrial locomotion.



This species, along with *Homo sapiens*, is classified as follows:

order – Primate; superfamily – Hominoidea; tribe – Hominini.

a State a feature shared by primates. (1 MARK)

b Explain what is meant by arboreal locomotion. In your answer, identify one structural feature of *A. sediba* that would have allowed it to use arboreal habitats effectively. (2 MARKS)

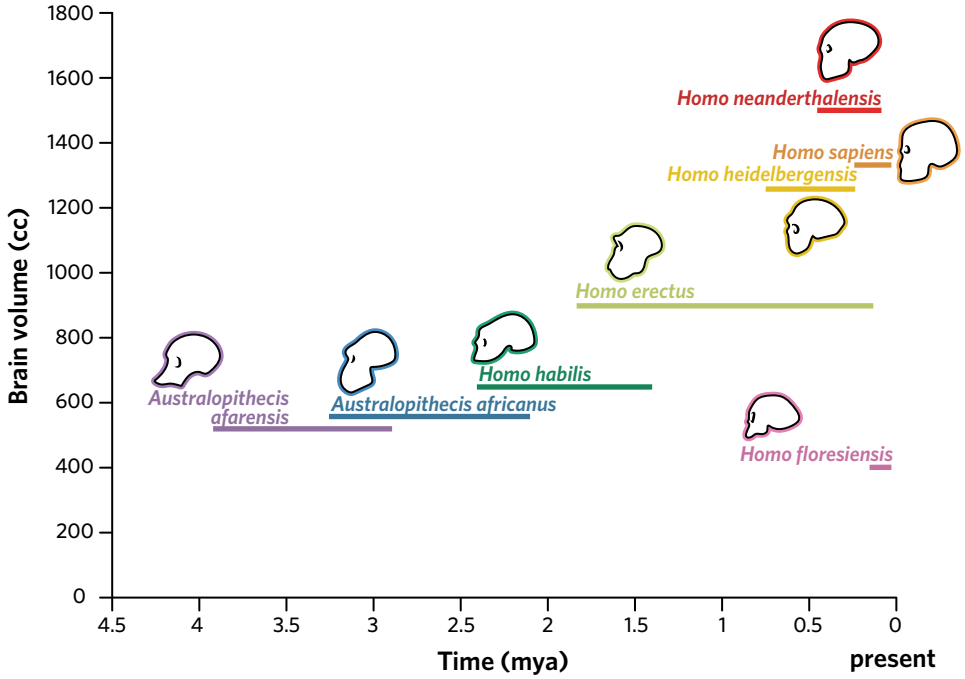
c Primates possess forward-facing eyes capable of 3D colour-vision. Suggest one reason why this adaptation would contribute to their evolutionary success. (1 MARK)

d Identify the key feature that separates members of the Hominini tribe from other members of the Hominoidea superfamily. (1 MARK)

e From the skeletal structure provided, choose one structural difference between *A. sediba* and *H. sapiens* and explain the significance of this change. (2 MARKS)

Question 17 (5 MARKS)

The following graph shows changes in average brain volume (cc) in different hominin species across time (mya).



a What is the average brain volume of *Homo sapiens*? (1 MARK)

b Define what it means to be human with reference to class, order, and genus. (2 MARKS)

c Identify two outliers in this graph that belong to the *Homo* genus. In your answer, explain the presence of the outliers with reference to the linearity of brain volume increase in hominins. (2 MARKS)

Question 18 (4 MARKS)

The following pictures show two primate skulls. One is a gorilla skull, the other is a *Homo sapiens* skull.

A

Image: uzuri /Shutterstock.com

B

Image: uzuri /Shutterstock.com

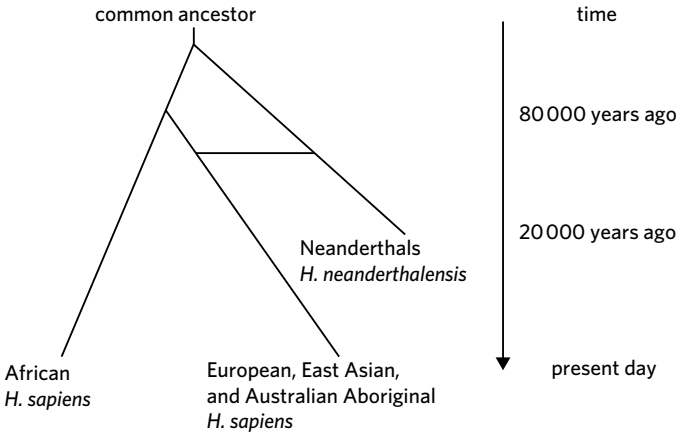
a Which of the two skulls is the gorilla skull? (1 MARK)

b Describe three features of the gorilla skull that distinguish it from that of *Homo sapiens*. (3 MARKS)

Adapted from VCAA 2009 Exam 2 Section B Q6

Question 19 (9 MARKS)

The diagram demonstrates a current theory regarding the relationship between different hominin species.



Recent DNA evidence suggests that the genomes of living humans of European, East Asian, and Aboriginal Australian descent all contain small amounts of Neanderthal DNA (1–4%).

a Scientists have found that the genome of living humans of African descent does not contain Neanderthal DNA. Which theory regarding the geographical origins and migration patterns of *Homo sapiens* does this finding support? Explain this theory with reference to the DNA evidence provided. (3 MARKS)

b According to the given diagram, these hominin groups each shared a common ancestor some time prior to 80 000 years ago.

i Name two differences you would expect to see in the skull of this common ancestor compared to modern human populations. (2 MARKS)

ii What are two structural features besides the skull that the hominin would need to demonstrate in order to be classified in the genus *Australopithecus* and not in the genus *Homo*? (2 MARKS)

c Recent studies have demonstrated that Aboriginal Australians can be traced back to the first Out of Africa migration.

i What does the diagram suggest about the timing of migration of Aboriginal Australians into Australia? (1 MARK)

ii Suggest how these findings might challenge Connection to Country for Aboriginal Australians. (1 MARK)

Adapted from VCAA 2015 Section B Q11

UNIT 4

AOS3

How is scientific inquiry used to investigate cellular processes and/or biological change?

Students undertake a student-designed scientific investigation in either Unit 3 or Unit 4, or across both Units 3 and 4. The investigation involves the generation of primary data relating to cellular processes and/or how life changes and responds to challenges.
The investigation draws on knowledge and related key science skills developed across Units 3 and 4 and is undertaken by students in the laboratory and/or in the field.

When undertaking the investigation students are required to apply the key science skills to develop a question, state an aim, formulate a hypothesis, and plan a course of action to answer the question, while complying with safety and ethical guidelines. Students then undertake an investigation to generate primary quantitative data, analyse and evaluate the data, identify limitations of data and methods, link experimental results to scientific ideas, discuss implications of the results, and draw a conclusion in response to the question. The presentation format for the investigation is a scientific poster constructed according to the structure outlined on pages 11 and 12 of the study design. A logbook is maintained by students for record, assessment, and authentication purposes.

Outcome 3

On completion of this unit the student should be able to design and conduct a scientific investigation related to cellular processes and/or how life changes and responds to challenges, and present an aim, methodology and methods, results, discussion, and a conclusion in a scientific poster.

Reproduced from VCAA VCE Biology Study Design 2022-2026

Image: Halfpoint/Shutterstock.com

HOW TO DESIGN AND CONDUCT A SCIENTIFIC INVESTIGATION

As part of your assessment for Unit 4, you will be asked to design and conduct your own scientific investigation and then present your investigation in the form of a scientific poster. The investigation must relate to something you have learned over the course of Units 3 and 4 regarding (1) cellular processes, and/or (2) how life changes and responds to challenges.

This task is set by the VCAA to allow students to demonstrate their ability to: (1) design their own experiment, (2) generate their own primary quantitative data, and (3) come to a conclusion as to what their generated evidence suggests about their chosen areas of study.

This sounds like a daunting task, we know. That's why we have included this lesson in your textbook to help you work through the process from start to finish. We are here to help you every step of the way, and have broken down this 'how-to' into three key stages to mirror page 37 of the study design:

1. *Investigation design*
2. *Scientific evidence*
3. *Scientific communication*

We will even conduct a brief investigation of our own, and use that as an example throughout this guide for you to refer to. The first thing to do is decide on a research question. The research question we have chosen to explore is: ***'Does the growth of an indoor mini fiddle-leaf fig (*Ficus lyrata bambino*) increase when exposed to more sunlight?'***

You can read this guide from start to finish, or simply refer back to certain sections of the guide whenever you are stuck on the corresponding section of your own investigation. You may also wish to refresh your knowledge of the key science skills from lesson 1A, or sharpen the tools in your bioethical toolkit from lesson 1B – good luck

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By the end of this investigation you should be able to design and conduct your own scientific investigation relating to cellular processes and/or how life changes and responds to challenges.

A high quality investigation will demonstrate:

- *the generation of primary quantitative data*
- *a full analysis and evaluation of the generated data*
- *identification and discussion of any limitations of both the data and/or the methodology*
- *a discussion of the relationship between the experimental results and broader scientific ideas, including a discussion of the implications of the results*
- *a final conclusion in response to the original research question.*

Stage 1: Investigation design

Stage overview

First things first, you must design your experiment. This is where we demonstrate the creative capabilities that are inherent to the key science skills (KSS's), which we learned about in chapter 1. This stage involves several individual steps, from deciding on a research question, to defining relevant concepts and theory, and finally considering different techniques for generating data. We will look at these steps in detail in this section.
A lot of the information you develop in this stage will work well in the introduction and methodology sections.

A summary of the investigation design process is seen in the flow chart (Figure 1).

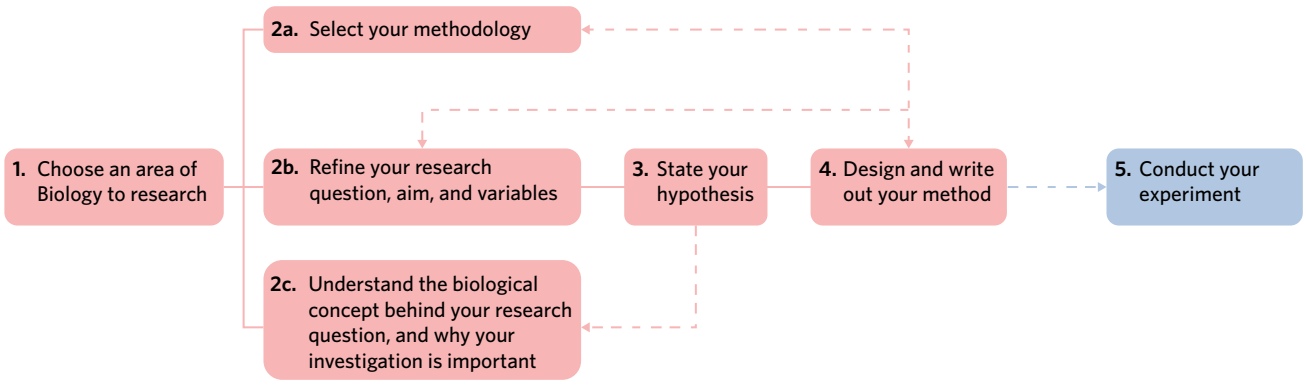


Figure 1 A flow chart showing the design process of your investigation. Note that we often return back to earlier steps in an effort to refine our investigation. The design process is incredibly important. Once you begin your research, it is much harder to change and revisit your methodology, so make sure to take your time with this stage!

Stage checklist

Across each stage of this guide, we will include the dot points that are explicitly mentioned by the VCAA on page 37 of the study design. As you work through this assessment, revisit these checklists to make sure you are including all of the required information at each stage.

For this section of your investigation you need to include discussion of each of the following:

- the biological concepts specific to the selected scientific investigation and their significance, including definitions of key terms
- characteristics of the selected scientific methodology and method
- the appropriateness of the use of independent, dependent, and controlled variables in the selected scientific investigation
- techniques of primary quantitative data generation relevant to the selected scientific investigation
- the accuracy, precision, reproducibility, repeatability, and validity of measurements
- the health, safety, and ethical guidelines relevant to the selected scientific investigation.

Step 1: Develop a research question

As mentioned, this task requires you to design your own scientific investigation in order to explore something you have learned over Units 3 and 4, specifically in regards to cellular processes, and/or how life changes and responds to challenges. Before you start, you must think of a research question you want to explore.

To do this, think back to the theory you've learned throughout the 11 chapters of this textbook. Is there anything you enjoyed that you'd like to explore in more detail? Was there something that you learned that you thought was really interesting? Or, did you have a question about something you learned that you'd like to find an answer to? It is important to remember the criteria of a good research question. The criteria were explored in lesson 1A and included measures such as being testable, achievable, and specific.

Some topics to think about which relate to Units 3 and 4 include:

- gene regulation or expression
- factors that affect enzyme activity
- DNA manipulation techniques, such as CRISPR, PCR, and genetic engineering
- factors that affect the rate of photosynthesis and/or cellular respiration
- immunity and dealing with pathogens, including the different lines of defence
- how species evolve and respond to the existence of different selection pressures
- evidence of relatedness between species, including structural morphology.

You also want to choose an area of research that lends itself well to a controlled experiment that is achievable in the classroom, as this is most likely going to be the scientific methodology that your teacher will have you conduct.

Let's compare these two research questions:

1 'What selection pressures lead to the evolution of longer neck length in species X compared to species Y?'

2 'At what temperature does plant species X photosynthesise most efficiently?'

We can see that while both questions fall under the topics covered in Units 3 and 4, the second question is going to be easier to create primary quantitative data and is more likely to lend itself to a controlled experiment that we can do in the classroom. The first question, by contrast, looks more to evolutionary data from the past and may be better suited to a correlational study. To refamiliarise yourself with the scientific methodologies and what makes a good research question, please revisit lesson 1A.

Finally, you also want to choose a research question that is testable and binary, as this makes it easier to arrive at a specific conclusion later in your investigation. For example, asking, 'At what temperature does plant species X photosynthesise most efficiently?' is a good research question as it allows you to come to a final judgement/conclusion based on your research – what temperature is best? In fact, this might serve a better purpose than a more general research question which asks, 'What are the factors that affect photosynthesis in plant species X?' Can you spot the difference here? Both questions require you to investigate the factors that affect photosynthesis, but the first is more specific and therefore provides more scope for reaching a judgement/conclusion at the end of your report.

Example

COME UP WITH A RESEARCH QUESTION

Here in the Biology Team at Edrolo, we've had a problem over the past year. We've been trying to grow some nice indoor plants on our desks at Edrolo headquarters, however, no matter what we do they always seem to shrivel up and die. What makes things worse is that we can look over at the Psychology Team and see some indoor plants on their desks, and theirs are thriving. As the supposed experts on all things living, you can imagine this is quite embarrassing for us.

We noticed that Team Psych's desks were right in front of the office windows and that their plants were receiving a lot more direct sunlight than ours. We were reminded that plants get their energy from photosynthesis, a process that requires sunlight, and believed this to be the reason for our miserable plant growth. We wondered whether sunlight affects indoor plant growth, and if so, to what extent. To formulate a testable, achievable, and specific research question, we identified the type of plant we wanted to grow in the office, and came up with the following research question:

'Does the growth of an indoor mini fiddle-leaf fig (*Ficus lyrata bambino*) increase when exposed to more sunlight?'

Step 2: Understand the biological concepts behind the research question

An important part of designing your investigation is to clearly define the scope of your area of study by discussing any key biological concepts that are relevant to your research question. This is both for your benefit, and that of your reader. From your point of view, not only does it demonstrate that you've been paying attention in class, but defining key concepts also helps you better understand what is important to investigate, and what goes beyond your chosen research question. In other words, it helps you more effectively analyse the data you obtain, and decide what should be included in your final assessment. For your reader, on the other hand, who may not be as familiar as you with the chosen area of research, defining key terms and explaining the biological concepts that underpin your research will help them engage with and better understand your investigation.

It will also help you in the coming steps which involve selecting your method and formulating a hypothesis. For example, if we are investigating the factors that affect photosynthesis, it is important that we explain these concepts clearly in our final presentation. That is, we would need to explain to our reader what photosynthesis is, why it is important, how it occurs, and why different factors impact photosynthesis in different ways. But keep in mind your word count! Start by clearly defining any key terms from the research question that might be important for your reader to know, as well as a brief summary of where the research is currently at and any limitations that might exist. Try thinking to yourself, 'are there things we don't yet know?'.

Example

UNDERSTAND RELEVANT BIOLOGICAL CONCEPTS

You might decide to leverage information from other parts of the textbook, or even from previous studies.

'Previous research has demonstrated that healthy office plants increase average employee morale in a workplace setting (Larsen et al., 1998). It is therefore important to understand the conditions in which indoor office plants flourish and grow. The mini fiddle-leaf fig (*Ficus lyrata bambino*) is a plant native to Africa that is widely used as an office plant in Australia. It is photosynthetic, meaning it uses energy from sunlight and turns it into usable chemical energy. The current understanding is that *F. lyrata bambino* grows best under indirect natural sunlight. This research hopes to determine at what distance from direct sunlight is best for vertical growth in the plant species.'

Step 3: Select the appropriate methodology and define your variables

Now that you've got your research question, it's time to figure out how to actually get to the answer by conducting a scientific investigation! This will require you to select the appropriate scientific methodology, which will help you in designing your specific method (the actual steps in your experiment). There are a range of methodology types, including case studies, correlation studies, literature reviews, and simulations. Given that you are being asked to generate your own primary quantitative data in the classroom, it is likely that you will be conducting a controlled experiment. For the purposes of this guide, we will be assuming that you are using a controlled experiment. For more detail on the other methodology types, please refer to lesson 1A again (and speak to your teacher about your options).

An important part of your methodology is clearly defining the variables under investigation. This is especially true of controlled experiments, which help us understand cause and effect by investigating the impact of an independent variable (IV) on a dependent variable (DV) whilst keeping all other variables constant (controlled variables). Make sure to clearly state each variable in your investigation. To remind you, the variable that is being affected is the dependent variable (DV), while the variable that is being manipulated is the independent variable (IV). For example, we might investigate the impact of changing temperature (IV) on the rate of photosynthesis in a particular plant species (DV). Here, we would set up an experiment where different specimens are exposed to different temperatures whilst ensuring that all other variables are controlled for and kept the same (such as UV exposure, water exposure, air pressure etc.).

Example

SELECT THE APPROPRIATE METHODOLOGY AND DEFINE YOUR VARIABLES

Because we are examining the impact of a single variable on another, we decided the best methodology to follow was a controlled experiment. We could have instead conducted fieldwork and observed plants outside, perhaps comparing the growth of plants in sunny patches with the growth of plants in shady spots. However, this method would have introduced a number of confounding variables that we couldn't control. Given that we wanted to be certain of the influence of light on plant growth, we decided to conduct a controlled experiment. It is important to discuss the reason for this decision in your final presentation.

We also defined our variables:

- **Independent variable (the thing we were going to change)** = the amount of sunlight our plants were exposed to.
- **Dependent variable (the thing that we were going to measure)** = the amount of plant growth.
- **Controlled variables (things we need to keep constant during our investigation)** = other factors that influence photosynthesis and plant growth, including water exposure, any fertilisers used, soil quality, and temperature.

Note that it is also important to define any units of measurement when describing your variables. We will return to this point in the communication section of this guide. We measure the amount of sunlight as (none, 10 m away, direct sunlight) while we measure the growth of the plant as height in millimetres (mm).

Step 4: State your hypothesis

Your hypothesis is a testable statement that predicts how your independent variable will affect your dependent variable. Based on your knowledge of the topic and your research, you should be able to come up with an idea of how altering your independent variable will influence your dependent variable. At the end of your experiment, the results you obtain will either be as you expected and support your hypothesis, or differ from what you expected and refute your hypothesis.

Example

HYPOTHESIS

Based on our knowledge about plants and photosynthesis, we made the following hypothesis:

'The growth of a mini fiddle-leaf fig will increase when exposed to more sunlight.'

Step 5: Design your experiment

Now that you've selected the most appropriate methodology to answer your research question, it's time to actually figure out what you're going to do in your investigation. Exactly what your investigation's design is will depend on what methodology you've chosen. If you've chosen to do a controlled experiment, consider the following key components:

1. What will you be measuring in your experiment and how will you be measuring it? This includes how long your experiment will run for and how big your sample is.
2. What different conditions are you going to have in your experiment? How will you design your experimental groups to ensure these conditions are tested?
3. How will you be controlling for uncontrolled variables to ensure they don't impact your results? In other words, list your controlled variables.
4. How will you be addressing replication?
5. How will you prevent errors from occurring? Or, at the very least, how will you conduct your experiment so that their influence on your result is minimal?
6. What is the best way to generate a sample?
7. What ethical and safety guidelines do you need to be aware of before conducting your investigation?

Once you've addressed each of these questions, you can use your answers to help write your method. Use our example as inspiration for how to construct your method.

Lesson link

If you've decided to use a different methodology, turn back to ***lesson 1A*** to see what things you need to consider when designing your experiment.

Example

DESIGN YOUR EXPERIMENT

We answered the above questions as shown:

1. We will be measuring vertical plant growth using a tape measure to measure how high the plants are, from the surface of the soil to their stem tip, after two weeks of growth. We will have fifteen plants in total, with five plants exposed to each light condition.
2. We are going to manipulate our independent variable by having a variety of different light conditions – some plants will be placed in a cupboard and receive no light (Group A), some will be placed ten metres away from a window (Group B), and others will be placed directly in front of a window (Group C). Group A will serve as our control group.
3. We identified relevant controlled variables. For example, (1) we will ensure that the plants receive the same amount of watering, (2) they will be watered at the same time of day, (3) we will not provide any of the plants with fertiliser, and (4) we will ensure that all the plants are the same species and are of similar size and health.
4. As mentioned, there are five plants exposed to each light condition. This means we have five replicates per each light condition.
5. We will use the same tape measure and have the same person measuring all the plants. We will ensure the tape measure has clear 1 mm markings.

cont'd

Example

DESIGN YOUR EXPERIMENT – CONTINUED

6 We will select plants at random from a pool of plants that are all of similar size and health and place them randomly into each light condition group.

7 Fortunately, we feel our investigation is quite safe. However, we do need to be careful to check if anyone is allergic to the plant we have chosen. We know that the sap of *Ficus lyrata bambino* is irritating to skin so we will need to provide our team with personal protective equipment (PPE) such as gloves to use while handling the plants.

Using these answers, we designed the following methodology for our scientific investigation. Note that not each question needs to correspond to a step in your methodology.

1 Randomly select 15 *Ficus lyrata bambino* plants of similar height and size.

2 Divide these plants into three groups – Group A, Group B, and Group C – and assign each plant a number within each group – e.g. plants A1, A2, A3, A4, and A5 are all in Group A, while plants B1–B5 are in Group B, and the same for Group C.

3 Measure the height from soil level to the tip of the stem of each plant using a tape measure and record these measurements. Ensure gloves are worn to protect from the plant's sap.

4 Water each plant with 20 mL of tap water.

5 Place all the plants from Group A into a cupboard with the door closed so they are in darkness, place the Group B plants on a table 10 metres away from a north-facing window, and place the Group C plants on a table directly in front of the same north-facing window.

6 After two weeks, have the same person measure and record the heights of each plant.

Step 6: Considering internal measures

The final step in designing your investigation is to consider the relevance of certain internal measures that are important for completing scientific investigations. It is important to think about how well your investigation satisfies these measures at the start of your process, prior to conducting your investigation, as well as at the end of the process, once you get back your results. We will remind you of some of these now, and invite you to return to these when it is time to present your findings. It is also encouraged to discuss these early in the piece, especially in your logbook, to demonstrate your preparedness to your teacher.

1 Accuracy: refers to how close your results are to the 'true' value of the quantity being measured. For example, if we know that plant species X typically photosynthesises optimally at a temperature of 35 °C, then we would expect our primary data to be as close to this value as possible (Figure 2).

2 Precision: refers to how close your results are to each other. Precise results indicate that your method is valid and reliable, and that you may be able to assume the same results would be found in a larger sample. If you get a wide spread of values across replicates, then results are imprecise. If replicates get similar results, your results are precise. For example, we would hope that each time we conducted the experiment, each replicate would be around the same value so as to indicate that our method is reliable.

3 Reproducibility: refers to how reproducible your results are. A reproducible experiment means that other scientists could follow your method and get the same results over and over again. For example, if another group of your classmates were given your methodology and asked to reproduce your experiment, they would be able to get the same results.

4 Repeatability: refers to how repeatable your results are. A repeatable experiment means that you personally could repeat your experiment and get the same results over and over again. In other words, if you did the exact same method again, you would get the same results each time.

5 Validity: refers to how valid your experimental design is. A valid experiment actually measures what it claims to be measuring. For example, if we are aiming to measure the effect of different temperatures on the photosynthesis rate of plant species X, then that is what we should measure, rather than looking at UV exposure, or different plant species.

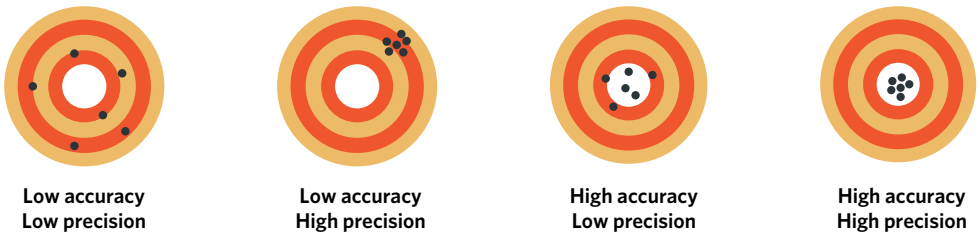


Figure 2 Accurate results are close to the true value, whereas precise results have very little spread around the mean value.

When discussing these internal measures, it is also important to identify any health, safety, and/or ethical guidelines that might be relevant to your selected investigation. This might include things like the sterilisation of your work station, the safe storage of equipment and materials, the storage of data and information about participants, obtaining informed consent, and/or any other relevant considerations you think it might be important to mention. This can also be a good opportunity to introduce some of the bioethical concepts and approaches that were introduced in lesson 1B, and is a great opportunity to demonstrate to your teacher a full and considered investigation design.

Stage 2: Scientific evidence

Stage overview

Having followed the steps perfectly and designed a beautiful scientific investigation, it's now time to collect some scientific evidence to answer your research question. To do this, there are some steps we need to follow once more, and some individual points we need to ensure that we are discussing in our final presentation. Remember: by the end of this section you should have generated your own qualitative and/or quantitative data.

Stage checklist

For this section of your investigation you need to discuss and include each of the following:

- the nature of evidence that supports or refutes a hypothesis, model or theory
- the ways of organising, analysing and evaluating primary data to identify patterns and relationships including sources of error and uncertainty
- authentication of generated primary data through the use of a logbook
- assumptions and limitations of investigation methodology and/or data generation and/or analysis methods

Step 1: Establish a logbook

Before doing anything, it is important to set up a logbook that can be used while researching and collecting data. This helps you to look back on your research and identify the sources you have used. It also gives you a good place to record all of your results and write down any interesting findings/thoughts you have along the way. There is no set format for using a logbook, so it is important to identify what works well for you. You could take handwritten notes, or type up your research with links.

Take a look at what the VCAA has to say about the use of a logbook:

Example

THE VCAA ON LOGBOOKS

The use of a logbook reflects standard scientific practice. Students undertaking this study must maintain a logbook of practical activities in each of Units 1 to 4 for recording, authentication, and assessment purposes. All items in the logbook must be dated and clearly documented.

The logbook is submitted as a requirement for satisfactory completion in each of Units 1 to 4. Teachers must regularly sight and monitor the logbook, particularly for the student-designed practical and/or research investigations in Outcome 3 of Units 1 and 2, and Outcome 3 of Unit 4.

The logbook may be maintained in hard copy or electronic form. However, to avoid falsification and/or alteration of results, for assessment tasks it is recommended that students maintain a hard copy, as is commonly the practice in scientific research.

Step 2: Collect your data

It is important that you find a reliable, clear, and valid way to collect and record your data while conducting your experiment. Be sure to note down all the results you obtain in your logbook, even if they seem strange or odd. Additionally, it is important to record anything 'out of the ordinary' that happened while conducting your experiment – these events may influence your results, so you need to be aware of what they were and when they occurred.

When collecting your data, and later presenting it in your final presentation, it is also important to briefly demonstrate an understanding of the different types of evidence. In this investigation, you are being asked to generate primary quantitative data, which we have already defined as data which is collected from experiments, interviews, or surveys undertaken by the researcher themselves. The quantitative nature of this evidence refers to it being in the form of numerical or empirical data, such as those with a given quantity, amount, or range. This data contrasts to secondary and qualitative data, which are sometimes seen as less robust.

Finally, it's important to make it clear that not all evidence is created equally. When we think of strong scientific evidence, we are typically referring to primary and/or secondary data that is empirical and measurable, and under the control of a formal research environment. Some scientific evidence might be stronger than others – for example, a controlled clinical trial with a large and randomised sample is considered stronger than a case study that uses a small sample. It is important to identify the strength of your own study in terms of sample size, and how representative it might be of a wider population or subset.

Example

COLLECT YOUR DATA

We measured the height of each plant in the three groups once at the start of the investigation, and then after two weeks of being in their respective positions. We recorded our findings in a table like shown:

Plant	Initial height (mm)	2-week height (mm)
A1	210	212
A2	214	217
A3	204	205
A4	212	212
A5	202	206
B1	220	231
B2	210	219
B3	213	224
B4	208	221
B5	211	222
C1	222	229
C2	214	234
C3	207	226
C4	215	235
C5	217	240

Observations

During initial measurement, plant C1 was knocked over and fell out of its pot. It was re-planted immediately and remained in the experiment.

Stage 3: Scientific communication

Stage overview

Having designed your experiment and collected your own primary data, it is now time to communicate your research and present the results in your final poster. You will use the data you've collected in Stage 2 to answer your research question and either confirm or reject your hypothesis.

Stage checklist

For this section of your investigation you need to demonstrate each of the following:

- conventions of science communication: scientific terminology and representations, symbols, formulas, standard abbreviations, and units of measurement
- conventions of scientific poster presentation, including succinct communication of the selected scientific investigation and acknowledgements and references
- the key findings and implications of the selected scientific investigation

It's now time to put together your poster. The VCAA have provided guidelines for how you should structure your poster (Figure 3), as well as what they wish to see in each section (Table 1).

The poster may be produced electronically or in hard-copy format and should not exceed 600 words. The centre of the poster will occupy between 20 to 25 per cent of the poster space and will be a one-sentence summary of the major finding of the investigation that answers the investigation question.

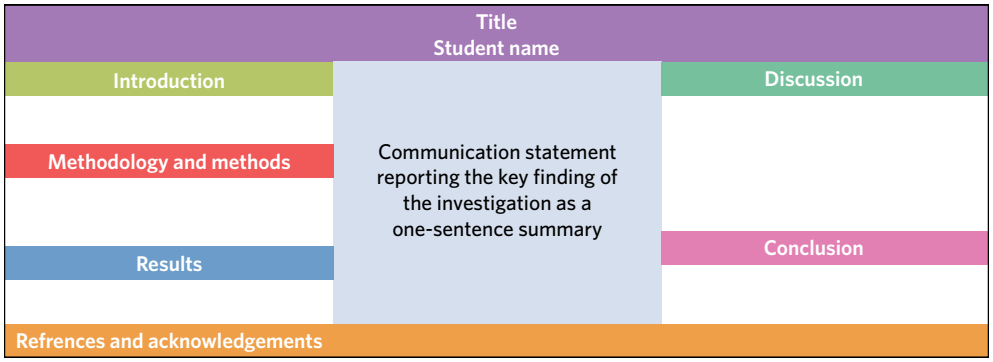


Figure 3 The VCAA suggested format for the scientific poster

Table 1 VCAA guidelines for the required content to be presented in a scientific investigation

Section	Content
Title	The question under investigation
Introduction	Explanation or reason for undertaking the investigation, relevant background biological concepts, a clear aim, and a hypothesis
Method	• A summary that outlines the methodology and steps used in the investigation and is authenticated by logbook entries • Identification and management of relevant risk, including the relevant health, safety, and ethical guidelines followed in the investigation
Results	Presentation of collected data/evidence in an appropriate format to illustrate trends, patterns, and/or relationships
Discussion	• Analysis and evaluation of primary data • Linking of results to relevant biological concepts • Identification of outliers and their subsequent treatment • Identification of limitations in data and methods, and suggested improvements
Conclusion	A conclusion that provides a response to the question
References and acknowledgements	Referencing and acknowledgment of all quotations and sourced content as they appear in the report

Title

Your title can be your original research question, or a slight re-wording of it if you want to be fancy. It doesn't need to be long or complicated – in fact, the shorter and simpler the better!

Example

TITLE

Does sunlight affect the growth of *Ficus lyrata bambino*?

Abstract

An abstract is a short summary of your investigation that helps garner the attention and interest of your reader. The VCAA says it isn't compulsory to have an abstract, but it can be a good idea as it gives your audience a quick snapshot of what your report is about. Check with your teacher if you should be writing an abstract. One simple way to write an abstract is to summarise the main components of your report into a sentence each, and then work these together to make one cohesive paragraph.

Example

ABSTRACT

Office plants have a number of mood-boosting effects for employees, and sunlight exposure is known to affect plant growth. The effect of different amounts of sunlight on *Ficus lyrata bambino*, a popular office plant, was studied. Three groups of plants were each exposed to one of three different light conditions – full sunlight, a moderate amount of sunlight, and no sunlight at all. The plants in full sun grew far more in the space of two weeks than the other two groups. These results suggest that office plant placement should consider available sunlight to ensure optimal plant growth and therefore maximum benefit to workers.

Introduction

Your introduction should include a few different components – we'll look at each of these now.

Explanation or reason for undertaking the investigation

At the start of your introduction, you should begin by justifying and explaining the reason for your investigation. Why do your results matter? Why should anyone care about what you did? For example, our research question was to help us grow better office plants. Think back to why you were interested in the research, and go from there.

Linking your explanation to prior research is a good idea as it shows that you have thought about your investigation in the wider context of biology as a whole, and can prove that your investigation and findings are important. Based on your research, you might also be able to point out flaws with previous investigations that suggest your research is important and relevant.

There should be no doubt after reading your introduction that your investigation is one of the most important pieces of research that has ever been done... just like our study into optimal office plant growth which will change the way office plants are configured for the rest of time!

Example

EXPLANATION OR REASON FOR UNDERTAKING THE INVESTIGATION

It is well understood that having indoor plants in an office space increases employees' mood and perceived comfort levels whilst at work (Larsen et al., 1998). Perhaps more importantly, however, it has also been shown that an increased number of plants in the workplace decreases worker stress and reduces the amount of sick leave taken (Bringslimark et al., 2007). As such, it is important to understand the conditions in which indoor office plants flourish and grow.

Background biological concepts

We mentioned in the investigation stage the importance of discussing background biological concepts relevant to your research. The introduction is likely where you will put it. Introduce the key aspects of theory from the course to demonstrate the relevance of your question to VCE Biology.

One way to think about which aspects of theory should be included is to think back to when you were designing your experiment – what aspects of theory did you use to formulate your prediction? Introduce these to your audience and link them to your research so that they can understand the biological theories and concepts relevant to your investigation.

Example

BACKGROUND BIOLOGICAL CONCEPTS

One key environmental factor that influences plant growth is the amount of light that plants are exposed to. This is because plant cells obtain the glucose they use for cellular respiration via a process known as photosynthesis. In photosynthesis, plant cells utilise light energy to transform carbon dioxide and water into glucose, oxygen, and water. The simplified formula for photosynthesis is as follows:

$$6\,CO_2 + 6\,H_2O \xrightarrow{\text{Light}} C_6H_{12}O_6 + 6\,O_2$$

Aim and hypothesis

After you've justified the reason for your investigation and provided the background information required to understand what you've explored, you can now introduce the aim of your investigation and the hypothesis you generated prior to commencing it. When you state your aim you can also introduce what the independent and dependent variables are, and your hypothesis will show the relationship you expected there to be between the two before you started your investigation. Note the emphasis on before – you should not be changing your aim or hypothesis once you've started.

It's important to remember that everything in your introduction is usually written in the present or future tense. This is because when you are presenting your investigation to an audience you are 'walking them through' what you did and found – as if you are conducting your whole investigation again but this time with them tagging along beside you. As such, when you are talking about your aims and hypothesis, you are talking as though these are things you haven't done yet rather than things that were already considered before you conducted your investigation.

Example

AIM AND HYPOTHESIS

This investigation aims to measure the effect of sunlight on the growth of the office plant *Ficus lyrata bambino*. Given the importance of light in the process of photosynthesis, it is hypothesised that increased amounts of sunlight exposure will cause plants to grow more compared to plants that are exposed to less sunlight or no sunlight at all.

Method

This section of your report is like a cooking recipe – it outlines the steps you undertook while carrying out your investigation and should be written in such a way that someone else could read it and replicate what you did. You can write your method in paragraphs or using dot points and can include diagrams to illustrate complex setups. Methods are usually written in past tense, but this is something you should check with your teacher.

Materials

Start by including a description of all the items you used to complete your investigation. You want to describe everything that another person would need to have in order to conduct the same experiment. You can write your materials either as a list or as a paragraph.

Example

MATERIALS

For this investigation, we used 15 healthy *Ficus lyrata bambino* plants selected at random from a larger population, a tape measure with millimetre markings, gloves, safety goggles, a cupboard, and a watering jug.

Methodology

From there, it is now time to write out the full methodology of your investigation. You can write your methodology in paragraphs or using dot points and can include diagrams to illustrate complex setups. Methods are usually written in past tense, but this is something you should check with your teacher. Be sure to mention any risks or ethical issues you encountered and how you addressed them while conducting your investigation.

Example

METHODOLOGY

We randomly allocated the 15 selected plants into three groups – Group A, Group B, and Group C. Each plant was assigned a number within each group (e.g. Group A consisted of plants A1, A2, A3, A4, and A5). We then had one person measure the height of all the *Ficus lyrata bambino* from the surface of the soil to their stem tip using the tape measure. Given that the sap of *F. lyrata bambino* is a known irritant, we ensured that the measurer was wearing gloves and eye protection at all times.

Once the heights of all the plants had been measured and recorded, each plant was watered with 20 mL of tap water. All Group A plants were then placed in a cupboard with the door closed, all Group B plants were placed on a table 10 metres away from a north-facing window, and all Group C plants were placed on a table directly in front of the same north-facing window.

The plants were left in their respective positions for two weeks. After two weeks, we had the same person measure and record the heights of the *F. lyrata bambino* plants.

Results

Transform and present your data

The results section is where you can finally present your audience with your findings and observations. Importantly, though, do not interpret or explain these results yet – this is saved for the next section of the report. You are only presenting your data here as a way to highlight trends or patterns for your reader later in the discussion.

To do this effectively, the data that you present in your results section should be transformed – you should not be presenting raw data in your report. In order to figure out how to transform your data, think back to your research question – what do you need to know in order to answer it? Transformed data can be presented in a table or graph. Remember from lesson 1A that different types of data tend to suit different forms of graphical representation:

- Line graphs and scatter plots are useful when presenting numerical data
- Bar graphs and pie graphs are useful when presenting categorical data
- Scatter plots are useful when comparing two variables.

Remember that typically, your x-axis will be your independent variable and your y-axis will be presenting your dependent variable measurements. Don't forget to give your tables/graphs clear titles and a figure number.

Example

RESULTS

Figure 4 shows the average initial and average 2-week heights of each group of plants. Group A had an initial average height of 208.4 mm which increased to 210.4 mm after two weeks. Group B had an initial average height of 212.4 mm which increased to 223.4 mm. Group C had an initial average height of 213.25 mm which increased to 233.75 mm.

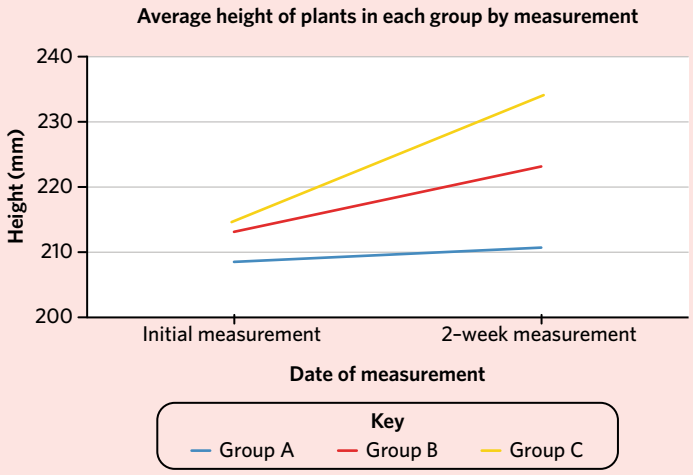


Figure 4 The average height of plants in each group by measurement date

Table 2 depicts the average increase in height in each group as a percentage relative to the group's initial average height. It can be seen that plants in Group A grew an average of 0.96% relative to their initial height, plants in Group B grew an average of 5.18%, and plants in Group C grew an average of 9.61%.

Table 2 Recorded height measurements and the average percentage growth per group

Plant	Initial height (mm)	Average initial height (mm)	2-week height (mm)	Average 2-week height (mm)	Average percent growth of group relative to original height (%)
A1	210	208.4	212	210.4	0.96
A2	214		217		
A3	204		205		
A4	212		212		
A5	202		206		
B1	220	212.4	231	223.4	5.18
B2	210		219		
B3	213		224		
B4	208		221		
B5	211		222		
C1	222	N/A	229	N/A	N/A
C2	214	213.25	234	233.75	9.61
C3	207		226		
C4	215		235		
C5	217		240		

Discussion

The discussion of your report is typically the longest component and contains a number of different sections. We'll go through each of these here in detail.

Analysis and evaluation of primary data

Now that you have transformed and organised your primary data effectively, it is time to analyse and evaluate the strength of that transformed data in terms of your research question. In this first section, you want to restate your hypothesis, and suggest whether the data you've obtained supports or refutes your hypothesis. To do this, it is important to demonstrate discussion of the following:

Analysis

To analyse data you must compare the purpose of your investigation with the type of data you obtained – what is your research question and what type of data is needed to arrive at a meaningful conclusion? Assuming you designed your investigation effectively, the data you source should be able to lend itself well to the research question at hand. Keep in mind that you are being asked to create quantitative primary data. While other data (and other sources) may be leveraged to assist in answering the question, it is important to discuss the different types of data in your investigation and analyse which is most suited to answering your question. For instance, if my question relates to the optimal temperature for photosynthesis in plant species X, the most effective data is likely to be primary measurements taken during my experiment of photosynthetic outputs from my sample.

Evaluation

To evaluate the data you will judge the strength and limitations of your research based on the internal measures you considered earlier in this guide, as well as looking for any errors or uncertainties in both your method and the data you collect. Uncertainty might arise if the experiment is not repeatable, reproducible, or valid, or if you use other research that has not been peer-reviewed. Make sure to familiarise yourself with this terminology and use it throughout your final investigation.

Some common errors include:

- personal errors – mistakes made by the experimenter, such as counting incorrectly, rounding to the wrong decimal place, or labelling samples incorrectly
- systematic errors – affect the accuracy of the findings and occur when results differ from the true value by a consistent amount each time, typically due to faulty equipment or calibration
- random errors – affect the precision of the findings and are caused by unpredictable variations in the measurement process.

Some common types of bias include:

- confirmation bias – the tendency for researchers to only include information that supports their hypothesis or aim
- selection bias – the selection of participants is not randomised and the sample isn't representative of the wider population
- publication bias – when the outcome of a study determines whether it is published or not. Typically, journals only publish studies with 'positive' findings, and often fail to publish studies that report a negative result.

Example

ANALYSIS AND EVALUATION OF PRIMARY DATA

It was hypothesised that increased sunlight exposure would result in increased plant growth. The results of this investigation support this hypothesis, with plants in Group C growing significantly more, relative to their original height, compared to plants in Groups B and A. Additionally, plants in Group B that were exposed to some sunlight grew more than plants in Group A that were not exposed to any sunlight.

Identification of outliers

Part of evaluating your data is identifying any outliers. If you noticed that any of your results appeared to be outliers, then you should comment on this in your discussion. If you chose to disregard these values during your analysis, make sure you state this clearly. Alternatively, if these outliers are still included in your analysis make sure your reader is aware that there are outliers that may alter the validity of your findings.

Example

IDENTIFICATION OF OUTLIERS

Plant C1 did not grow as much as the other plants in Group C. This may be because, unlike all the other plants, plant C1 was knocked over during the experiment, resulting in all the soil and the plant itself falling out of the pot. This introduced an uncontrolled variable into the experiment, making it hard to compare the results obtained from plant C1 with the results of all the other plants. As a consequence, the results from plant C1 are no longer useful for answering the research question and have therefore been excluded from analysis in this investigation.

Linking of results to relevant biological concepts

In your introduction, you should have discussed the biological concept/s that are relevant to your investigation. It is important to raise these again in your discussion and use them to explain why you obtained the results that you recorded. This is called cross-referencing your results against relevant biological concepts. If you've done research on previous studies that are similar to your own and your findings support or differ from them, it is also a good idea to comment on this here.

Example

LINKING OF RESULTS TO RELEVANT BIOLOGICAL CONCEPTS

Given the importance of light in the process of photosynthesis, it is unsurprising that plants that are exposed to higher amounts of sunlight grew more in our investigation. It seems that the extra energy they were able to generate enabled their cells to grow and replicate at a greater rate compared to plants that were kept in darkness, resulting in greater growth during the recorded period of time.

Identification of limitations in data and methods, and suggested improvements

After you've claimed whether your results support the hypothesis, it's important to take a step back and evaluate whether or not your results are reliable and can be trusted. Consider if the method was flawed and, if so, whether extraneous or uncontrolled variables may have impacted the results. Can you think of any errors that may have occurred during the investigation? If so, what were they? This is part of the evaluation stage of your report, and demonstrates your ability to interact with your own generated primary data in an impartial and scientific manner.

After you've identified any potential limitations and the effect they may have had on your results, it's important to state how they could be addressed in future reproductions of your investigation. What could you change about the method that would make your results more accurate and precise? How could errors be avoided in the future?

Finally, it's good to finish your discussion by weighing up these potential limitations with the strengths of the investigation. Conclude by stating clearly whether or not your results and, therefore, your answer to your research question, can be relied upon.

Example

IDENTIFICATION OF LIMITATIONS AND SUGGESTED IMPROVEMENTS

It is important to note that a number of factors may have influenced our result in this investigation. For example, the tape measure that was used, whilst having 1 mm markings, may not have been the most precise measuring tool available. As such, it may have introduced an element of random or personal error into our results, given the added difficulty of accurately measuring the plants. For future research, it would be advisable to use a more accurate measuring device, such as a laser measure or digital calipers, to get more exact measurements.

Additionally, plant height may not be the best way to measure the effect of increased photosynthesis on plants. Plants have extensive root systems, and it may have been that the plants that didn't grow as much vertically grew more roots instead. Additionally, plants may have been growing outwards rather than upwards, or, the stems of the plants may have been increasing in diameter. A different way of measuring the plants, perhaps one based on weight instead of height, may give a more accurate understanding as to the effect of sunlight exposure on plant growth. Furthermore, only one species of plant was studied in this investigation. It is possible that other types of plants may respond differently to different amounts of light.

Despite these limitations, the clear trend in our results suggests that exposing *Ficus lyrata bambino* to more sunlight does indeed increase its growth, and therefore we suggest it would also increase the growth of other types of office plants.

Conclusion

Lots of people worry about writing conclusions, but they really aren't that hard! Your conclusion should begin by restating your research question, and then state whether or not your hypothesis was supported or refuted by the data you collected. You then need to suggest avenues of further research based on your findings and how future study could address the limitations of your investigation. Finally, you should end your report by showing how your findings are important to society/the environment/the world in general – just like you did in the introduction. After your conclusion, your audience should be left feeling like your findings and conclusions are important and have ramifications in thereal world.

Example

CONCLUSION

This investigation sought to understand the effect of variable amounts of sunlight exposure on the growth of *Ficus lyrata bambino*. It was hypothesised that plants exposed to higher levels of sunlight would grow more compared to plants exposed to less sunlight. This hypothesis was supported by the results, which showed that *Ficus lyrata bambino* plants exposed to more sunlight grew faster. Future research should aim to refine the measuring techniques used to measure the growth of plants, and potentially explore how other environmental factors influence plant growth. Future research might also explore plant growth in other plant species suitable for office life, as well as the effect of healthy plant life on office worker morale.

Nevertheless, it does seem that when placing plants in an office, consideration ought to be given to the amount of sunlight plants will be exposed to, as sunlight availability seems to be a key determinant of plant growth. Ensuring plants are provided with the ideal conditions to thrive is important, as their presence contributes greatly to the workplace atmosphere and employee mood.

References and acknowledgements

It is important to cite any sources that you used in your investigation at the conclusion of your report. Referencing can be complex, and there are many different styles you can use. Check with your teacher which style of referencing they would prefer you to use.

For VCE Biology, the APA and Harvard referencing systems are two of the most commonly used referencing styles. When referencing, it's important to note that these two styles have two types of citation styles – in-text citations and reference lists:

- In-text citations are used in the actual body of your investigation directly after you've referenced a source. You only need to have in-text citations if you are referencing a specific thought, claim, or finding from another source. For the most part, in-text citations are mostly found in your introduction and discussion, as this is where you explore other people's research.
- A reference list is a list of all the sources you've cited in your investigation. That means that you don't need to include every single website or book that you have read as part of your investigation, but only those which you actually used and cited.

Examples of APA and Harvard referencing are shown. For more detail about referencing styles, you can visit The University of Melbourne citation website at: library.unimelb.edu.au/recite

You can also choose to have an acknowledgements section at the end of your investigation. This isn't compulsory, but you can use it to thank anyone who has helped you conduct your investigation. Remember, though, this isn't your Oscars speech, so if you are going to have an acknowledgement section keep it brief!

Example

REFERENCES

APA style – in-text citation

'It is well understood that having indoor plants in an office space increases employees' mood and perceived comfort levels whilst at work (Larsen et al., 1998).'

APA style – reference list entry

Larsen, L., Adams, J., Deal, B., Kweon, B. S., Tyler, E. (1998). Plants in the workplace: the effects of plant density on productivity, attitudes, and perceptions. *Environment and Behaviour, 30(3)*, 261–281.

Harvard style – in-text citation

'It is well understood that having indoor plants in an office space increases employees' mood and perceived comfort levels whilst at work (Larsen et al., 1998, p.261).'

Harvard style – reference list entry

Larsen, L, Adams, J, Deal, B, Kweon, B. S & Tyler, E 1998, 'Plants in the workplace: the effects of plant density on productivity, attitudes, and perceptions', *Environment and Behaviour, vol. 30*, no. 3, pp. 261–281.

What does a successful scientific poster look like?

Ok, now that we've walked through each stage of conducting your investigation, let's look at what putting together a successful poster might look like. Here we show you the following:

1. A suggested poster format with summaries of what to include in each section (Figure 5)
2. A completed poster showing how we conducted our experiment (Figure 6)
3. A rubric that summarises all of the requirements set by the VCAA (Table 3)

Abstract

The abstract is optional on poster presentations

Introduction

The introduction should be very brief.
It should include:

- 1–2 sentence overview of the purpose of the investigation and why the research question is of interest.
- A few sentences explaining background theory, definitions, formulae, prior investigations, and a hypothesis.
- You could put images or illustrations here (unlike in reports).

Methodology

The materials, apparatus, and method should be described well enough for reproduction, but should still be succint.

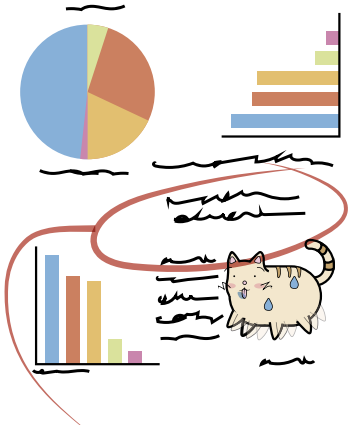
The detail required in formal practical reports is not required.

You may wish to repace detailed steps with flow charts, diagrams, or photographs.
If necessary, you should justify why you chose to investigate the question using this method.

Title

The title must be written as a question that describes the main thing you are trying to determine. Include your name.

Results



Succinct figures should be accompanied by a written description of the main result or trend, and if it supports they hypothesis.

All figures should have titles, labels, and be numbered. Only provide a sample calculation for repeat calculations.

Discussion

The discussion on a poster is similair in size and depth to that of a practical report.
Here you should:

- Identify if the results support, partly support, or refute the hypothesis.
- Find logical explanations for issues with the data – do not ignore problematic data!
- Analyse experimantal design.
- Identify possible sources of error.
- Compare findings to other research.
- Suggest ways to overcome problems.

Conclusion

The conclusion here will be similar to one in a practical report. It should include:

- The main findings, and if they support the hypothesis.
- How the results relate to broader biological concepts.
- Limitations and improvements.
- Possible future work.

References and acknowledgements

Specific contributors should be named and referencing should be in harvard or APA style.

Figure 5 A suggested layout for a poster presentation, and brief descriptions of what to include in each section. Note that more detailed descriptions are given earlier in the piece, and that this is to be used as a guide only.

Does sunlight affect the growth of *Ficus lyrata bambino*?

Introduction

It is well understood that having indoor plants in an office space increases employees' mood and perceived comfort levels whilst at work (Larsen et al., 1998). Perhaps more importantly, however, it has also been shown that an increased number of plants in the workplace decreases worker stress and reduces the amount of sick leave taken (Bringslimark et al., 2007).

As such, it is important to understand the conditions in which indoor office plants flourish and grow.

Image: Ros Fraser/Shutterstock.com

Methodology

- 15 healthy *Ficus lyrata bambino* plants were selected at random from a larger population.
- The 15 selected plants were randomly allocated into three groups – Group A, Group B, and Group C. Each plant was assigned a number within each group (e.g. Group A consisted of plants A1, A2, A3, A4, and A5). We then had one person measure the height of all the *Ficus lyrata bambino* from their soil height to their stem tip using the tape measure.
- All Group A plants were placed in a cupboard with the door closed, all the Group B plants were placed on a table 10 metres away from a north-facing window, and all the Group C plants were placed on a table directly in front of the same window.
- Plants were left in their respective positions for two weeks. After this time, we had the same person measure and record the heights of the *Ficus lyrata bambino* plants.

Discussion

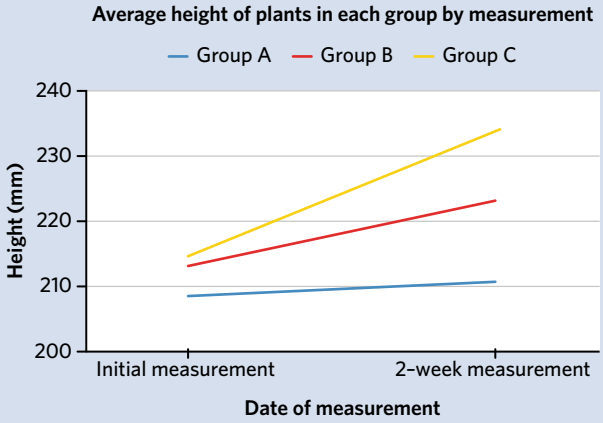
It was hypothesised that increased exposure to sunlight would result in increased plant growth. The results of this investigation support this hypothesis, with plants in Group C growing significantly more relative to their original height compared to plants in Groups B and A. Additionally, plants in Group B that were exposed to more sunlight grew more than plants in Group A that were exposed to no sunlight at all.

Given the importance of sunlight in the process of photosynthesis and respiration, it is unsurprising that plants that are exposed to higher amounts of sunlight grew more in our investigation. It seems that the extra energy they were able to generate enabled their cells to grow and replicate at a greater rate compared to plants that were kept in darkness, resulting in greater growth during the recorded period of time.

It is possible that the tape measure that was used, whilst having 1 mm markings, was not the most precise measuring tool that could have been used and may have introduced an element of random error given the difficulty of measuring the plants with it. Additionally, plant height may not be the best way to measure the effect of increased photosynthesis on plants. Despite these limitations, however, the clear trend in our results suggests that the amount of sunlight a plant is exposed to does indeed affect its growth.

Results

The results show that plants in Group A grew an average of 0.96% relative to their original height, whilst plants in Group B grew an average of 5.18%, and plants in Group C grew an average of 9.61%.



Conclusion

This investigation sought to understand the effect of variable amounts of sunlight exposure to *Ficus lyrata bambino* on their growth. It was hypothesised that plants exposed to higher levels of sunlight would grow more compared to plants exposed to less sunlight. This hypothesis was supported by the results obtained, suggesting that plants exposed to more sunlight grow faster. Future research should aim to refine the measuring techniques used to measure the growth of plants exposed to different levels of sunlight, and potentially explore how other environmental factors influence plant growth.

References

Larsen, L., Adams, J., Deal, B., Kweon, B. S., Tyler, E. (1998). Plants in the workplace: the effects of plant density on productivity, attitudes, and perceptions. *Environment and Behaviour, 30(3)*, 261–281.

Bringslimark, T., Hartig, T., Patil, G. G. (2007). Psychological benefits workplaces: putting experimental results into context. *Americal Society for Horticultural Science, 42(3)*, 581–587.

Figure 6 A poster of Team Bio's findings in regards to the growth of *Ficus lyrata bambino* in different light conditions

Rubric

While it is important to follow the set structure for your poster, it can be hard to be sure you've included everything your teacher wants to see. Always defer to the information provided by your teacher, but cross-check your poster against the following rubric, which has been adapted straight from the dot points provided by the VCAA. Notice that the VCAA separate the investigation into three broad categories, with specific expectations in each.

Table 3 A working rubric to use when finishing your poster. Note that these points are adapted straight from the VCAA.

Investigation design	• I have discussed the biological concepts relevant to my scientific investigation, including explaining their significance and defining any key terms. • I have discussed the characteristics of my selected scientific methodology and method, including why it is appropriate for my research question. • I have defined the independent, dependent, and controlled variables in my investigation. • I have demonstrated techniques of primary quantitative data generation in my investigation. • I have discussed the accuracy, precision, reproducibility, repeatability, and validity of my investigation. • I have discussed the health, safety, and ethical guidelines relevant to my investigation.
Scientific evidence	• I have discussed the nature of my evidence, and whether it supports or refutes my hypothesis. • I have organised my primary data in an appropriate way. • I have analysed and evaluated my primary data to identify patterns and relationships including sources of error and uncertainty. • I have authenticated my primary data by maintaining a complete, up-to-date logbook during my investigation. • I have discussed the possible assumptions of my investigation with reference to any limitations in my method.
Science communication	• I have demonstrated consistent scientific conventions throughout my poster, including proper scientific terminology, symbols, formulas, standard abbreviations, and any relevant units of measurement. • I have included any relevant acknowledgements and references in my final poster. • I have commented on the overall findings and implications of the selected scientific investigation, including suggestions for future research.

Scientific investigations

2.1 Extracting DNA from strawberries

3.1 Enzymes and bubbles

3.2 Investigating the rate of enzyme-catalysed reactions

4.1 Exploring DNA technology

5.1 Effect of lightwaves on photosynthesis

5.2 Photosynthesis in algae

6.1 Yeasty boys

6.2 To seed or not to seed

7.1 Blood typing

8.1 Back off bacteria

8.2 Shake 'n' incubate

9.1 Natural selection in mice

9.2 Simulating natural selection

10.1 Amphibian phylogeny

11.1 Retracing the human odyssey

Students undertake scientific investigations across Units 3 and 4 of VCE Biology. Scientific investigations may be undertaken in groups, but all work for assessment must be completed individually. The use of a logbook reflects standard scientific practice. Students undertaking VCE Biology must maintain a logbook of practical activities in each of Units 3 and 4 for recording, authentication, and assessment purposes. All items in the logbook must be dated and clearly documented.

Image: New Anawach/Shutterstock.com

2.1 EXTRACTING DNA FROM STRAWBERRIES

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 2: Nucleic acids and proteins

INTRODUCTION

Every living organism contains DNA – the molecule which tells an organism how to function, develop, and survive. Since DNA is so critical, it is found in nearly every cell. Each cell contains a copy of the entire set of genetic instructions, which is referred to as the genome. Scientists study an organism's genome for many reasons, including solving forensic investigations, developing novel therapies, genetically modifying crops, or establishing the evolutionary history of different species.

In order to obtain and visualise DNA, scientists have established a method to extract, isolate, and observe DNA from thousands of cells at a time. The first DNA extraction experiment was performed in 1869 by Friedrich Miescher.

In this investigation, you will extract, isolate, and observe DNA for yourself. You will be using strawberries because they possess eight copies of each chromosome (octoploid) and therefore yield more DNA than any other fruit! In addition, strawberries are easy to mash and contain pectinases and cellulases (types of enzymes) which help to break down the cell wall when acted upon by mechanical force.

Resources

Risk assessments, lab tech notes, and answers are available online.

Image: Ian Cruz/Shutterstock.com

Figure 1 Strawberry extractions are useful for observing DNA

AIM

To extract, isolate, and observe DNA from strawberries.

MATERIALS

- personal protective equipment (gloves, lab coat, and goggles)
- 1 × 50 mL lysis buffer falcon tube
- 1 × 45 mL measuring cylinder
- 1 × 5 mL disposable pipette
- 1 × sealable plastic bag
- 1 × glass beaker
- filter paper
- 1 × funnel
- 5 g of table salt (NaCl)
- 45 mL water
- 5 mL of liquid detergent
- 1 × ripe strawberry
- 25 mL of isopropyl alcohol (rubbing alcohol)

METHOD

Part A: Making the lysis buffer

1. Add 5 g of table salt to the lysis buffer falcon tube.
2. Add 45 mL water and 5 mL liquid detergent to the lysis buffer falcon tube.
3. Place the cap securely on the tube and mix by gently inverting the tube several times. This is the lysis buffer you will be using in Part B.

Part B: Making the strawberry lysate

4. Place the ripe strawberry in a sealable plastic bag. Remove all the air from the bag before sealing it.
5. Mash the strawberry through the bag with your fingers. Be careful not to break the bag. Record what you observe in Table 1.
6. Unseal the bag and add the lysis buffer to the bag.
7. Once again, remove the air, seal the bag and continue to mash the strawberry with your fingers. The mixture at this stage is referred to as strawberry lysate.
8. Record what you observe in Table 1.

Part C: Filtering the lysate

9 Place the funnel over the glass beaker and insert the filter paper into the funnel.

10 Carefully pour the strawberry lysate from the plastic bag into the funnel and wait until all the liquid has dripped from the funnel into the glass beaker. The liquid in the beaker is called the filtrate.

Part D: Precipitating and observing DNA

11 Remove the funnel from the glass beaker.

12 Measure out and slowly add 25 mL isopropyl alcohol into the glass beaker.

13 Observe the boundary between the isopropyl alcohol and strawberry filtrate layer. Record your observations in Table 2.

RESULTS

Table 1 Appearance of strawberry lysate before and after the addition of lysis buffer

	Lysis buffer	
	Before	**After**
Lysate		

Table 2 Appearance of DNA before and after the addition of isopropyl alcohol

	Isopropyl alcohol	
	Before	**After**
DNA		

DISCUSSION QUESTIONS

1 List three real-life applications that show why it is important for scientists to be able to extract and examine DNA.

2 Describe the main steps involved in DNA extraction.

3 Identify three factors that could affect the outcome of DNA extraction in other fruit or vegetables.

4 Draw and describe the appearance of the precipitated DNA in your test tube at the conclusion of the experiment.

5 Identify the cellular components of the strawberry filtrate and identify where the filtrate is located in your test tube.

6 The following table shows the steps you performed in this DNA extraction experiment. Identify the purpose of each step.

Procedure	**Purpose**
A. Mash the strawberry in the sealable plastic bag	
B. Add the DNA lysis buffer to the mashed strawberry	
C. Pour the strawberry lysate through the funnel which contains filter paper	
D. Add isopropyl alcohol to the strawberry filtrate	

7 Imagine someone is standing 100 meters away from a single cotton thread which is not visible. If the person winds thousands of threads together in a rope however, the threads become visible. Is this statement comparable to what occured when you performed DNA extraction in this investigation? Justify your answer.

8 Explain the role of detergent and isopropyl alcohol in this investigation.

9 Identify an appropriate control for this investigation and explain your answer.

10 Imagine a student obtained 127 mg of DNA from 1.25 g strawberry lysate. Calculate the percentage yield of extracted DNA. Note that 1 g = 1000 mg

11 Identify whether cells are broken before or after the lysis buffer is added. Justify your response.

12 Considering your method, what steps could you add in or modify to increase the yield of extracted DNA?

13 Identify any possible errors that may have affected your results. Be sure to state whether they were personal, systematic, or random errors.

14 There are many different variables that influence whether DNA is successfully extracted from fruit or vegetables. Select one of these variables, and design a method to test the effect this variable has on DNA extraction. Provide details of the following aspects of your experiment:

- **a** What is the hypothesis?
- **b** What are the independent and dependent variables?
- **c** What is the control group?
- **d** How will errors be minimised?
- **e** How will you maximise accuracy and precision?
- **f** How will you address replication?

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- whether the aim was achieved by referring to your results
- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

3.1 ENZYMES AND BUBBLES

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 3: Enzymes

INTRODUCTION

Hydrogen peroxide (H_2O_2) is a molecule formed in the cells of many living organisms. Its presence, however, can cause serious damage to an organism's cells and molecules, meaning it must be immediately broken down into less harmful compounds. Catalase is one of the enzymes responsible for the breakdown of H_2O_2 into water (H_2O) and oxygen (O_2), as represented by the equation in Figure 1.

Figure 1 Catalase catalyses the breakdown of hydrogen peroxide.

Catalase enzymes in different organisms (i.e. a human vs a plant) will have differences in functionality, as they evolved over time to be best suited to that specific organism and its optimal environmental conditions. Still, the role of catalase in breaking down the potentially harmful H_2O_2 into much safer H_2O and O_2 remains the same.

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To observe the enzymatic activity of catalase in a range of different samples.

MATERIALS

- 9 × test tubes
- hydrogen peroxide solution (H_2O_2) (3% solution)
- lab coat, goggles, gloves
- small, test-tube size samples of the following:
  - sliced raw potato
  - baked potato
  - ground, young leaves
  - ground, old, dried leaves
  - yeast cells
  - liver sample (e.g. from a sheep)
  - ground, raw meat
  - cooked meat
  - Note that your samples do not have to be identical to those listed above, but for the purposes of this methodology they will be used, as long as there is a good variety of living and non-living material selected.

METHOD

1. Label eight test tubes, each with the name of one of the samples.
2. Label a ninth test tube as a control.
3. Fill each test tube approximately one-third full with hydrogen peroxide solution.
4. Carefully add a small amount of sample to its corresponding test tube, ensuring you do not cause a splash.
5. Observe the test tube for a minute or two and note whether or not bubbles are produced. Record your results in Table 1.
6. Repeat steps 4–5 for all of your samples, with the control test tube having no sample material added.

RESULTS

Record your observations in Table 1.

Table 1 Observations of experimental results

Sample	Bubbles (Y/N)
Raw potato	
Baked potato	
Young leaves	
Old, dried leaves	
Yeast cells	
Liver	
Raw meat	
Cooked meat	
Control	

DISCUSSION QUESTIONS

1. Identify the dependent and independent variables in this experiment.
2. Did any of the results surprise you? If so, why?
3. Categorise the eight types of sample as living or non-living material.
4. From your observations, what can be concluded about the presence or absence of catalase in living and non-living material?
5. Considering your method, what steps could you add in or modify to increase the precision of your experiment?
6. Studies have found that the optimal temperature of potato catalase is 35 °C, and the optimal pH of potato catalase is 8.2. A low concentration H_2O_2 solution has a slightly acidic pH. If the experiment were to be run again, identify a change to the conditions of the raw potato sample that would produce a greater rate of bubble production. Justify your response.
7. Identify any possible errors that may have affected your results. Be sure to state whether it was a personal, systematic, or random error.

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

3.2 INVESTIGATING THE RATE OF ENZYME-CATALYSED REACTIONS

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 3: Enzymes

INTRODUCTION

Oxygen is a double-edged sword. We need it to survive, but if it reacts and forms a 'superoxide' it can destroy molecules in our cells and hasten the aging process. Because of this, all cells have an enzyme called superoxide dismutase that converts superoxides into hydrogen peroxide (H_2O_2). Unfortunately, if left to its own devices, H_2O_2 will break down into hydroxyl radicals. While less damaging than superoxides, hydroxyl radicals can still breakdown DNA and proteins. Enter catalase: the enzyme that deals with problematic oxygen, once and for all.

Catalase converts hydrogen peroxide into harmless oxygen and water:

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

Figure 1 The chemical equation showing hydrogen peroxide being converted into oxygen and water via the action of catalase.

You should remember from chapter 3 that enzymes are organic catalysts. They speed up the rate of chemical reactions but are not used up or changed by the process. They work by binding with substrates at the active site, lowering the activation energy, then releasing the products of the reaction. You'll also recall the that efficiency of enzymes can be affected by a number of factors such as:

- temperature
- pH
- substrate concentration
- enzyme concentration
- chemical inhibitors.

In Part 1 of this investigation, you will investigate the effect of substrate concentration on the rate of the reaction catalysed by catalase, which is found in potatoes (as well as many other living tissues). In Part 2 of the investigation, you will use your key science skills to improve the accuracy and precision of the method and reduce the risk of errors whilst investigating the influence of another factor on the rate of reaction.

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To measure the rate of an enzyme-catalysed reaction at different substrate concentrations.

MATERIALS

- 3 × 20 mL fresh puréed potato
- 2 mL distilled water
- 2 mL 1% hydrogen peroxide solution
- 2 mL 3% hydrogen peroxide solution
- 2 mL 5% hydrogen peroxide solution
- 1 × 125 mL conical flask
- 1 × 50 mL measuring cylinder
- clamp stand, boss, and clamp (optional)
- 1 × stopwatch
- 1 × 1-holed rubber stopper that fits a 125 mL conical flask
- 30–50 cm rubber tubing to fit the rubber stopper hole
- 1 × ice cream container or deep dish

METHOD

Part 1

1 Pour 20 mL of the potato into the conical flask. You may need to use a stirring rod to ensure all the potato sits on the base of the flask.

2 Insert your rubber tubing into the rubber stopper and ensure the fit is airtight.

3 Fill your ice cream container about half full with tap water. Fill your measuring cylinder all the way to the top with water. Carefully place your hand over the top of the measuring cylinder so that no water can escape, and turn it upside down. Put the mouth of the measuring cylinder underneath the water in the ice cream container (while still covering it with your hand), then remove your hand. The measuring cylinder should still be full of water, with no air at the top (Figure 2).

4 You may secure your measuring cylinder with a clamp stand, boss, and clamp, or get one person in the group to hold it for the entire experiment. Ensure that the measuring cylinder is neither sitting on the bottom of the ice cream container nor rising above the surface water.

5 Place the end of the rubber tubing that is not secured to the stopper underneath the water in the ice cream container, and up into the measuring cylinder. While doing this, ensure that the measuring cylinder stays underwater and doesn't collect air bubbles.

6 The next step must be done quickly and carefully. You may wish to practice it first. Pour 2 mL of 1% hydrogen peroxide solution into the conical flask containing the potato. Quickly place the rubber stopper (that is connected to the rubber tubing) on top of the conical flask to seal. At the same time, get a second person to start the stopwatch.

7 After 30 seconds, measure the volume of gas in the measuring cylinder. Record this in Table 1.

8 Empty and rinse the conical flask well. Repeat steps 1–7 with the other concentrations of hydrogen peroxide and the distilled water.

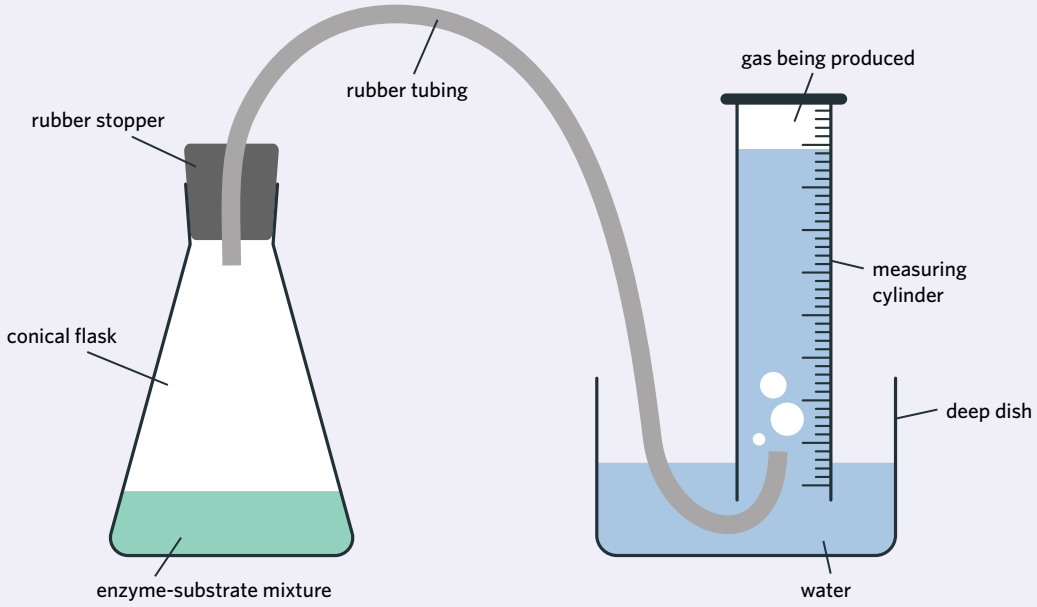


Figure 2 Collecting gas to measure the rate of an enzyme-catalysed reaction

Part 2 – student-directed

9 Identify changes to the method in Part 1 that would improve the accuracy and precision of the data obtained, and also minimise personal, random, and systematic errors.

10 Choose a new research question about how different factors might affect the rate of an enzyme-catalysed reaction. Design and undertake an experiment to answer this research question. When you write out your method, make sure you answer the following questions:

- What are your independent and dependent variables?
- What are your control group/s and your experimental group/s?
- What factors will you keep constant?
- How can you minimise error when collecting your data?
- To what degree is your investigation replicated?

RESULTS

Part 1

Table 1 Raw data showing the amount of gas produced

The concentration of hydrogen peroxide (%)	Amount of gas produced (mL)
0 (distilled water)	
1	
3	
5	

Use Table 1 to draw a graph that demonstrates your results.

Part 2

Record your results, then present them in the most appropriate format.

DISCUSSION QUESTIONS

1 Describe the general structure of an enzyme.
2 Explain how enzymes catalyse reactions.
3 Discuss why enzymes such as catalase are important for living things.
4 Use a model to explain how catalase and hydrogen peroxide interact to produce water and oxygen.
5 Identify the gas(es) produced in the measuring cylinder.
6 Describe and explain your results from Part 1.
7 Identify the controlled variables in this experiment.
8 Identify two errors or limitations in Part 1 of the experiment, and suggest how they could be overcome.

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- whether the hypothesis was supported by referring to the results
- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

4.1 EXPLORING DNA TECHNOLOGY

Scientific investigation type: Simulation

This experiment relates to Chapter 4: DNA manipulation

INTRODUCTION

In the real world, scientists frequently manipulate DNA for a wide variety of purposes, including in biotechnology research, to solve forensic investigations, and to identify disease- causing mutations. The DNA manipulation techniques you will focus on in this investigation are DNA extraction, the polymerase chain reaction and gel electrophoresis. In the process of DNA extraction, DNA is extracted from cells and released so that it can be separated from other cell organelles and intracellular proteins. The polymerase chain reaction amplifies the DNA of interest and gel electrophoresis separates the DNA according to molecular size and charge.

Imagine that you are a forensic detective who is trying to identify the perpetrator of a crime or a researcher seeking to determine the gene(s) responsible for a serious illness. Your first objective would be to obtain a biological sample from the individual of interest. This could be sourced from their hair, fingernails, or a cheek swab (amongst other things). Then, you would be required to extract and isolate the target DNA from this biological sample (Figure 1), make many copies of the DNA (Figure 2), and subsequently separate the DNA according to molecular size (Figure 3). There are three virtual laboratory simulations for you to work through – DNA extraction, the polymerase chain reaction, and gel electrophoresis. Good luck on your mission!

Resources

Risk assessments, lab tech notes, and answers are available online.

Image: HQuality/Shutterstock.com

Figure 1 DNA extraction technique

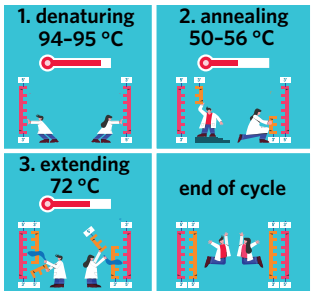


Image: M. PATTHAWEE/Shutterstock.com

Figure 2 Polymerase chain reaction technique

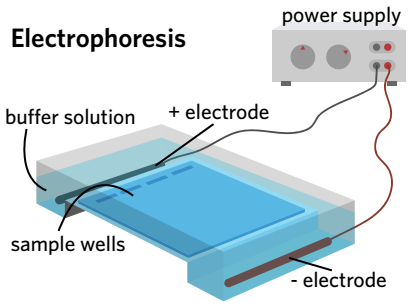


Image: Ormalternative/Shutterstock.com

Figure 3 Gel electrophoresis technique

AIM

To understand DNA extraction, the polymerase chain reaction, and gel electrophoresis using virtual laboratory simulations.

MATERIALS

- computer or laptop
- access to the three links below in the following order:
  1. learn.genetics.utah.edu/content/labs/extraction/
  2. learn.genetics.utah.edu/content/labs/pcr/
  3. learn.genetics.utah.edu/content/labs/gel/
- VCE Biology notebook

METHOD

1. Navigate to the Learn Genetics DNA extraction lab via the first link and work through the simulation. Identify the purpose of DNA extraction, summarise each step and list both the reagents and equipment used.
2. Navigate to the Learn Genetics polymerase chain reaction lab via the second link and work through the simulation. Identify the purpose of the polymerase chain reaction, summarise each step and list both the reagents and equipment used.
3. Navigate to the Learn Genetics gel electrophoresis lab via the third link and work through the simulation. Identify the purpose of gel electrophoresis, summarise each step and list both the reagents and equipment used.

RESULTS

Draw Table 1 in your VCE Biology notebook and record your results.

Table 1 DNA extraction, polymerase chain reaction and gel electrophoresis simulation results

Technique	DNA extraction	Polymerase chain reaction	Gel electrophoresis
Summary of main steps			
Reagents			
Equipment			

DISCUSSION QUESTIONS

1. Briefly describe each stage of the polymerase chain reaction.
2. Explain the purpose of gel electrophoresis.
3. Explain each stage of DNA extraction that you were shown in the extraction simulation.
4. In the DNA extraction simulation, you were introduced to the lysis solution. Name the two key reagents in the lysis solution and describe how they facilitate DNA extraction.
5. In the DNA extraction simulation, you saw the microcentrifuge tube being placed into a warm water bath. What was the purpose of this step?
6. What was the purpose of the salt solution you were shown in the DNA extraction simulation?
7. The extracted DNA was spun down twice. Identify the outcome of each spin.
8. When undertaking the polymerase chain reaction, scientists often amplify a region of interest within the genome. Explain how this is achieved.
9. Taq polymerase isolated from *Thermus aquaticus* bacteria is used in the polymerase chain reaction instead of human DNA polymerase. Explain why this is the case.
10. The polymerase chain reaction is frequently used to make copies of introns and exons. Briefly explain what introns and exons are and comment on their functional significance.
11. In the gel electrophoresis simulation you were shown that in order to make gels, salt buffer and agarose powder must be mixed together. Explain the role of each in facilitating gel electrophoresis.
12. State why it is important to use a DNA standard in gel electrophoresis. In your answer, identify which well the DNA standard is loaded into during electrophoresis.
13. Explain the pattern of migration of DNA fragments through the gel you observed in the simulation.
14. After running gel electrophoresis, the gel was removed from the buffer chamber and stained. Identify the dye that was used and explain why it is needed to locate bands on the gel.
15. Consider the three techniques you have learned about in this investigation and identify a factor for each that could reduce the reliability of the results. Suggest two strategies for each technique to enhance the reliability of the results.

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- limitations of each technique
- potential ways to maximise the accuracy of each
- broader implications of each technique or further areas of exploration that stem from undertaking the simulations.

5.1 EFFECT OF LIGHTWAVES ON PHOTOSYNTHESIS

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 5: Photosynthesis

INTRODUCTION

When plant cells undertake photosynthesis, they produce oxygen that typically leaves the cell and eventually exits the plant altogether. In C3 plants, mesophyll cells within leaves are responsible for the bulk of the photosynthesis, and, therefore, the production of oxygen that exits the leaves via the function of the stomata.

The amount of oxygen produced (and therefore the rate of photosynthesis) can be inferred by the buoyancy of leaf tissue when the leaves are suspended in water. This is because when land plants are placed in water, they struggle to expel the oxygen formed from photosynthesis as efficiently and it instead remains within the spaces inside the leaf. This is why buoyancy can be used to infer the rate of photosynthesis: the greater the rate of photosynthesis, the greater the amount of oxygen produced and the more buoyant the leaf tissue will be.

Two properties of light that have the potential to affect the rate of photosynthesis are the intensity of light, and the wavelength (or colour) of light that a plant is exposed to.

Today, you will investigate which colour of light is best for photosynthesis. For this experiment, you will add leaf discs to water that contains sodium bicarbonate (a source of carbon dioxide), and expose the water to light, thus satisfying the major requirements for photosynthesis. Plants will be exposed to green, red, and blue light using green, red, and blue cellophane. This will allow you to conclude which colour of light allows for the greatest rate of photosynthesis.

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To determine which colour of light leads to the greatest rate of photosynthesis.

MATERIALS

- leaves (choose leaves that are thin, smooth, and ideally dark on the top surface but paler underneath)
- 4 × large syringes (without the needle)
- 4 × 250 mL beakers
- tweezers
- single hole punch
- 6 × pieces of coloured cellophane (two each of green, red, and blue)
- cling wrap
- 2% sodium bicarbonate solution (with a small amount of dishwashing detergent added to reduce the surface tension between the water and the leaf surface)
- 1 × stopwatch
- 1 × glass stirrer
- 1 × lamp

METHOD

This investigation is best conducted in groups of 3–4.

1. Cut 40 leaf discs from the leaves, using the single hole punch. Try to pick smooth and thin parts of the leaf whilst also avoiding any major veins.
2. Prepare four small beakers by wrapping them in pieces of cling wrap or cellophane. Wrap the first beaker in a piece of cling wrap, wrap the second in a piece of green cellophane, the third in red cellophane, and the fourth in blue cellophane. Also, set aside a second piece of each material to use as a 'lid' for each beaker later on in the experiment. However, for now, you can leave the top of the beakers open.
3. Remove the plungers from the syringes and use tweezers to carefully pick up and drop 10 leaf discs into each syringe barrel.
4. Replace the plunger in each syringe, being careful not to squash any leaf discs.

5 Fill the syringes with 2% sodium bicarbonate solution, drawing up the solution by lifting up the plunger.

6 Hold a finger over the opening of the syringe (where the needle would be if it had a needle) and pull firmly on the plunger for 30 seconds (Figure 1). After 30 seconds release the plunger to decrease the pressure of the fluid inside the syringe. This drop in pressure will draw the sodium bicarbonate solution into the leaf spaces of the leaf tissue, bathing the mesophyll cells with water and carbon dioxide.

- It may help to shake the syringe while you are pulling on the plunger to help agitate any gas particles that are in the leaf tissue.

7 Continue to pull on the plunger and release it at 30-second intervals. The aim is for the leaf discs to sink to the bottom. If they do not sink to the bottom, you will need to repeat the procedure. Continue to pull on the syringe plunger and release it at 30-second intervals, until the leaf discs sink in the syringe. When they sink, you will know that the leaf spaces are filled with fluid. Repeat this step for all four syringes.

- If you are still finding it difficult to get the discs to sink after three attempts, it is usually because there is not enough detergent in the solution. Add a few more drops of detergent to the solution and continue pulling the remaining disks to the bottom of the syringe.

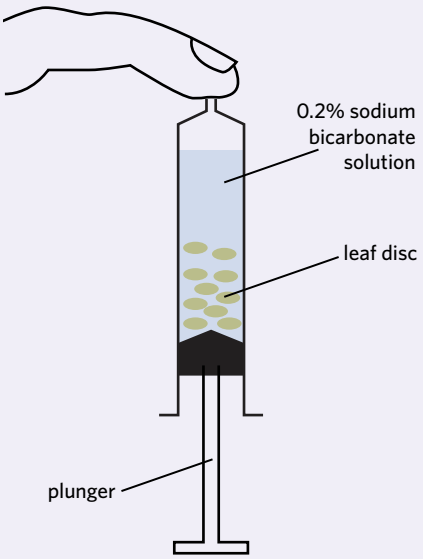


Figure 1 Initial setup of the plunger

8 Have the beakers ready for the leaf disc solutions to be transferred. Depending on the volumes of your syringes and beakers, some sodium bicarbonate solution can be added to the beakers to ensure they are moderately full when the syringe volumes are added.

9 Transfer the leaf disc solutions to their own beaker. The leaf discs should sink to the bottom of the beaker.

10 Use a glass stirrer to gently spread the discs out so that they do not interfere with each other. Add the appropriate cellophane or cling wrap 'lid' to each beaker (Figure 2).

11 Place the beakers under the lamp. Using a stopwatch, record how long it takes for five of the discs to rise to the surface in each of the four beakers (Figure 2). Make sure to keep the stopwatch running until all four of the beakers are finished.

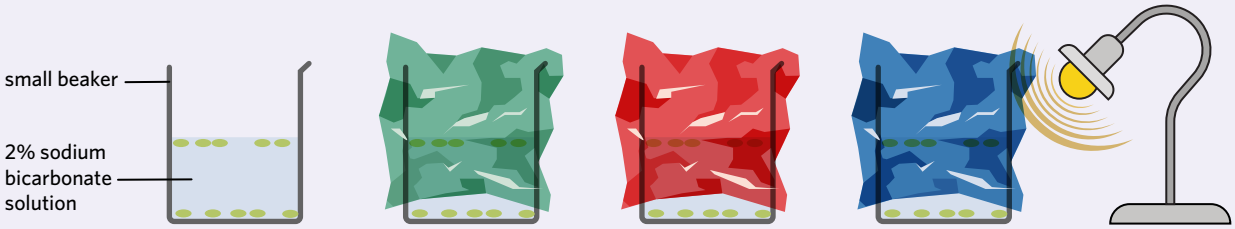


Figure 2 Experimental setup of the beakers

RESULTS

Part 1

Table 1 Time for five leaf discs to rise to the surface

Beaker	Time
Cling wrap	
Green cellophane	
Red cellophane	
Blue cellophane	

DISCUSSION QUESTIONS

1 What are the inputs of photosynthesis?

2 Name and briefly describe the two stages of photosynthesis.

3 How does light intensity impact the rate of photosynthesis?

4 Identify the independent and dependent variables in this experiment.

5 Identify any controlled variables in this experiment.

6 Why was one beaker wrapped in cling wrap? What was the purpose of this trial?

7 Differences in timing between the four simultaneous trials within this experiment can influence the results. Discuss any major observations regarding the timing of the trials. Were any trials exposed to greater/lesser amounts of time in syringes or beakers? How could this have impacted your results?

8 What was the purpose of waiting for five leaf discs to rise to the surface in each beaker rather than all ten?

9 There are many different variables that influence the rate of photosynthesis. Select one of these variables, and design a method to test the effect of this variable on the rate of photosynthesis. Provide details of the following aspects of your experiment:

   a How will you address replication?

   b What are the independent and dependent variables?

   c What is the control group?

   d What is the hypothesis?

   e How will errors be minimised?

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- whether the aim was achieved by referring to the results
- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

5.2 PHOTOSYNTHESIS IN ALGAE

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 5: Photosynthesis

INTRODUCTION

Algae are a group of photosynthesising protists found in aquatic environments. When measuring the rate of photosynthesis in algae, scientists often measure changes in CO_2 levels in their environment. This is because CO_2 is a direct input in the photosynthesis process, meaning that environmental levels should decrease as photosynthesise levels increase.

In this investigation, you will follow these same basic principles to observe algae undergoing photosynthesis in your classroom. The aim of this investigation is to determine whether the effect of algal density influences the rate of photosynthesis. As discussed, to measure the rate of photosynthesis, we will measure the changes in the CO_2 levels directly surrounding our algae sample. In order to visually observe this change in CO_2 levels, a pH indicator will be added to the algae. The pH indicator will change colour to tell you whether the pH surrounding your algae has increased or decreased. An increase in pH indicates a decrease in the amount of CO_2 surrounding the algae which in turn indicates an increase in the rate of photosynthesis.

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To determine whether the algal density affects the rate of photosynthesis.

MATERIALS

- 100 × algal balls
- 5 × 7 mL empty dram vials
- 10 mL of hydrogen carbonate indicator
- 1 × pH colour chart
- 2 mL of distilled water
- 2 × 2 mL plastic pipettes
- light source
- 1 × strainer
- 1 × spoon
- 1 × tape measure

METHOD

1. Label your five vials with one of the following: A, B, C, D, and E.
2. Using your pipette, add 2 mL of distilled water into vial A.
3. In the vial labelled B, add 10 algal balls. Straining the balls may make it easier to distribute them.
4. Add 20 algal balls to vial C, 30 algal balls to vial D, and 40 algal balls to vial E (Figure 1).

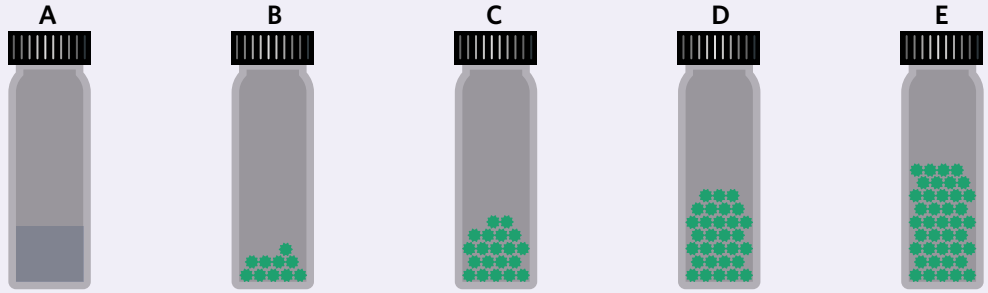


Figure 1 Water sample control and algal bloom set up

5 Using your pipette, add 2 mL of hydrogen carbonate indicator into all five vials then secure their lids.
6 Prior to any light exposure, estimate the pH of the solution within each vial using the pH colour chart and record both the colour and pH in the first column of Table 1 .
7 Place each of your vials at an equal distance from your light source using the tape measure and record the colour and pH of each solution after 10, 20 and 30 minutes in Table 1.

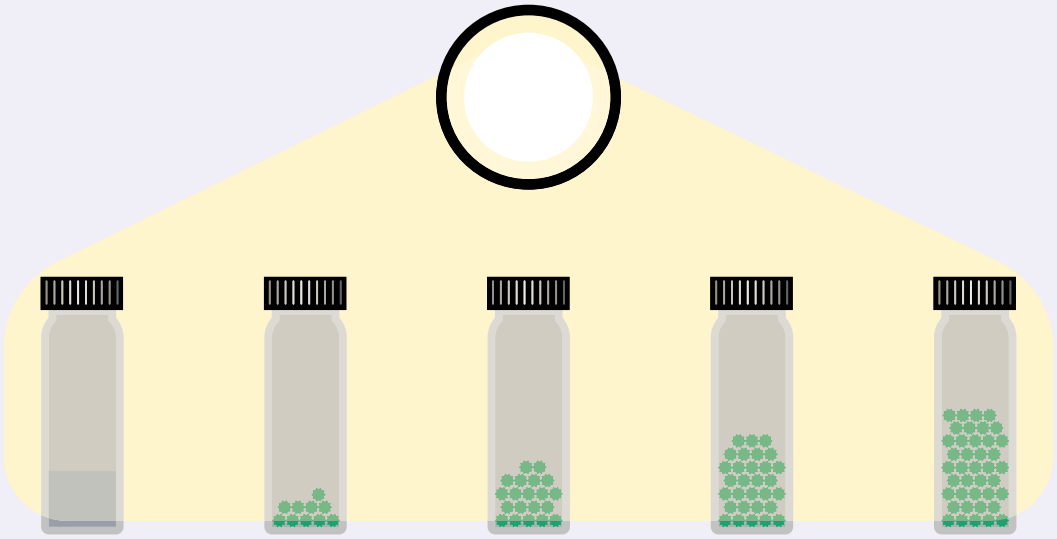


Figure 2 Set up of vials in front of the light source

RESULTS

Draw Table 1 in your VCE Biology notebook and record your results.

Table 1 Results of the colour and pH of each vial

Vial	Prior to light exposure	10 minutes after light exposure	20 minutes after light exposure	30 minutes after light exposure
A				
B				
C				
D				
E				

DISCUSSION QUESTIONS

1 Write out both the simplified chemical and worded equations for photosynthesis.
2 Identify the inputs, outputs and locations of the light dependent and light independent stages of photosynthesis.
3 List the main functions of glucose in plant cells.
4 Identify the overall trend in colour and pH change over the course of the experiment.
5 Using your results, explain whether there is a link between the number of algal balls and the rate of photosynthesis.
6 Identify the independent and dependent variables in this experiment.
7 Explain why each vial was placed at an equal distance from the light source.
8 Considering your method, what steps could you add in or modify to increase the accuracy and precision of your experiment?

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- potential ways to improve the simulation
- broader implications of your research or further areas of exploration that stem from your findings.

6.1 YEASTY BOYS

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 6: Cellular respiration

INTRODUCTION

Yeast are eukaryotic, single-celled microorganisms that are classified as members of the fungi kingdom. As yeast are unable to photosynthesise, they must acquire the food molecules they need for respiration from their environment. Yeast do not require oxygen to respire; instead, they can undertake anaerobic cellular respiration according to the following equation:

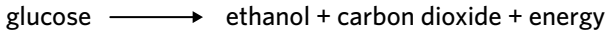


Figure 1 Equation for anaerobic cellular respiration in yeast

Anaerobic cellular respiration is also referred to as fermentation. The energy produced is in the form of adenosine triphosphate (ATP). The reaction shown depicts anaerobic cellular respiration in plants and yeast, but in humans it is different. When we undertake anaerobic cellular respiration we produce lactic acid rather than ethanol and carbon dioxide. Consider this a good thing – if we produced ethanol whenever we anaerobically respired, we would get drunk whenever we exercised which would be a nightmare during school cross country!

Many different kinds of yeast exist in nature. In the wild, they are typically found growing on the fruits or grains of plants, from where they obtain a variety of food molecules. Several strains of yeast have been domesticated for human use, mainly for baking and brewing purposes. The domesticated yeast we use in baking is typically fed refined sugar as a fuel source. As the dough rests, the yeast consumes the sugar and releases carbon dioxide bubbles, which causes the dough to rise.

In this activity, you will measure the rate of respiration in yeast that are provided with different food sources, or exposed to different temperatures and conditions. Some of the yeast will be provided with sugar, and some with artificial sweetener instead. Some will be kept at room temperature and others will be warmed to 32 °C, and some will be combined with water whilst others are combined with water and shampoo. How do you think these factors will influence the rate of respiration in yeast?

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To observe the rate of respiration in yeast with varying food sources and conditions.

MATERIALS

- 4 × screw cap tubes with lids e.g. falcon tubes (or any tubes that can be covered by a lid and also fit a balloon on the end – see Figure 2)
- 1 × tube rack
- 1 × digital scale
- watch glasses
- 1 × 10 mL pipette or measuring syringe
- 1 × 2 mL pipette or measuring syringe
- freeze-dried yeast
- sugar
- artificial sweetener e.g. stevia
- shampoo
- 4 × balloons
- 32 °C water bath

METHOD

1 Working in pairs or small groups, label four tubes with 1, 2, 3, and 4.
2 Use the watch glasses and scale to measure out and place 0.6 grams of freeze-dried yeast into each tube.
3 Use the watch glasses and scale to measure out and place 0.4 grams of sugar in tubes 1, 2, and 3. Don't put any sugar in tube 4.
4 Use the watch glasses and scale to measure out and place 0.4 grams of artificial sweetener in tube 4.
5 Use a pipette to add 2 mL of shampoo to tube 3. Don't put any shampoo in the other tubes.
6 Use a pipette to add 8 mL of water to tube 3. Cover the tube by placing the lid on and give the contents a shake. Set aside in the rack for now.
7 Now use a pipette to add 10 mL of water to tubes 1, 2, and 4. Put the lids on the tubes and shake to mix the contents before setting them down in the tube rack.
8 One at a time, remove the lid of each tube and place a balloon over the top.
9 Place tubes 1, 3, and 4 in the 32 °C water bath.
10 Leave tube 2 in the tube rack on the bench.
11 After 1 hour has passed, observe your four tubes and measure the size of each balloon. Record your results in Table 2.

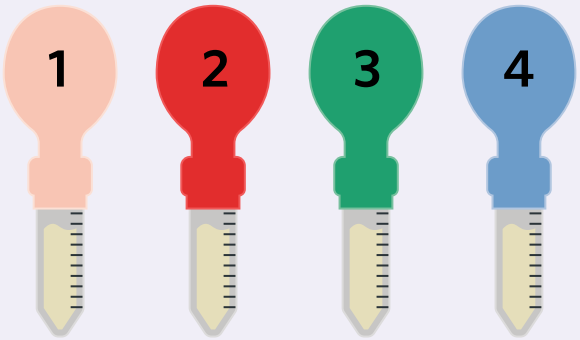


Figure 2 The four tubes containing yeast solutions covered in balloons to measure the respiration rate

Table 1 Summary of the components of the four tubes

	Tube 1	Tube 2	Tube 3	Tube 4
Freeze-dried yeast	✓	✓	✓	✓
Sugar	✓	✓	✓	–
Artificial sweetener	–	–	–	✓
Shampoo	–	–	✓	–
Water	✓	✓	✓ (8 mL)	✓
Placed in water bath	✓	–	✓	✓

RESULTS

Table 2 The diameter of balloons in each tube after one hour

	Tube 1	Tube 2	Tube 3	Tube 4
Size of balloon after one hour				

DISCUSSION QUESTIONS

1. What was your hypothesis for this experiment? Explain whether your results support your hypothesis.
2. Identify the main purpose of cellular respiration in organisms.
3. What is the difference between aerobic and anaerobic cellular respiration?
4. Which tube experienced the greatest rate of respiration?
5. Was there a difference between the results for tube 1 and tube 2? If so, account for this difference.
6. Why was only 8 mL of water added to tube 3 when all the others received 10 mL of water?
7. Was a control used in this experiment?
8. What do you think happened in tube 3 and why? Hint: shampoo is an emulsifier.
9. Considering the method, what steps could you add in or modify to increase the accuracy and precision of your experiment?
10. Yeast can use a range of fuel sources. They can utilise a variety of sugars and starches, not just the packet sugar or artificial sweetener used in this experiment. If the experiment was to be repeated with the initial four tubes, plus the addition of a fifth tube containing honey and a sixth tube containing energy drink, how would these additional tubes be designed? Make sure you indicate whether each of the components listed in Table 1 are included in the tubes or not.
11. Other than the type of sugar, temperature, and presence of shampoo, there are many more variables that influence the rate of anaerobic cellular respiration in yeast. Select one of these variables, and design a method to test the effect this variable has on yeast anaerobic cellular respiration rate. Provide details of the following aspects of your hypothetical experiment:
    - **a** How will you address replication?
    - **b** What are the independent variables?
    - **c** What is the control group?
    - **d** What is the hypothesis?
    - **e** How will errors be minimised?

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- whether the hypothesis was supported by referring to the results
- limitations of the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

6.2 TO SEED OR NOT TO SEED

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 6: Cellular respiration

INTRODUCTION

Aerobic cellular respiration is one of the main processes used by cells to extract energy (ATP) from glucose in the presence of oxygen (Figure 1). During this enzymatic process, carbon dioxide and water molecules are released as by-products. As you know, enzyme function is heavily dependent on temperature and pH. This means that the enzymes that catalyse aerobic cellular respiration also possess an optimal temperature and pH range. Below the optimal temperature, enzyme activity decreases, resulting in a lower rate of cellular respiration. Above the optimal temperature, however, enzymes can become denatured leading to the reaction slowing or stopping completely (Figure 2).

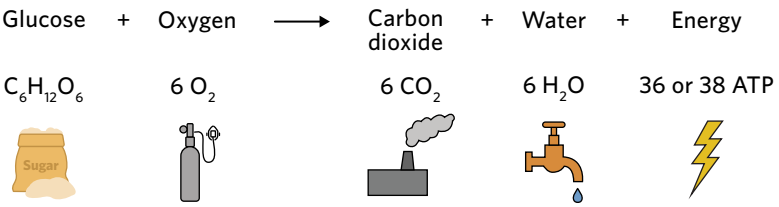


Figure 1 The overall equation for aerobic cellular respiration

When carbon dioxide reacts with water, it produces small amounts of carbonic acid (H_2CO_3) which then ionises and releases H^+ ions into the solution (Figure 3). This results in a decrease in the solution's pH.

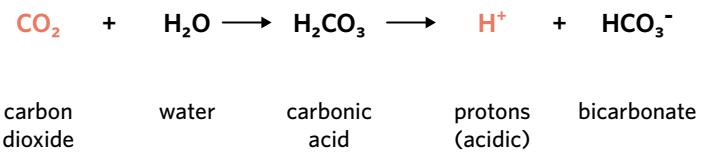


Figure 3 Carbon dioxide reacts with water, which leads to the formation of H^+ ions in the solution.

There are a number of ways to measure the pH of a solution, such as using indicators, which are often plant extracts or other compounds with acid-base properties. Universal indicator (UI) is formed when many different indicators are combined, giving it a wide spectrum of colours. For this reason, the colour of universal indicator can be used to determine the approximate pH of a solution (Figure 4).

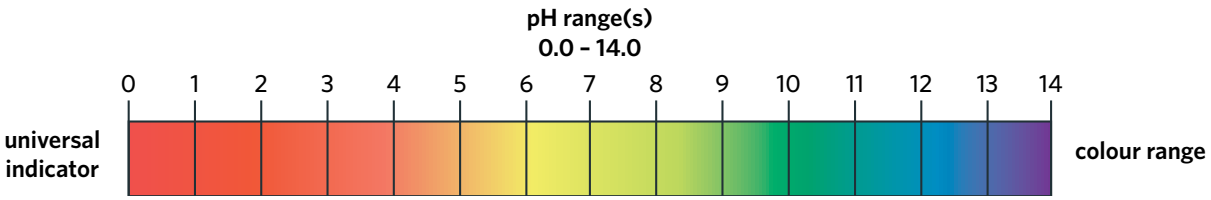


Figure 4 The colour of a solution that contains universal indicator can be used to estimate its pH.

In this activity, you will measure the effect of changes in temperature (an ice bath, room temperature (RT) and 60 °C) on the rate of aerobic cellular respiration in germinating and ungerminated bean seeds. This is accomplished by adding a few drops of universal indicator to our solutions and analyzing any color changes over time.

Note that while germinating, bean seeds use a large amount of available nutrients contained within their endosperm storage tissue until they are able to support their own growth via photosynthesis. With this in mind, how do you think changes in temperature will influence the rate of aerobic cellular respiration in the different types of bean seeds (germinating and ungerminated)?

Resources

Risk assessments, lab tech notes, and answers are available online.

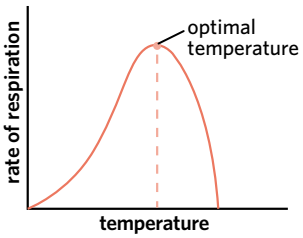


Figure 2 The effect of temperature on the rate of cellular respiration

AIM

To investigate the relationship between temperature and the rate of aerobic cellular respiration in germinating bean seeds.

MATERIALS

- 4 × 250 mL conical flasks
- 4 × rubber stoppers with associated delivery tubing
- 4 × test tubes
- 4 × trays to hold ice or water
- water (room temperature and 60 °C)
- 1 × hot plate or kettle
- 1 × thermometer
- ice
- paper towel
- cotton wool buds
- bean seeds (ungerminated and germinated)
- universal indicator
- stopwatch timer

METHOD

Part A: Preparing the bean seeds

1. Working in small groups, gather 30 germinated bean seeds and allow them to soak overnight in a conical flask (or multiple
if necessary).
2. Drain the conical flask(s) and cover the bean seeds with a wet paper towel for 48 hours. This will allow the bean seeds to germinate.
3. Select groups of 10 germinated bean seeds and place them on top of a layer of cotton wool buds in conical flasks labeled "1G – ice", "2G – RT" and "3G – 60 °C".
4. Prepare a fourth conical flask containing a further 10 ungerminated bean seeds. Label this conical flask as "4UG".

Part B: Analysing aerobic cellular respiration

5. Heat water to 60 °C using the hot plate and thermometer.
6. Prepare trays containing an ice bath, room temperature (RT) water and 60 °C water. Place inside each tray the conical flasks labeled "1G – ice," "2G – RT," and "3G – 60 °C" inside each tray respectively. For the 60 °C water bath tray, continue to maintain the heat of the tray with the hot plate and monitor it carefully to ensure a stable temperature across the duration of the experiment.
7. Prepare and label four test tubes in the same manner as the conical flasks in Step 3. Add deionised water to each test tube in addition to five drops of universal indicator.
8. Place rubber stoppers over each conical flask and connect the delivery tube to the corresponding test tube solution, including for the conical flask labeled "4UG". An example of how the completed setup should look is shown in Figure 5.
9. Allow each conical flask and connected test tube to sit for 45 minutes.
10. After 45 minutes, observe and record the colour of the universal indicator solution in each of the four test tubes. Use this information and Figure 4 to infer and record the approximate pH levels of the four test tube solutions.

11 Record each of these observations in Table 1

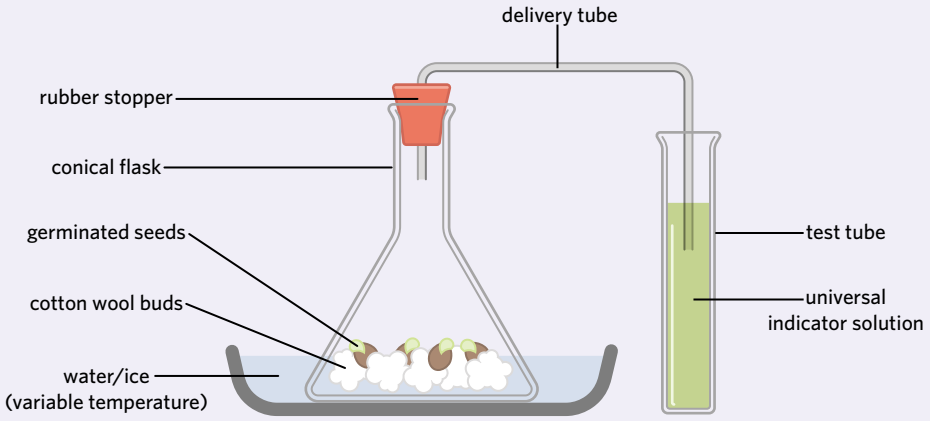


Figure 5 Experimental setup used for Part B

RESULTS

Table 1 The colour and approximate pH of the universal indicator solution in each test tube after 45 minutes

	1G – ice	2G – RT	3G – 60 °C	4UG
Colour of the UI solution				
Approximate pH				

DISCUSSION QUESTIONS

1. State your hypothesis for this experiment. Identify whether your results support your chosen hypothesis.
2. Identify the dependent and independent variables in this experiment.
3. State the products of aerobic cellular respiration.
4. In an aerobic environment, which organelle allows the maximum amount of ATP to be obtained from one molecule of glucose?
5. Consider the following statement: "As the temperature of a cell's environment increases, the rate of aerobic cellular respiration will increase due to enzyme activity increasing." Evaluate the accuracy of this statement.
6. Identify which group of bean seeds experienced the greatest rate of aerobic cellular respiration in your experiment. Explain your reasoning, making sure to justify your answer with reference to the pH and carbon dioxide production.
7. Ungerminated and early germinating bean seeds are unable to photosynthesise, and so do not consume carbon dioxide as part of their metabolic processes. Explain whether the varying levels of aerobic cellular respiration due to temperature in a photosynthetic plant could be measured using this experimental setup.
8. Identify any possible errors that may have affected your results. Classify any error(s) as a personal, systematic, or random error.
9. Identify any uncontrolled variables in this experiment, and suggest how they may have affected your results. Then, explain how you could change the method to deal with these uncontrolled variables.
10. Considering your method, what steps could you add in or modify to increase the precision of your experiment?
11. Did any of the results surprise you? If so, why? What could be a possible explanation for any irregular or surprising results?

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- whether the aim was achieved by referring to the results
- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

7.1 BLOOD TYPING

Scientific investigation type: Classification and identification

This experiment relates to Chapter 7: Dealing with disease

INTRODUCTION

There are a number of different antigens found on the surface of human red blood cells (RBCs). Two of the most important are the ABO antigens and the Rhesus factor (RhD) antigen, which are crucial in determining an individual's blood type.

ABO Blood Groups

The ABO blood group system refers to a single class of antigens found on the surface of human red blood cells.

- Type A individuals display the A antigen on the surface of their RBCs.
- Type B individuals display the B antigen on the surface of their RBCs.
- Type AB individuals display both the A and B antigens on the surface of their RBCs.
- Type O individuals display neither the A nor B antigens on the surface of their RBCs.

Just like any antigen, red blood cell antigens are recognised by the immune system and can stimulate the production of antibodies. For example, an individual with type A antigens will have anti-B antibodies in their plasma. If they are transfused blood that is type B, the anti-B antibodies in their blood will agglutinate the introduced type B red blood cells. Agglutination involves the clumping together of blood cells. The following diagram shows the antigens and antibodies present in each blood type.

	Type O	Type A	Type B	Type AB
Red blood cell type				
Antibodies in plasma	 anti-A + anti-B	 anti-B	 anti-A	none
Antigens on red blood cells	none	antigen A	antigen B	 antigen A + antigen B

Figure 1 Antigens and antibodies present in each blood type

Rhesus Blood type

The Rhesus antigen is another antigen involved in the classification of blood types and is controlled by a gene which is either activated or inactivated. Individuals can either be Rhesus positive (Rh+) when the Rhesus antigen is present or Rhesus negative (Rh-) when the Rhesus antigen is absent. A patient who is Rh+ can receive a transfusion of Rh- blood because Rh- blood lacks the RhD antigen so it will not trigger an immune response in the patient. However, Rh+ blood cannot be given to an Rh- patient as the RhD antigen is foreign to the patient and will cause an antibody response.

When we combine the ABO and Rhesus blood type systems, this means that there are eight possible blood types:

- A+
- A–
- B+
- B–
- O+
- O–
- AB+
- AB–

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To determine the blood type of four unknown samples.

MATERIALS

There are many different blood typing kits which can be used to conduct this experiment. Most of these kits will contain four different samples of synthetic blood which each represent one of the different blood types, as well as anti-A, anti-B, and anti-D antibody serums.

- sample 1 synthetic blood
- sample 2 synthetic blood
- sample 3 synthetic blood
- sample 4 synthetic blood
- synthetic anti-A serum (blue)
- synthetic anti-B serum (yellow)
- synthetic anti-D (anti-RhD) serum (white)
- paddle pop sticks
- microscope slides
- pipettes

METHOD

1. Transfer two drops of Sample 1 synthetic blood into three different areas on a microscope slide (Figure 2).
2. Transfer one drop of the anti-A serum into the first area on the microscope slide.
3. Repeat Step 2 for the anti-B serum and anti-D serum for the second and third areas.
4. Using different paddle pop sticks, mix each of the three areas and observe any changes. If agglutination occurs, the mixture will lose its smooth consistency and become granular, indicating a reaction between the antibody serum and the synthetic blood.
5. Repeat steps 1–4 using Samples 2–4 of synthetic blood, carefully rinsing and drying the microscopic slide after each replicate to prevent contamination.
6. Record your results in Table 1, indicating whether agglutination occurred or not. Use your findings to determine the blood type of each sample.

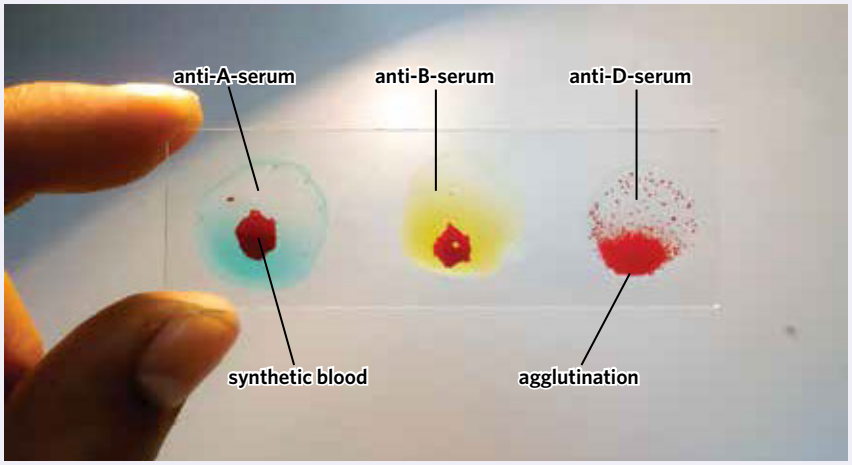


Image: Saiful52/Shutterstock.com

Figure 2 Antigens and antibodies present in each blood type

RESULTS

Table 1 Observation of experimental results

Antibody serum	Sample 1	Sample 2	Sample 3	Sample 4
Anti-A				
Anti-B				
Anti-D				
Blood type				

DISCUSSION QUESTIONS

1. Describe the purpose of self-antigens.
2. Describe the process of agglutination by antibodies.
3. Describe how the immune system can initiate an adaptive immune response against a foreign pathogen.
4. Explain why a person with AB+ blood can receive a blood transfusion of A– blood.
5. Explain why a person with A– blood cannot receive a transfusion of AB+ blood.
6. List the blood types that can be safely given to a person who has AB– blood.
7. Explain why O– blood is in highest demand by blood banks.
8. Considering your method, what steps could you add in or modify to increase the accuracy and precision of your experiment?
9. Identify any possible errors that may have affected your results. Be sure to state whether it was a personal, systematic, or random error.

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

8.1 BACK OFF BACTERIA

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 8: Immunity

INTRODUCTION

Bacteria are a group of single-celled, prokaryotic organisms that commonly live symbiotically in, and on, our bodies. When a species of bacteria is introduced to an area that it doesn't normally occupy, the impacts can be large. If a foreign species of bacteria is introduced to the human body via an injury or the eating of a not-so-fresh oyster, bacteria can make humans very unwell. When this occurs, the bacteria is said to be 'pathogenic', and we describe a person with an invasion of pathogenic bacteria as having an 'infection'.

Prior to the 20th century, people frequently died from such bacterial infections. In 1928, Alexander Fleming discovered modern-day penicillin, a drug derived from *Penicillium* moulds. Penicillin is a type of antibiotic, a medication with antimicrobial effects against bacteria. Unfortunately for us, not all antibiotics work against all bacteria. If a person presents to a doctor with an infection it is common practice to prescribe the patient with a broad-spectrum antibiotic – that is, an antibiotic that is known to affect a wide variety of bacteria. If this broad-spectrum drug does not resolve the infection, further testing can be undertaken in order to find out which antibiotics the persistent bacteria will be sensitive to.

What exactly does this testing involve? You're about to find out! In this practical investigation, you will be presented with samples of bacteria obtained from two different patients – Patient A, who has a nasty urinary tract infection (UTI) caused by *Escherichia coli*; and Patient B, who has developed a *Staphylococcus epidermidis* infection that needs treatment as soon as possible. It's up to you to determine which antibiotic each patient should be prescribed with!

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To investigate the effect that antibiotics have on bacterial growth and survival.

MATERIALS

- live broth cultures of *Escherichia coli* and *Staphylococcus epidermidis*
- 3 × sterile nutrient agar plates
- 2 × sterile Mastrings (or equivalent antimicrobial susceptibility disc)
- 2 × sterile 5 mL pipettes
- forceps
- 1 × Bunsen burner
- sticky tape
- 1 × ruler
- 1 × marking pen
- 1 × incubator set to 37 °C
- lab coats
- safety glasses
- gloves

METHOD

Part A: preparation of agar plates

1. Label the underside of the three agar plates with the marking pen. Label one plate 'Patient A', another plate 'Patient B', and the third plate 'No patient'. Also, label each plate with the date and your initials.
2. Use one of the pipettes to remove 0.1 mL of the live *Escherichia coli* broth culture from the container and apply it to the surface of the agar plate labelled 'Patient A'. Dispose of the pipette when done.
3. Replace the lid, and spread the broth evenly across the surface of the agar by gently swirling the plate in your hands ensuring that you hold the lid in place to prevent any spillage.
4. Using a fresh pipette, remove 0.1 mL of the live *Staphylococcus epidermidis* broth culture from the container and apply it to the surface of the agar plate labelled 'Patient B'. Dispose of the pipette when done.

5 Once again, replace the lid and spread the broth evenly across the surface of the agar by gently swirling the plate in your hands. Leave both 'Patient A' and 'Patient B' plates on the bench for two minutes to allow time for the bacteria to penetrate into the nutrient agar.

6 Hold the end of the forceps in the flame of the Bunsen burner until they are glowing red, then allow them to cool.

7 Using the forceps, place one Mastring onto the centre of the 'Patient A' plate.

8 Repeat steps 6–7, this time placing one Mastring onto the centre of the 'Patient B' plate and the 'No patient' plate.

9 Mastrings are used to determine the sensitivity of an organism to a variety of antibiotics. Each arm of the ring is impregnated with a different antibiotic that is identifiable either by a code or colour. Ask your teacher to examine the box the Mastrings came in to see which antibiotics your Mastring has in it.

10 Place the lids on all three plates and seal them with sticky tape. Place the plates in an incubator set to 37 °C for 24–36 hours (your teacher will determine the exact time frame).

Part B: measuring the zone of inhibition

11 After incubating your dish for the time arranged by your teacher, carefully examine the growth of bacteria on each plate. Note the pattern of growth of each bacteria for each type of antibiotic in the Mastring. Use Figure 2 in the Results section to sketch what you see in each Petri dish.

12 Measure the zone of inhibition of each antibiotic using a ruler. Do so by measuring the diameter of the area around a disc where no bacteria has grown (Figure 1). This reflects the degree to which the bacteria on the dish are sensitive to the antibiotic – antibiotics with a larger zone of inhibition are more effective against that specific type of bacteria than antibiotics with smaller, or no, zone of inhibition. Record your measurements in Table 1.

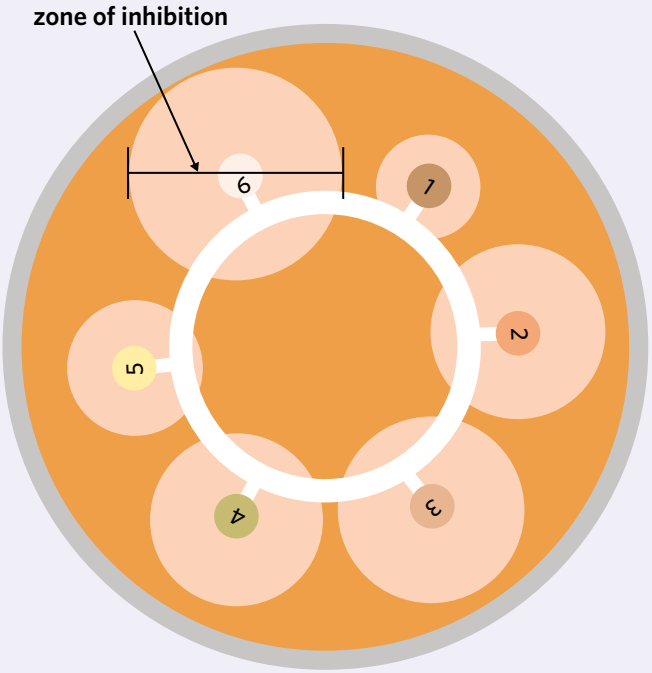


Figure 1 A Petri dish with a Mastring showing the zone of inhibition for each antibiotic

RESULTS

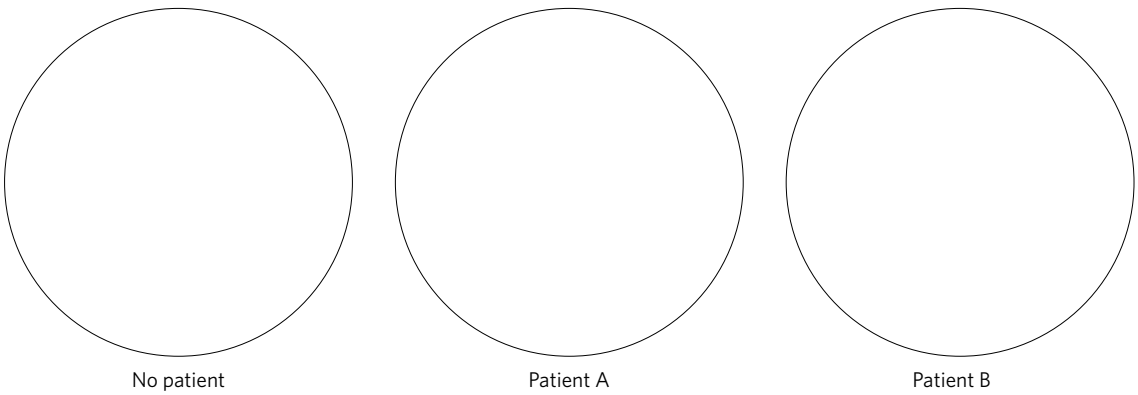


Figure 2 Growth of bacteria after incubation

Table 1 Zone of inhibition measurements

Antibiotic	Diameter of zone of inhibition (mm)		
	No patient	Patient A	Patient B

DISCUSSION QUESTIONS

1. Identify three differences between prokaryotic cells such as bacteria and eukaryotic cells such as blood cells or nerve cells.
2. Describe the difference between bacteriostatic and bactericidal antibiotics.
3. Identify one key target of antibiotics.
4. Propose which antibiotic should be prescribed to each patient.
5. Based on the results in this experiment, which antibiotic would you prescribe to a patient if you were unable to conduct tests to determine the organism causing their infection? Justify your answer.
6. Explain why it is important to heat the forceps before using them.
7. State the purpose of the 'No patient' dish in this experiment.
8. Identify the independent and dependent variables in this experiment.
9. Considering your method, what steps could you add in or modify to increase the accuracy and precision of your experiment?
10. Identify any controlled variables in this experiment.

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- whether the aim was achieved by referring to the results
- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

8.2 SHAKE 'N' INCUBATE

Scientific investigation type: Controlled experiment

This experiment relates to Chapter 8: Immunity

INTRODUCTION

The surface of our body is covered in bacteria, particularly our hands. Fortunately, these... handy... bacteria don't usually cause us harm. However, occasionally a pathogenic bacteria that can cause illness finds its way onto our hands and, from here, it may enter into our body. We frequently touch things in the environment that are covered in pathogens, and then inadvertently introduce these into our bodies by touching our mouth, eyes, nose, or food. One of the worst sources of hand contamination is the bathroom – it might horrify you to know that a study in America found that only 83% of people washed their hands after using the toilet. Think of that next time you go to shake someone's hand!

It has been proven that frequent handwashing is effective in combating a wide variety of diseases. In one study it was shown that washing hands with soap and water reduces the incidence of diarrhoeal diseases by approximately 47%. The study also found that interventions encouraging handwashing could save up to a million lives that would otherwise have been lost due to diarrhoea. Furthermore, handwashing also appears to reduce the incidence of respiratory infections by approximately 45%.

There are a wide variety of products that claim to 'clean' our hands of bacteria – but which is the best? In this scientific investigation, you will explore which common skin cleaning agent – water, soap, antibacterial soap, or hand sanitiser – is most effective by using a yeast organism to model pathogenic bacteria.

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To determine the effectiveness of different skin cleaning agents.

MATERIALS

- 12 × poured sterile nutrient agar plates
- 1 × packet of yeast
- 1 × 250 mL beaker
- 1 × measuring spoon
- sugar
- 1 × marking pen
- 1 × sterile pipette
- 12 × sterile cotton swabs
- gloves
- sticky tape
- 10 mL regular hand soap
- 10 mL antibacterial hand soap
- 10 mL liquid hand sanitiser
- 1 × incubator set to 37 °C
- 10% bleach solution
- lab coat and goggles/safety glasses

METHOD

Part A: preparation of agar plates

1. Get into groups of 5 and assign each person to one of the following roles:
   - Person 1 – the 'disease spreader'
   - Person 2 – washing with water
   - Person 3 – washing with regular soap
   - Person 4 – washing with antibacterial soap
   - Person 5 – washing with liquid hand sanitiser
2. Measure 230 mL of warm water into a 250 mL beaker. Add one teaspoon of sugar and the yeast packet and gently stir the solution to dissolve all of the components. Leave for a few minutes – the solution should start to foam.
3. While the solution is resting, have all the people who will be washing their hands (Persons 2–5) grab three agar plates each. Each person should label the base of one of their plates 'pre-exposure', another plate 'post-exposure', and their third plate 'post-wash'. Have each person also label their plates with the method of handwashing they will be using, as well as their initials and the date.
4. Persons 2–5 should remove the lid of their 'pre-exposure' plate. Holding a sterile cotton swab in their left hand, have them each swab their right hand by rubbing the swab over their palm and fingers multiple times. Each person should then gently rub the swab on the surface of their 'pre-exposure' plate, ensuring they don't break the surface of the agar. Apply the lid to each plate and apply sticky tape to seal them.
5. Have Person 1 apply a glove to their left hand. Use a pipette to apply 8 drops of the yeast solution to the palm of the gloved hand, and then have Person 1 rub their hands together to spread the yeast over their right hand. Remove and dispose of the glove.
6. Have Person 1 shake hands with Person 2's right hand for 10 seconds straight.
7. Swab the surface of Person 2's right hand with a sterile cotton swab, and then gently rub the swab on the surface of Person 2's 'post-exposure' plate. Replace the lid of the plate and seal it with sticky tape.
8. Have Person 2 wash their hands with water from the tap (but no soap), scrubbing for 20 seconds. Allow their hands to 'air dry' without touching anything.
9. Swab the surface of Person 2's right hand once again with a sterile cotton swab, this time rubbing the swab on the surface of Person 2's 'post-wash' plate. Replace the lid of the plate and seal it with sticky tape.
10. Repeat steps 5–9 twice for Persons 3 and 4, with Person 3 washing their hands for 20 seconds with normal hand soap and water and Person 4 washing their hands for 20 seconds with antibacterial soap and water.
11. Repeat steps 5–9 with Person 5. Have them apply the hand sanitiser to their hands and rub them until they are dry.
12. Incubate all of the sealed dishes at 37 °C for 24–36 hours (your teacher will determine the exact time frame).

Part B: examination of plates

13. After incubating your dishes for the time arranged by your teacher, carefully examine the growth on each plate. Observe the level of bacterial growth and count the number of colonies that grew on the 'pre-exposure', 'post-exposure' and 'post-wash' plates and record the results in Table 1 using the following notation. Ensure that you also record the number of colonies, if countable:
   - 0 = no growth
   - + = a small amount of growth (<5 colonies)
   - ++ = moderate growth (6–20 colonies)
   - +++ = heavy growth (20–50 colonies)
   - ++++ = extremely heavy growth, no individual colonies, growth is a 'lawn' and therefore uncountable
14. Once you have obtained your results, don some gloves, a lab coat, and safety goggles and carefully pour 5 mL of 10% bleach solution onto each plate, making sure to cover the entire surface of the plate. Incubate the dishes for 20 minutes, then pour the contents of each down the sink with running water. Seal each plate again and dispose of them in the bin provided by your teacher.

RESULTS

Table 1 Growth on plates

Treatment	Swab	Amount of growth and number of colonies
Water only	Pre-exposure	
	Post-exposure	
	Post-wash	
Normal soap	Pre-exposure	
	Post-exposure	
	Post-wash	
Antibacterial soap	Pre-exposure	
	Post-exposure	
	Post-wash	
Hand sanitiser	Pre-exposure	
	Post-exposure	
	Post-wash	

Use the data from Table 1 to create a graph of your results displaying the changes to bacterial growth under each treatment type.

DISCUSSION QUESTIONS

1. Bacteria and yeast are types of cellular pathogens. Describe what is meant by the term 'cellular pathogen'.
2. Many pathogenic bacteria are spread via the faecal-oral route. Explain how transmission via the faecal-oral route occurs.
3. Describe the difference between disinfectant and antiseptic agents.
4. Explain the purpose of the 'pre-exposure' swabs in this experiment.
5. What evidence from this experiment suggests that pathogens can be transmitted by hand-to-hand contact? Justify your answer using the data you obtained in your experiment.
6. Which handwashing agent was the most effective at removing the 'pathogen' from your participants' hands? Justify your answer using the data you obtained in your experiment.
7. Identify the independent and dependent variables in this experiment.
8. Identify a possible error that may have affected your results. Be sure to state whether it was a personal, systematic, or random error, and suggest how the error could be avoided in a future repetition of this experiment.
9. Some people think that washing your hands with hot water is more effective at removing pathogens than washing your hands with cold water. Design an experiment to test the impact water temperature has on the effectiveness of hand washing. Provide details of the following aspects of your experiment:
   - **a** Will you address replication? If so, how?
   - **b** What are the independent and dependent variables?
   - **c** What is the control group?
   - **d** What is the hypothesis?
   - **e** How will errors be minimised?
   - **f** How will you ensure that the sample is large and randomly collected?

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- whether the aim was achieved by referring to the results
- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

9.1 NATURAL SELECTION IN MICE

Scientific investigation type: Simulation

This experiment relates to Chapter 9: How are species related over time?

INTRODUCTION

Natural selection is the process by which organisms that are better adapted to their environment have an increased chance of surviving and passing their alleles on to the next generation. This process relies on the variation in alleles within the original population so that advantageous alleles, which allow individuals within the population to better survive in their environment, are present. In other words, individuals who possess the advantageous alleles within their specific environment are conferred a selective advantage.

The original sources of these advantageous alleles are typically mutations, which involve changes to the DNA sequences of individuals. Without a high degree of variation, the chances of advantageous alleles being present decrease, rendering a population vulnerable to extinction due to an inability to adapt to new environmental selection pressures.

Mice

The deer mouse (*Peromyscus maniculatus*) is a species of mouse which is found throughout North America, primarily living in woodlands, but also in desert areas (Figure 1). In these desert areas, the colour of the sand can either be dark-brown or light-brown.

In this simulation, there is a population of 30 mice living in light-brown coloured sand. Each year, they mate once at random and each pair produces one offspring. After each mating season, there are a range of different predators that prey upon the deer mice, including snakes, hawks (Figure 2), and owls, eating one-third of the population (15 mice). Therefore, at the end of each year, there will be a population of 30 mice left.

There are two types of deer mice including dark-brown mice and light-brown mice. The dark-brown colour (B) is dominant to the light-brown colour (b), with dark-brown mice being more obvious in the light-brown sand when compared to the light-brown mice. Therefore, the dark-brown mice are more easily seen by predators in light-brown sand.

AIM

To simulate natural selection and observe some of its consequences within a population of mice.

Resources

Risk assessments, lab tech notes, and answers are available online.

ImageL: Karel Bock/Shutterstock.com

Figure 1 A dark-brown deer mouse (*Peromyscus maniculatus*) in light-brown sand

ImageL: Justin Buchli/Shutterstock.com

Figure 2 A predator of deer mice – a hawk

MATERIALS

- six-sided die
- set of 30 mouse cards with 10 BB (dark-brown), 10 Bb (dark-brown), and 10 bb (light-brown)
- 20 extra mouse cards (a mixture of genotypes to be used as offspring)

METHOD

Part A: Light-brown sand

1 Shuffle the 30 mouse cards to simulate random mating and deal the cards into 15 pairs, placing one on top of another (don't worry about the sex of the mice).

2 Determine the genotypes of the offspring as follows (use the six-sided die when required):
   - BB × BB = BB
   - bb × bb = bb
   - BB × bb = Bb
   - BB × Bb = roll the die. Even number is BB; odd number is Bb
   - Bb × Bb = roll the die. One is BB; two or three is Bb; four is bb; five or six roll again
   - Bb × bb = roll the die. Even number is Bb; odd number is bb.

3 Once you have finished mating the mice as described above, roll the die 15 times. Each time you roll the die:
   - remove a dark-brown mouse if a one, two, three, four, or five is rolled
   - remove a light-brown mouse if a six is rolled.

4 Repeat the steps above another four times to simulate five years. Record your results in Table 1.

Part B: Dark-brown sand

Nearby in another location, a separate population occupies an area of land covered by dark-brown sand instead of light-brown sand. In this region, light-brown mice are more easily seen by predators.

5 Starting a new population (10 BB, 10 Bb, and 10 bb), repeat steps 1–4 of procedure A and record your results in Table 2. However, this time when you roll the die:
   - remove a dark-brown mouse if a one is rolled.
   - remove a light-brown mouse if a two, three, four, five, or six is rolled.

RESULTS

Table 1 Natural selection of mice within light-brown sand

Genotype	Number of mice					
	Starting population	Year 1	Year 2	Year 3	Year 4	Year 5
BB (dark)	10					
Bb (dark)	10					
bb (light)	10					
Total	30	30	30	30	30	30

Table 2 Natural selection of mice within dark-brown sand

Genotype	Number of mice					
	Starting population	Year 1	Year 2	Year 3	Year 4	Year 5
BB (dark)	10					
Bb (dark)	10					
bb (light)	10					
Total	30	30	30	30	30	30

DISCUSSION QUESTIONS

1 Describe the process of natural selection.
2 Explain whether natural selection increases or decreases genetic diversity.
3 Explain the importance of genetic diversity with respect to the survivability of a species.
4 Identify the environmental selection pressure(s) acting on the mice in this simulation.
5 Explain why the number of light-brown mice increased in the light-brown coloured habitat.
6 Explain why the number of light-brown mice decreased in the dark-brown habitat.
7 Identify the independent and dependent variables in the simulation.

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- potential ways to improve the simulation
- broader implications of your research or further areas of exploration that stem from your findings.

9.2 SIMULATING NATURAL SELECTION

Scientific investigation type: Simulation

This experiment relates to Chapter 9: How are species related over time?

INTRODUCTION

Species evolve and change over time via the process of natural selection. According to the theory of natural selection, organisms that are better adapted to their environment have an increased chance of surviving environmental selection pressures and passing on their alleles to the next generation. Consequently, in subsequent generations, there will be a higher frequency of organisms that have a selective advantage against that selection pressure due to possessing favourable alleles.

Evolution via natural selection is perhaps the most unifying principle in biology and understanding how it works in practise is incredibly important. The problem is that the process of natural selection takes a long time and occurs over several generations, meaning that it can sometimes be difficult to study and visualise. To counteract this, we can make use of simulations in order to study the mechanism of natural selection on a smaller time-scale.

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To observe natural selection using a simulation.

MATERIALS

- a computer, laptop, iPad, or any other device connected to the internet
- the following simulation: phet.colorado.edu/sims/html/natural-selection
  - Once you open the simulation, select the 'Lab' option.

METHOD

For this exercise, we will divide the method into eight separate simulations to test the action of different selection pressures on different populations. Before starting any of the simulations, make sure to familiarise yourself with the software (Figure 1).

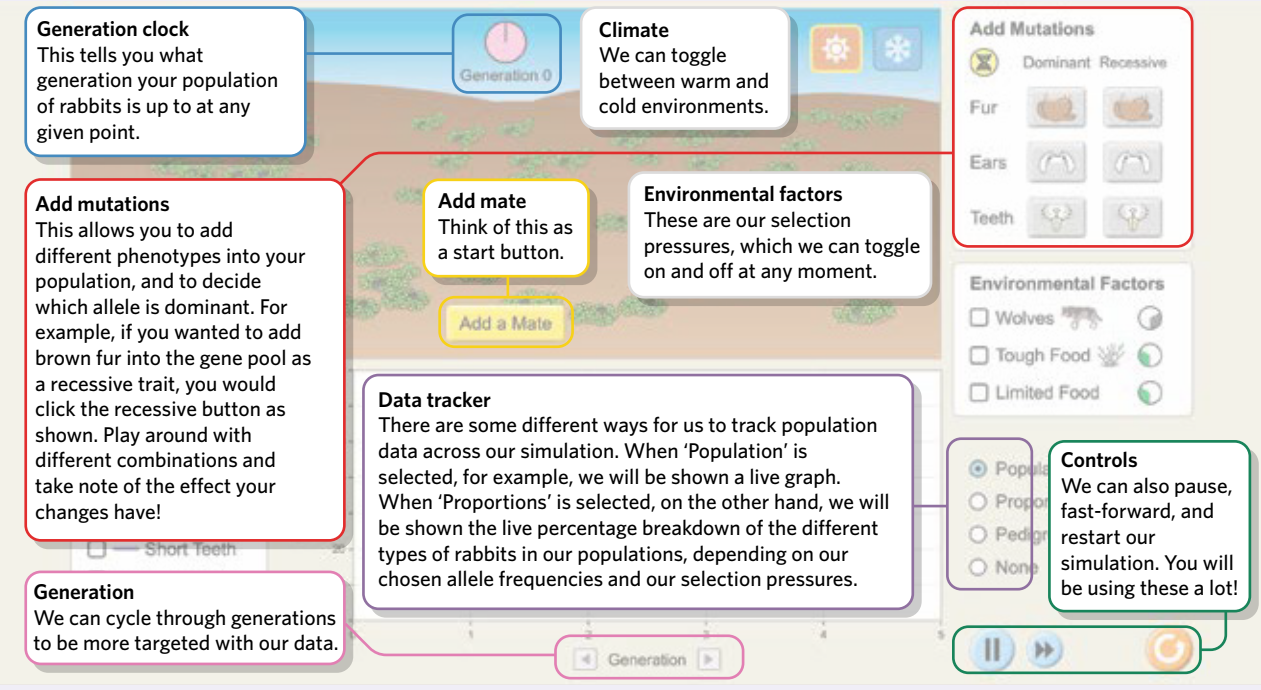


Image: PhET Interactive Simulations, phet.colorado.edu/sims/html/natural-selection/latest/natural-selection_en.html

Figure 1 This is the simulation we will be running. Take time to familiarise yourself with the different variations and all of the controls – things can move quite quickly when we get started!

Before beginning the simulation sets, please ensure the conditions are set to default – warm climate, no mutations and no environmental factors. If you have been playing around with different settings, clicking the reset button will bring you back to this default.

Simulation 1

1. With conditions at default, click 'Add a Mate' to start your simulation.
2. Hold down the fast-forward button and record how many generations it takes for the population of white rabbits to overrun the planet in Table 1.

Simulation 2

1. Hit the reset button.
2. Before beginning the simulation, select Limited Food as an environmental factor.
3. Click 'Add a Mate' to start your simulation.
4. Hold down the fast-forward button and record your results in Table 1.

Simulation 3

1. Hit the reset button.
2. Click 'Add a Mate' to start your simulation.
3. At the start of Generation 5, click pause to stop the simulation.
4. Select Tough Food as an environmental factor and then resume the simulation.
5. Hold down the fast-forward button and record your results in Table 1.

Simulation 4

1. Hit the reset button.
2. Before beginning the simulation, select the allele for Long Teeth and make it dominant. The simulation will say 'Mutation Coming'.
3. Click 'Add a Mate' to start your simulation.
4. At the start of Generation 5, click pause to stop the simulation.
5. Select Tough Food as an environmental factor and then resume the simulation.
6. Hold down the fast-forward button and record your results in Table 1.

Simulation 5

1. Hit the reset button.
2. Before beginning the simulation, select the allele for Long Teeth and make it recessive. The simulation will say 'Mutation Coming'.
3. Click 'Add a Mate' to start your simulation.
4. At the start of Generation 5, click pause to stop the simulation.
5. Select Tough Food as an environmental factor and then resume the simulation.
6. Hold down the fast-forward button and record your results in Table 1.

Simulation 6

1. Hit the reset button to start the simulation over again.
2. Click 'Add a Mate' to start your simulation.
3. At the beginning of Generation 5, click pause to stop the simulation.
4. Select Wolves as an environmental factor and then resume the simulation.
5. Hold down the fast-forward button and record your results in Table 1.

Simulation 7

1. Hit the reset button to start the simulation over again.
2. Select Brown Fur as a recessive trait, while keeping all other conditions as default.
3. Click 'Add a Mate' to start your simulation.
4. At the beginning of Generation 5, click pause to stop the simulation.
5. Select Wolves as an environmental factor and then resume the simulation.
6. Hold down the fast-forward button and record your results in Table 1.

Simulation 8

1. Hit the reset button to start the simulation over again.
2. Select Brown Fur as a recessive trait, while keeping all other conditions as default.
3. Change the climate to Cold.
4. Click 'Add a Mate' to start your simulation.
5. At the beginning of Generation 5, click pause to stop the simulation.
6. Select Wolves as an environmental factor and then resume the simulation.
7. Hold down the fast-forward button and record your results in Table 1.

RESULTS

Table 1 The results of each simulation

Simulation	Climate	Mutations	Selection pressure	Result
1	Warm	None	None	The rabbits override the planet in _____ generations.
2	Warm	None	Limited food	
3	Warm	None	Tough Food introduced after Generation 5	
4	Warm	Long Teeth (dominant)	Tough Food introduced after Generation 5	
5	Warm	Long Teeth (recessive)	Tough Food introduced after Generation 5	
6	Warm	None	Wolves introduced after Generation 5	
7	Warm	Brown Fur (recessive)	Wolves introduced after Generation 5	
8	Warm	Brown Fur (recessive)	Wolves introduced after Generation 5	

DISCUSSION QUESTIONS

1. Define what is meant by the term 'selection pressure'.
2. Do selection pressures act the same on all individuals of a population? Why or why not?
3. Explain the purpose of Simulation 1.
4. Explain the results seen in Simulation 2.
5. Despite sharing the same climate and selection pressure, Simulation 3 and 4 show very different results. Suggest why we see such differences in these two simulations.
6. State why the population took longer to override the planet in Simulation 5 compared to Simulation 4.
7. Consider Simulation 5 in detail. We see the rabbit population grow quickly over the first two generations. At the end of Generation 2, you should notice that all of the rabbits have short teeth, despite us choosing long teeth as a recessive trait. By Generations 3 (or 4), however, you should see a small proportion of rabbits with long teeth in your populations.
   - **a** How is it possible for rabbits with long teeth to show up in our population by Generation 3, despite the absence of any rabbits with long teeth in earlier generations?
   - **b** Select one of the rabbits with long teeth in your population by clicking on them. Using the Pedigree tool, identify the genotypes of their parents.
8. Suggest one reason why brown fur appears to confer a selective advantage in Simulation 7.

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

10.1 AMPHIBIAN PHYLOGENY

Scientific investigation type: Classification and identification

This experiment relates to Chapter 10: How we are related

INTRODUCTION

Determining the relatedness between different species can be done in a multitude of ways. One such method is through the use of genome-sequencing and comparing DNA and amino acid sequences. The similarities and differences between separate species obtained from these methods allow us to create phylogenetic trees, which can visually demonstrate potential degrees of relatedness and evolutionary divergences between the species under examination.

In this exercise, you will be provided with some information about different amphibian species. You will then be asked to use this information to complete a phylogenetic tree and infer different degrees of relatedness between the species. Your phylogenetic tree will serve as a visual representation of possible evolutionary relationships between the species of the amphibian class.

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To understand the evolutionary relationships between a group of amphibian species by creating a phylogenetic tree.

MATERIALS

Table 1 Species information for seven seperate amphibian species

Scientific name	Order name	Family name	Length of genome assembly (Mb)	Guanine-cytosine content	Protein-coding genes
Bufo bufo	Anura	*Bufonidae*	5,044.74	44.52	38,109
Engystomops pustulosus	Anura	*Leptodactylidae*	2,555.52	41.97	48,168
Microcaecilia unicolor	Gymnophiona	*Caeciliidae*	4,685.94	44.07	37,109
Ambystoma mexicanum	Urodela	*Ambystomatidae*	28,206.9	40.30	unlisted
Rhinatrema bivittatum	Gymnophiona	*Rhinatrematidae*	5,319.24	44.44	49,282
Xenopus laevis	Anura	*Pipidae*	2,742.47	39.35	72,913
Xenopus tropicalis	Anura	*Pipidae*	1,451.3	40.75	45,099

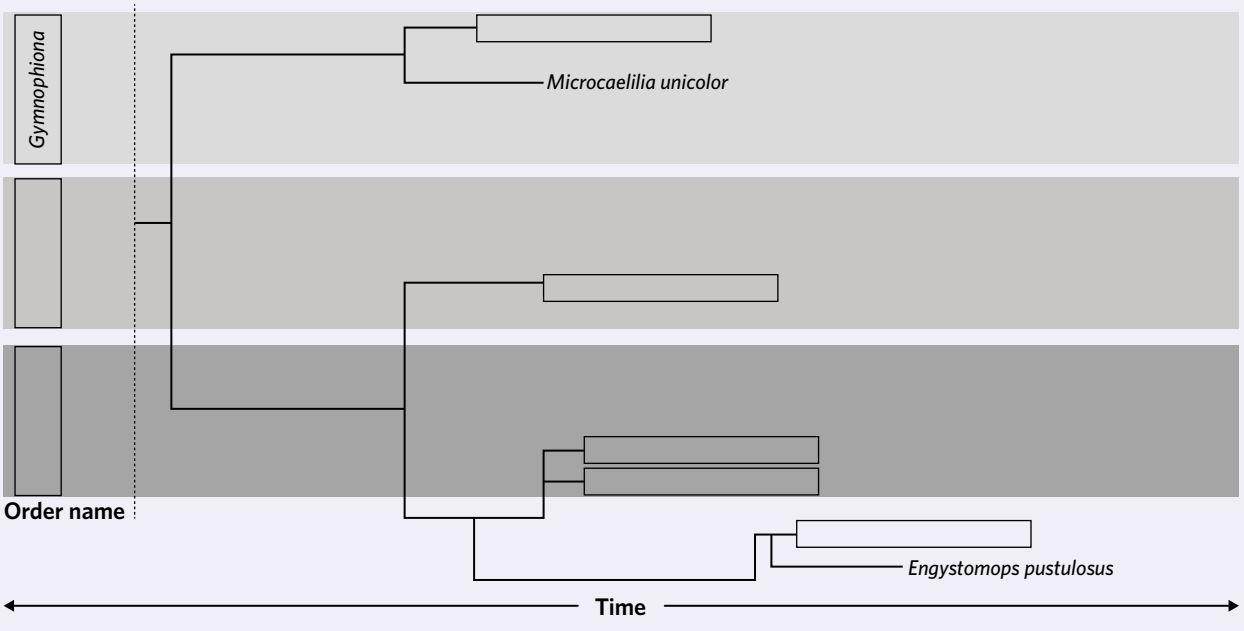


Figure 1 Incomplete phylogeny showing relatedness between the amphibian species in Table 1. Note: (1) colour segmentation is used to demonstrate separate orders, and (2) that this phylogeny is highly simplified and may not accurately represent true evolutionary relationships.

METHOD

Using Table 1, fill in the blank spaces in the phylogenetic tree provided (Figure 1).

Either write directly in your textbook or recreate the phylogeny in your workbook.

RESULTS

DISCUSSION QUESTIONS

1 Define what is meant by a phylogenetic tree.

2 Which of the seven species of amphibians evolved first?

3 According to the phylogenetic tree, which two species share a direct ancestor-descendant relationship?

4 Which species' genome contains the highest thymine-adenine content? Justify your response.

5 With reference to your phylogenetic tree, explain whether *Ambystomatidae* is more closely related to *Pipidae* or *Caeciliidae*.

6 The unit 'Mb' refers to 'megabases', where 1 Mb is equal to 1 million nucleotides.

a Which species has the longest genome assembly according to the data used in Table 1?

b According to the information presented in Table 1, what is the approximate length, in nucleotides, of *Rhinatrema bivittatum*'s genome assembly?

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

11.1 RETRACING THE HUMAN ODYSSEY

Scientific investigation type: Classification and identification

This experiment relates to Chapter 11: Becoming human

INTRODUCTION

Over the course of chapter 11, we learned about the incredible journey our ancestors went on in the long process of becoming human. Using evidence from archaeological sites, and the genetic material gathered from ancient fossils, researchers have been able to paint a picture of our ancient migratory patterns and the trends that defined our evolutionary pathway.

Even though interpreting the fossil record can be incredibly difficult, many different methods can be used to confirm, consolidate and/or change our ever-expanding understanding of how it was that we humans came to be. In this exercise, you will cross-reference some key moments in our evolutionary past as a species against a well-defined timeline that demonstrates our path to occupying around 85% of the world's physical surface (according to some estimates). This task involves two scientific methodologies: (1) classification and identification, and (2) a simulation.

Resources

Risk assessments, lab tech notes, and answers are available online.

AIM

To classify and identify significant archaeological findings by cross-referencing them with a publicly available simulation program.

MATERIALS

For this practical, you will need:

- Access to the following website: pbslearningmedia.org/resource/interactive-human-migration-map/interactive-map/
- Table 1

Table 1 An incomplete classification table taken from key moments in the interactive timeline

Timing	Discovery	Location	Significance for *Homo Sapiens*
195 000 years ago		Ethiopia	
		Skhul Cave	Evidence of the first migratory wave of modern humans Out of Africa, as well as early signs of ritual burial
	Extreme drought		
55 000 years ago			After reaching this region, some populations moved towards Australia while others continued deeper into Northern Asia
	Sundra Land Bridge	Indonesia	
			Fossils suggest that early humans first reached Australia around this time
	Extremely well-preserved Neanderthal fossils	Croatia's Vindija Cave	Evidence of Neanderthal-human interbreeding deep into colder regions of Northwestern Europe
40 000 years ago		Siberia's Denisova Cave	
	Human artefacts		Currently the oldest known evidence of modern humans in North America

METHOD

1 Navigate to the website provided and launch the 'Interactive Human Migration Map'. A new window will open showing the beginning of the interactive timeline (Figure 1).

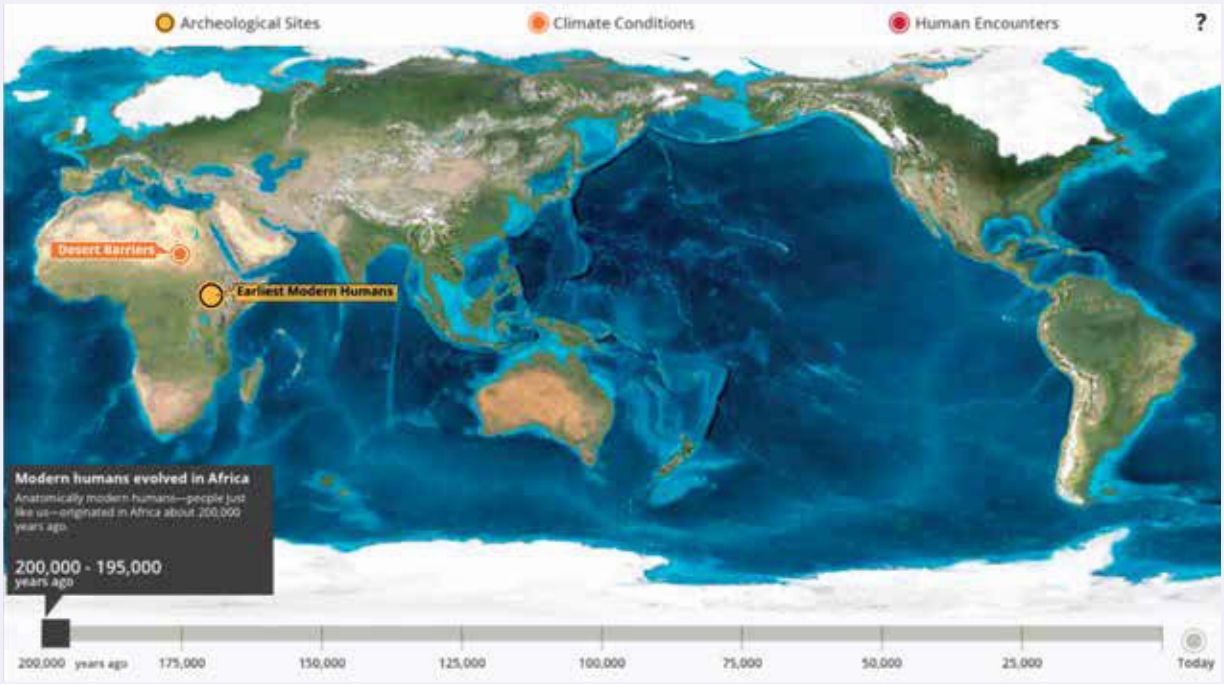


Figure 1 The interactive timeline at stage 1 (200 000 years before today). You should note the key along the top of the map, which indicates the three different coloured reference points and the corresponding information they represent.

2 Make your way through the interactive timeline from stage 1 until today, taking note of the important sites, climate conditions, and human encounters along our journey.

3 Use the information in the simulation to identify and classify each of the key moments represented in Table 1. You should be able to fill in the missing information relating to the timing, location, and significance of different discoveries.

DISCUSSION QUESTIONS

1 Define what it means to be 'human' from a biological perspective.

2 Contrast modern humans with one other species of the tribe Hominini that is shown in the timeline. In your answer, suggest one structural difference you would expect to see between the skulls of the two species.

3 Based on the information in the timeline, suggest one way in which climate conditions have influenced the migratory patterns of ancient humans.

4 According to the timeline, ancient humans ventured far north into cold arctic climates.

- **a** Identify the location and timing of the northernmost evidence of human migration as shown in this timeline.
- **b** In your answer, classify this point on the timeline as either an archeological site or a human encounter. Justify your response.
- **c** Are stone tools an example of a fossil? Justify your response with reference to the definition of a fossil.

5 With reference to your understanding of the conditions needed for fossilisation, suggest one reason why fossils are often found in ancient waterbeds or lakes.

CONCLUSION

Write a concluding paragraph to summarise your investigation. Be sure to include:

- limitations in the experiment
- potential ways to improve the experiment
- broader implications of your research or further areas of exploration that stem from your findings.

ANSWERS

1A Key science skills

Theory review questions

1 A

2 A

3 C

4 B

5 I; II; III

6 random; systematic; accuracy; precision

7 D

8 III; IV; II; I

SAC skills questions

9 B **10** C **11** C **12** B

Exam-style questions

Within lesson

13 C **14** D

15 a [The independent variable is the distance between GM and non-GM fields. The dependent variable is the percentage of seeds produced at various positions as a result of cross-pollination.[1]]

✓ ✗ I have correctly identified the independent variable and the dependent variable.[1]

b [A control group was not used in this experiment.[1]] [Control groups are not exposed to the IV. An example of a control group would be setting up two fields of non-GM crops next to each other, and measuring the percentage of seeds produced at various positions as a result of cross-pollination.[2]]

Other acceptable responses include:

- A control group could be a non-GM crop set up in an isolated space e.g. a greenhouse.

✓ ✗ I have stated that there was no control group.[1]

✓ ✗ I have outlined what a control group could look like in this experiment.[2]

✓ ✗ I have used appropriate biological terminology such as: IV, control, percentage.

c [The general trend is that the further the distance between crops, the less cross-pollination.[1]] [The most cross-pollination occurred when there was no gap between plots (10% cross-pollination at edge of crop, 2% 10 m into crop).[2]] [There was little difference between placing the crops 5 and 7 m apart (both had 1% cross-pollination at edge of plot), except the plots 7 m apart had only 0.3% cross pollination 10 m into the non-GM crop (as opposed to 0.5%).[3]]

✓ ✗ I have outlined the general trend of the data.[1]

✓ ✗ I have stated where cross-pollination was most common.[2]

✓ ✗ I have stated where cross-pollination was least common.[3]

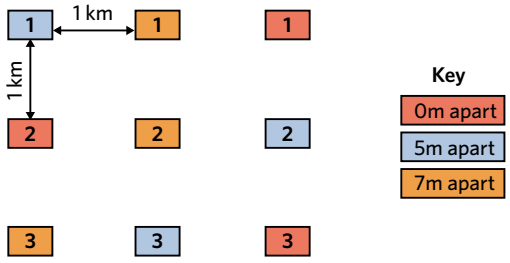
✓ ✗ I have used data from the table to support my response.

d i [Replication allows you to take a mean of a group, so outliers or results influenced by random error have less impact on your results.[1]]

✓ ✗ I have stated that replication reduces the impact of outliers and random error.[1]

✓ ✗ I have used appropriate biological terminology such as: random error, outliers, precision.

ii



[If they wished to replicate each experimental group three times, the farmers would need more plots and land.[1]] [They could set up each group a great distance (e.g. 1 km) apart from each other to ensure they are not affecting each other, and plant three of each plot type.[2]]

✓ ✗ I have explained an appropriately replicated experiment.[1]

✓ ✗ I have recognised that the replicates may affect each other, and attempted to overcome this issue in the design.[2]

e [By having the trials run at different times, different treatments may be exposed to different weather conditions (e.g. rain, light, temperature, wind).[1]] [This could reduce the accuracy of the results and be a potential uncontrolled variable.[2]]

✓ ✗ I have identified factors that may be uncontrolled over time.[1]

✓ ✗ I have stated this could make the results less accurate.[2]

f [Farmer Y is benefitting from GM crops, but Farmer X is the individual who pays for the GM crops.[1]]

Other acceptable responses include:

- Despite their initial agreement, Farmer Y is being forced to use GM crops. Even if she isn't too bothered by this, there may be effects on Farmer Y's crop of which she is not yet aware.
- If Farmer Y's crop is becoming GM, then even more farms nearby may be affected. The farmers should consider the impact of their actions on others.
- Farmer Y may still be advertising her crops as non-GM.

✓ ✗ I have identified one ethical issue.[1]

16 a [The independent variable is temperature[1]] [and the dependent variable is the percentage of nutrient agar covered by bacteria.[2]]

✓ ✗ I have stated that the temperature is the independent variable.[1]

✓ ✗ I have stated that the percentage of bacterial cover on the agar plates is the dependent variable.[2]

b [If the temperature is increased, then more bacterial growth occurs.[1]]

Other acceptable responses include:

- If temperature increases, then bacterial growth occurs more quickly.

✓ ✗ I have stated a testable hypothesis that is supported by the results.[1]

✓ ✗ I have stated how the independent variable affects the dependent variable in my hypothesis.

c [The amount of bacteria initially added to the agar plates[1]] [and the length of time kept at each temperature would need to stay constant across experimental groups.[2]]

Other acceptable responses include:

- the size of the nutrient agar plates
- the concentration of nutrient agar in the plates
- the light intensity to which the plates are exposed
- the strain of bacteria used in each sample

✓ ✗ I have identified a first variable to keep constant.[1]

✓ ✗ I have identified a second variable to keep constant.[2]

d [Mimi is correct,[1]] [because personal errors are fluctuations in data caused by human mistakes or imprecision. Averaging the percentage cover of bacteria will reduce the impact of poor estimates made by individual sets of human eyes.[2]]

✓ ✗ I have stated that Mimi is correct.[1]

✓ ✗ I have explained why Mimi's suggestion decreases the impact of personal errors.[2]

1B Ethics in Biology

Theory review questions

1 bioethics; metathinking; ethics

2 A

3 I: Virtues-based
II: Duty/rule based
III: Consequences-based

4 A

5 I–integrity; II–justice; III–beneficence; IV–respect; V–non-maleficence

SAC skills questions

6 C **7** B **8** A **9** D

10 A

Exam style questions

Within lesson

11 **a** [A literature review involves the collation and analysis of secondary data.[1]] [A literature review can be used to prepare for an investigation by drawing on multiple different resources.[2]]

✓ ✗ I have stated what a literature review is.[1]

✓ ✗ I have stated one benefit of using a literature review.[2]

b [Scientific evidence is primary and/or secondary data, whereas opinion does not require data.[1]]

✓ ✗ I have provided a distinction between opinion and evidence.[1]

c [The team only collated data from three sources, each of which was specialised to a certain disease type.[1]] [In the interest of integrity, and in order for the literature review to be robust in helping to decide how to allocate resources, the team ought to rely on secondary data from a multitude of sources, including different disease types.[2]]

✓ ✗ I have identified a weakness of the data.[1]

✓ ✗ I have explained my answer with reference to the bioethical concept of integrity.[2]

12 **a** [4.2 °C.[1]]

✓ ✗ I have correctly identified the predicted temperature change.[1]

b [–0.6 °C.[1]]

✓ ✗ I have correctly identified the retrospective temperature change.[1]

c [An RCP of 2.6 would cause global surface temperature changes to plateau and remain around 1 °C,[1]] [as shown in the period between 2050–2100.[2]]

✓ ✗ I have explained that an RCP of 2.6 would cause temperature change to plateau and remain at 1 °C.[1]

✓ ✗ I have made explicit reference to the period between 2050–2100.[2]

d [A literature review involves collecting and analysing secondary data from existing research,[1]] [while modelling involves making predictions and simulations based on the relationship between different variables.[2]] [In the case of the findings of the IPCC, the body conducted a literature review of the previous findings (1900–2018) of the CMIP5 experiments, and used those to model future global temperature changes (2018–2100) based on different levels of RCP.[3]]

✓ ✗ I have explained what is involved in a literature review.[1]

✓ ✗ I have explained what is involved in modelling.[2]

✓ ✗ I have made explicit reference to the findings of the IPCC to demonstrate this difference.[3]

e [The IPCC upholds the bioethical concept of integrity by acting as an impartial distributor of rigorous, peer-reviewed, scientific information relating to climate change.[1]]

✓ ✗ I have explained the significance of the IPCC for enabling improved public understanding.[1]

✓ ✗ I have signposted connections to the bioethical concept of integrity using terms such as: impartial, rigorous, peer-reviewed, information.

f i [The argument of the climate change sceptics suggests that the predicted temperature changes are exaggerated and that the potential for negative consequences are therefore overestimated.[1]]

Other acceptable responses include:

- The argument of the climate change sceptics suggests that the potential negative outcomes of man-made climate change do not outweigh the current benefits associated with our current emissions practices.

✓ ✗ I have made reference to the idea that the projections are exaggerated.[1]

✓ ✗ I have signposted connections to a consequences-based approach using terms such as: negative consequences, outweigh benefits.

ii [Future climate policies might recognise the moral obligation to reduce RCP in light of its potential consequences for low-socioeconomic countries, and aim to remove the unfair burden placed on these at-risk countries.[1]]

✓ ✗ I have explained the relevance of justice by reference to the unfair burden on low-socioeconomic countries.[1]

✓ ✗ I have signposted connections to the bioethical concept of justice using terms such as: unfair burden, at-risk countries.

Chapter 1 SAC practice

1 [Cholesterol is not dangerous at the right levels, as it is necessary for the digestion of lipids and the formation of hormones.[1]][However, if the amount of LDL is too high, then cholesterol can become dangerous for the body and increase the risk of stroke and cardiovascular disease.[2]]

✓ ✗ I have explained why cholesterol is not dangerous for the body at the right concentration.[1]

✓ ✗ I have explained how cholesterol can be dangerous for the body.[2]

2 [The man has recorded borderline level readings for his blood cholesterol in terms of both LDL and HDL.[1]][The doctor should encourage a decrease in the consumption of meat, dairy, and fried foods to decrease the risk of adverse health effects.[2]]

✓ ✗ I have stated what range his results are in.[1]

✓ ✗ I have outlined a course of action that could be taken by the doctor.[2]

3 [High risk level.[1]]

✓ ✗ I have stated which level this patient is presenting with.[1]

4 [Pharmaceutical industry-sponsored studies may have an inclination to publish favourable results for their intervention over a competitor,[1]][as this is likely to increase the reputation of their product.[2]]

✓ ✗ I have explained how bias may be introduced into the study.[1]

✓ ✗ I have explained how this can benefit those funding the study.[2]

5 [The concept of integrity centres around honest reporting of information.[1]][Therefore the student would be correct suggesting this study is focusing on integrity as it is focusing on how privately funded studies create their methodologies and present their results.[2]]

✓ ✗ I have defined integrity.[1]

✓ ✗ I have justified how it is relevant to this study.[2]

6 [The dosage of statin administered to both experimental groups[1]][and the time of day the drug was administered.[2]]

Other acceptable responses include:

- the frequency of doses administered
- that participants are not affected by any other diseases which might cause an alternative reaction
- the age, weight, and health of participants

✓ ✗ I have identified one controlled variable.[1]

✓ ✗ I have identified a second controlled variable.[2]

7 [Studies could purposely administer a less effective dosage of the comparison drug compared to the drug of interest, which could exaggerate the difference in effects between the drugs.[1]]

✓ ✗ I have identified one way which bias could be introduced into the study.[1]

8 [Model 1 is more valid as human brains may react differently to a stroke compared to animal brains.[1]]

Other acceptable responses include:

- Animal bodies may react differently to treatments.

✓ ✗ I have explained which model is more valid.[1]

9 [Model 2 should occur first to determine the safety of the treatment,[1]][then Model 1 should occur to assess the effectiveness of the treatment in humans.[2]]

✓ ✗ I have explained why Model 2 should be used first.[1]

✓ ✗ I have explained why Model 1 should be used second.[2]

10 [These studies involve inducing a stroke in an otherwise healthy animal can lead to serious brain damage and/or death.[1]][Therefore, to uphold respect and value the life of all living things, an ethics committee may choose to reject these studies.[2]]

✓ ✗ I have explained why this study may be unethical.[1]

✓ ✗ I have referred this to the bioethical concept of respect.[2]

11 [Non-maleficence focuses on preventing undue harm being caused in participants of an experiment.[1]][A doctor may reject an experimental treatment in case it is ineffective or causes serious side effects to the patient.[2]]

✓ ✗ I have defined non-maleficence.[1]

✓ ✗ I have explained how that concept may lead the doctor to reject the experimental drug.[2]

12 [This is relevant to the concept of respect, as participants should be entitled to provide informed consent to validate that their values and welfare are being considered.[1]]

✓ ✗ I have explained the relevant bioethical concept.[1]

Chapter 1 Exam practice

Section A

1 C	**2** D	**3** B	**4** D
5 B	**6** B	**7** B	**8** C
9 D	**10** A		

Section B

11 a [The independent variable is glucose concentration.[1]] [The dependent variable is the temperature change of yeast mixture.[2]]

✓ ✗ I have identified the independent variable.[1]

✓ ✗ I have identified the dependent variable.[2]

b [A control is compared with the treatment group/s, and any differences between the control and the treatment group are likely attributable to the IV.[1]] [A control for this experiment would be setting up a yeast mixture with no glucose added.[2]]

✓ ✗ I have explained the purpose of a control.[1]

✓ ✗ I have identified a possible control for this experiment.[2]

c i [Group 3 has the most precise results[1]] [as all results are within 0.5 °C of one another.[2]]

✓ ✗ I have identified the most precise group.[1]

✓ ✗ I have justified why it is the most precise.[2]

ii [Group 4 is the most accurate[1]] [as the average thermometer reading is 20 °C, which is equal to the true temperature.[2]]

✓ ✗ I have identified the most accurate group.[1]

✓ ✗ I have justified why it is the most accurate.[2]

iii [Reliability refers to the degree to which results can be relied upon to be accurate.[1]] [Testing the thermometers means we know if we can rely upon them to give accurate measurements, or if another tool should be used.[2]]

✓ ✗ I have defined what reliability refers to.[1]

✓ ✗ I have explained how testing the thermometers increases reliability.[2]

iv [The data is quantitative as it is expressed numerically.[1]] [Qualitative data is descriptive information describing results, and typically lacks numerical values.[2]]

✓ ✗ I have stated and explained that this is quantitative data.[1]

✓ ✗ I have contrasted this to qualitative data.[2]

d [Integrity is concerned with the accurate and honest reporting of results, whether favourable or unfavourable. For this reason, the students should present their true results despite not supporting their hypothesis.[1]]

✓ ✗ I have explained an appropriate course of action.[1]

12 a [The independent variable is sucrose concentration.[1]] [The dependent variable is the average final height of each group of plants.[2]]

✓ ✗ I have identified the independent variable.[1]

✓ ✗ I have identified the dependent variable.[2]

b [Controlled variables in this experiment include the initial height of every plant being approximately 2 cm,[1]] [the researchers watering every plant with 5 mL of water each day,[2]] [and that the experiment uses the same plant species for each group.[3]]

Other acceptable responses include:

- using 40 plants for each group
- having the same number of days of growth for each group

✓ ✗ I have identified a first controlled variable.[1]

✓ ✗ I have identified a second controlled variable.[2]

✓ ✗ I have identified a third controlled variable.[3]

c i [Saskia's graph is more correct. She has followed standard practice by plotting the independent variable on the horizontal axis and the dependent variable on the vertical axis,[1]] [unlike Gustave who has plotted the inverse.[2]]

✓ ✗ I have stated who has graphed the results correctly.[1]

✓ ✗ I have explained why the other person is incorrect.[2]

ii [Increasing sample size increases the reliability of results,[1]] [as it allows researchers to average across samples and makes it less likely that the results are affected by random outliers.[2]]

✓ ✗ I have outlined the effect of increased sample size on reliability.[1]

✓ ✗ I have explained why reliability is affected.[2]

d [Accuracy is a measure of how closely the experimentally obtained results align with the true result,[1]] [whereas validity is a measure as to whether the results measure what they claim to be measuring and exclude the effects of confounding variables.[2]]

✓ ✗ I have explained what is meant by the term accuracy.[1]

✓ ✗ I have explained what is meant by the term validity.[2]

✓ ✗ I have used comparative language such as: whereas.

13 a [Ecologists can sample particular locations that are representative of a larger area.[1]][From this, an estimate can be made of the total number of organisms across a specific, larger area.[2]]

✓ ✗ I have stated how ecologists could sample these areas.[1]

✓ ✗ I have explained that the value will be an estimate.[2]

b [Unbiased refers to results that are unaffected by prejudice or inclination towards a particular finding.[1]][Representative refers to accurately reflecting the characteristics of the entire population in question.[2]]

✓ ✗ I have explained what is meant by unbiased.[1]

✓ ✗ I have explained what is meant by representative.[2]

c [Judgement sampling will likely introduce bias to a study.[1]] [This is because the researcher has created criteria to guide their selections.[2]]

Other acceptable responses include:

- The sample will not be representative of the actual population.

✓ ✗ I have identified a limitation.[1]

✓ ✗ I have explained this limitation.[2]

2A Protein structure and function

Theory review questions

1 A

2 L-amino group; M-carboxyl group; N-central carbon; O-R-group

3 C

4 I-primary; II-tertiary; III-quaternary; IV-secondary

SAC skills questions

5 B **6** B **7** D **8** A

9 C

Exam-style questions

Within lesson

10 D **11** D **12** A **13** A

14 C

15 a [W-tertiary, X-secondary, Y-primary, Z-quaternary.[1]]

✓ ✗ I have identified the correct level of protein structure for diagrams W, X, Y, and Z.[1]

b [The functional 3D structure of a protein is formed primarily through the bonding and interactions between the R-groups of amino acids within the polypeptide chain.[1]]

✓ ✗ I have identified that bonds form between R-groups.[1]

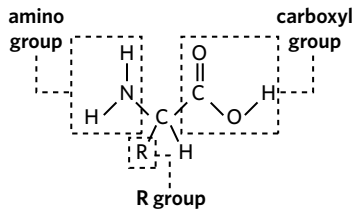
c [The functional diversity of proteins arises through the ability to create many combinations of amino acids and polypeptide chains of differing lengths, thereby allowing proteins to fold into different functional structures.[1]]

✓ ✗ I have suggested how the functional diversity of proteins arises.[1]

16 a [Peptide bond.[1]]

✓ ✗ I have identified the correct bond.[1]

b



✓ ✗ I have drawn the correct structure.

✓ ✗ I have identified the amino, carboxyl, and R-groups.

c [Oxytocin has a tertiary structure,[1]][which represents the folding of the polypeptide chain into a functional 3D structure.[2]]

✓ ✗ I have identified the structure level of oxytocin.[1]

✓ ✗ I have described tertiary protein structure.[2]

Multiple lessons

17 A

18 D

Key science skills and ethical understanding

19 a [Lipids are hydrophobic, so are not attracted to and do not interact with hydrophilic substances.[1]][The outer R-groups of albumin are hydrophilic, so albumin will not interact with and dissolve in lipids.[2]]

✓ ✗ I have stated that lipids are hydrophobic substances and do not interact with hydrophilic substances.[1]

✓ ✗ I have explained why albumin will not dissolve in lipids.[2]

✓ ✗ I have used key biological terminology such as: hydrophobic, hydrophilic, R-group, dissolve.

b [Proteins transport substances across membranes[1]][and defend against pathogens.[2]]

Other acceptable responses include:

- Receive and transduce cell signals.
- Chemical messengers/hormones.
- Storage of amino acids and ions.
- Motor/contractile.
- Structural.
- Enzymes.

✓ ✗ I have identified one function of proteins.[1]

✓ ✗ I have identified a second function of proteins.[2]

c i [Ten.[1]]

✓ ✗ I have identified the number of patients with albumin levels outside the normal range.[1]

ii [Systematic error,[1]][as the use of an uncalibrated scale will lead to a consistent difference in measurements.[2]]

✓ ✗ I have identified the correct type of error.[1]

✓ ✗ I have justified my response.[2]

d i [The patient's blood albumin levels vary widely (from 3.21 to 5.75 g/dL) both below and above the normal healthy range, so these are not precise measurements.[1]]

✓ ✗ I have used data from the scenario to justify why the data is not precise.[1]

ii [Non-maleficence involves the minimisation of preventable harm.[1]][Therefore, the doctor should not have unnecessarily taken multiple blood samples from the patient (a procedure involving pain and discomfort), as a single sample would likely have been sufficient to assess for albumin levels.[2]]

✓ ✗ I have described the bioethical concept of non-maleficence.[1]

✓ ✗ I have described the relevance of non-maleficence to the scenario.[2]

2B Nucleic acids

Theory review questions

1 polymers; monomers; deoxyribose; ribose; thymine; uracil; double-stranded; single-stranded

2 X-nitrogenous base; Y-five-carbon sugar; Z-phosphate

3 C

4 I-rRNA; II-mRNA; III-tRNA

SAC skills questions

5 A **6** A **7** B **8** D

Exam-style questions

Within lesson

9 D **10** A **11** B **12** C

13 C **14** A **15** C

Multiple lessons

16 B **17** A **18** B **19** B

20 a [Nucleic acid[1]][and protein.[2]]

I have identified one of the macromolecules shown.[1]

I have identified the second macromolecule shown.[2]

b [The monomers of a nucleic acid are nucleotides[1]][and the monomers of a protein are amino acids.[2]]

I have identified the monomer of the first type of macromolecule.[1]

I have identified the monomer of the second type of macromolecule.[2]

Key science skills and ethical understanding

21 a i [The primary structure of a protein, which is its sequence of amino acids.[1]]

I have described the level of protein structure which is informed by protein sequencing.[1]

ii [The sequence of nucleotides in a gene.[1]]

I have identified the information obtained from gene sequencing.[1]

b i [Bar X represents the nucleotide cytosine and bar Y represents the nucleotide thymine.[1]]

I have correctly identified bar X and Y.[1]

ii [Scientist B is correct. The graph is likely showing double-stranded DNA given that there are equal numbers of the nucleotides A and T and the nucleotides C and G.[1]] [If the sample were single-stranded DNA, it would be highly unlikely for there to be an equal number of complementary nucleotides.[2]]

I have explained why this is likely a sample of double-stranded DNA.[1]

I have explained why this is unlikely to be a sample of single-stranded DNA.[2]

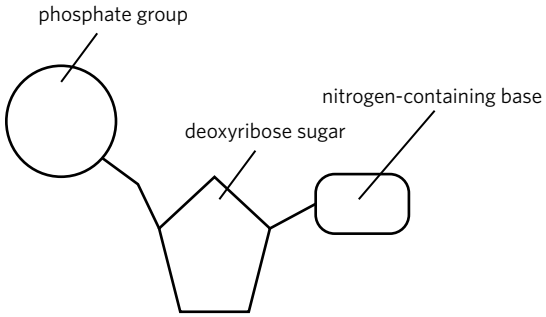
I have used appropriate biological terminology such as: nucleotide, complementary.

iii [230 base pairs.[1]]

I have correctly calculated the length of the DNA sample.[1]

I have recognised that this is a sample of double-stranded DNA, so the length must be half the total number of nucleotides.

iv



I have correctly drawn a nucleotide,the monomer of DNA.

I have labelled the diagram.

I have used key biological terminology such as: nitrogen-containing base, deoxyribose sugar, phosphate group.

c [Respect.[1]][This is because respect involves the consideration of autonomy, which requires the acquisition of consent prior to the extraction of DNA.[2]]

I have correctly identified the bioethical concept.[1]

I have justified the response by describing the bioethical concept of respect.[2]

2C Genes

Theory review questions

1 triplet; codon; amino acid

2 I-degenerate; II-unambiguous; III-non-overlapping; IV-universal

3 I-promoter; II-termination sequence; III-operator; IV-introns; V-exons

4 Prokaryotic: III
Eukaryotic: IV
Both: I; II

SAC skills questions

5 D **6** D **7** D **8** B

Exam-style questions

Within lesson

9 C **10** B **11** A **12** A

Multiple lessons

13 a i [Met Leu Ile Thr Gly Glu Ser Gly Ala.[1]]

I have identified the correct amino acid sequence.[1]

ii [TAC GAA TAA TGA CCC CTC AGA CCA CGG.[1]]

I have identified the correct DNA sequence.[1]

b [Quaternary structure.[1]][This is because myosin is a protein composed of multiple polypeptide chains.[2]]

I have identified the correct level of protein structure.[1]

I have justified my response.[2]

c [mRNA is single-stranded, while DNA is double-stranded.[1]] [mRNA contains a ribose sugar, while DNA contains a deoxyribose sugar.[2]][mRNA contains the nucleotide base uracil, while DNA contains thymine.[3]]

Other acceptable responses include:

- DNA has a double helix shape while mRNA has a linear shape.

I have identified one difference.[1]

I have identified a second difference.[2]

I have identified a third difference.[3]

14 a [Mutations may not lead to the production of a different amino acid due to the degenerate nature of the genetic code,[1]][which allows for multiple different codons or triplets to code for the same amino acid.[2]]

I have identified the degenerate nature of the genetic code.[1]

I have described the degenerate nature of the genetic code.[2]

b i [While introns are regions of non-coding DNA, exons are regions of coding DNA.[1]]

I have described a difference between introns and exons.[1]

ii [No, an operator region would not be found in the *p53* gene of humans, as operator regions are only found in prokaryotes, and not eukaryotes.[1]]

I have explained why an operator would not be found in the *p53* gene of a human.[1]

iii [The promoter region serves as the binding site for RNA polymerase, which denotes the starting position of transcription.[1]]

I have described the purpose of the promoter region.[1]

Key science skills and ethical understanding

15 a [The dependent variable is the number of mice that developed skin cancer.[1]][The independent variable is the exposure of the mice to UV radiation or not.[2]]

I have identified the dependent variable.[1]

I have identified the independent variable.[2]

b [If mice are exposed to UV radiation, then the number of mice that develop skin cancer will increase because UV radiation is known to cause mutations.[1]]

I have identified a possible hypothesis.[1]

c [The species of mice and[1]][the diet of the mice.[2]]

Other acceptable responses include:

- Environmental factors (e.g. temperature).

I have identified one controlled variable.[1]

I have identified another controlled variable.[2]

d [To increase precision, researchers can increase the sample size of the mice in each group, thereby increasing the number of replicates in the study and reducing the influence of random errors.[1]]

I have suggested how the researchers could increase precision.[1]

e [Beneficence involves the maximisation of benefits.[1]][Therefore, in this experiment, scientists may have decided to conduct the experiment on mice, despite the harmful nature of UV radiation, in order to further research and develop therapies to combat skin cancer for humans.[2]]

Other acceptable responses include:

- The concept of beneficence requires the scientists to support the wellbeing of the mice wherever possible, for example by regularly cleaning their cages and feeding them a nutritious diet.

I have described the bioethical concept of beneficence.[1]

I have described the relevance of beneficence to the scenario.[2]

2D Gene expression

Theory review questions

1 A

2 A

3 template strand; pre-mRNA; 3′ poly-A tail; codons

4 ribosome; anticodons; STOP codon

5 W-translation; X-tRNA; Y-amino acid; Z-anticodon

SAC skills questions

6 A **7** B **8** D **9** B

Exam-style questions

Within lesson

10 D **11** B **12** A

Multiple lessons

13 D

14 **a** [Six.[1]][This is because there are six exon DNA triplets in this gene, each of which will encode an amino acid in the resulting polypeptide, followed by the ATC triplet, which represents a stop codon and the termination of translation.[2]]

- I have identified how many amino acids would be present.[1]
- I have justified my response with respect to the codons present.[2]
- I have not included the stop codon or introns in my count.

b **i** [5′ AUG UGG CGA AUA AAA GUA GAA AGA CGU AUC CUA UAG 3′.[1]]

- I have written a complementary chain of RNA nucleotides.[1]
- I have identified the direction using 5′ and 3′.

ii [5′ AUG UGG CGA GUA AGA CUA UAG 3′[1]]

- I have written a complementary chain of RNA nucleotides without introns.[1]
- I have identified the direction using 5′ and 3′.
- I have included the stop codon in the mRNA strand.

iii [Met-Trp-Arg-Val-Arg-Leu.[1]]

- I have stated the order of the amino acids in the polypeptide.[1]

15 **a** **i** [Translation.[1]]

- I have stated which process Molecule Z is involved in.[1]

ii [The tRNA anticodon is complementary to the mRNA codon being read by the ribosome.[1]][The anticodon attaches to the mRNA codon, which then allows for the corresponding amino acid carried by tRNA to be joined to the growing polypeptide chain.[2]]

- I have stated how the tRNA anticodon is related to the mRNA codon.[1]
- I have described how the codon-anticodon attachment allows for translation to occur.[2]
- I have used key biological terminology such as: tRNA, complementary, mRNA, codon, amino acids, polypeptide chain.

b [Primary structure.[1]]

- I have identified which level of structure preproinsulin is.[1]

16 **a** [mRNA.[1]]

- I have identified the molecule.[1]

b [RNA polymerase.[1]]

- I have identified the correct enzyme.[1]

c [Transcription involves the unwinding of the DNA helix which allows for the binding of RNA polymerase to the promoter region.[1]][RNA polymerase then synthesises a strand of pre-mRNA with the use of complementary RNA bases.[2]][Transcription is terminated when RNA polymerase reaches a termination sequence and the pre-mRNA strand undergoes post-transcriptional modifications to become an mRNA strand.[3]]

- I have described the unwinding of the DNA helix and binding of RNA polymerase.[1]
- I have described the synthesis of pre-mRNA.[2]
- I have described the termination of transcription and the modification of pre-mRNA.[3]
- I have used key biological terminology such as: transcription, RNA polymerase, promoter, pre-mRNA.

d [Protein.[1]]

Other acceptable responses include:

- Polypeptide.

- I have identified the molecule type.[1]

e [Translation begins with the binding of an mRNA molecule to a ribosome, with the start codon initiating the process.[1]][tRNA molecules with anticodons complementary to the mRNA codons transport specific amino acids to the ribosome, which are added to the growing polypeptide chain via peptide bonds.[2]][Translation terminates once the stop codon is recognised and the polypeptide chain is released.[3]]

- I have described the initiation of translation when an mRNA molecule binds to a ribosome.[1]
- I have described the transportation of specific amino acids to a ribosome by tRNA molecules.[2]
- I have described the termination of translation.[3]
- I have used key biological terminology such as: translation, mRNA, codon, tRNA, complementary.

17 [A-DNA; B-pre-mRNA; C-mRNA; D-ribosome; E-tRNA; F-polypeptide; G-anticodon; Stage Y-transcription; Stage Z-translation.[1]]

✓ ✗ I have correctly identified the molecules and stages in the diagram.[1]

Key science skills and ethical understanding

18 a [The independent variable is the sequence of amino acids within the polypeptide and[1]][the dependent variable is the accumulation in the nucleus.[2]]

✓ ✗ I have identified the independent variable.[1]

✓ ✗ I have identified the dependent variable.[2]

b [Polypeptides N and E were easily able to cross the nuclear membrane,[1]][as they were able to accumulate in the nucleus.[2]]

✓ ✗ I have identified which polypeptides were able to cross the nuclear membrane.[1]

✓ ✗ I have stated why this is known.[2]

c [Polypeptide E* contains an Asp amino acid in the same position that polypeptide E contains a Gly. This change may affect the ability of the polypeptide to accumulate in the nucleus.[1]]

✓ ✗ I have stated a potential reason why polypeptide E* could not accumulate in the nucleus like polypeptide E.[1]

d [Precision refers to the spread of results due to random errors.[1]] [The scientists could repeat their experiment to minimise the effect of random errors, reducing the spread of results and improving precision.[2]]

✓ ✗ I have described what precision involves.[1]

✓ ✗ I have described how the scientists could improve the precision of their experiment.[2]

e [Based on the bioethical concept of beneficence, which involves maximising benefits,[1]][funding should be directed towards research projects with the greatest potential benefit. For example, that may include research projects investigating diseases affecting a large proportion of individuals.[2]]

Other acceptable responses include:

- Justice, where funding should be allocated based on needs.

✓ ✗ I have described a bioethical concept.[1]

✓ ✗ I have suggested a criterion that should be considered.[2]

2E Gene regulation

Theory review questions

1 structural genes; regulatory genes; repressor proteins; operator; RNA polymerase

2 D

3 W-regulatory promoter; X-regulatory gene; Y-structural promoter; Z-operator

4 low; allowing; transcribed; high; preventing

SAC skills questions

5 B **6** A **7** C **8** C

Exam-style questions

Within lesson

9 B **10** C

Multiple lessons

11 B

12 a [Process X is transcription[1]][and involves the production of pre-mRNA from the nucleotide code of the DNA template strand.[2]]

✓ ✗ I have identified Process X.[1]

✓ ✗ I have described the purpose of Process X.[2]

b [Process Z is translation[1]][and involves the interpretation of the mRNA strand at a ribosome to form a polypeptide chain.[2]]

✓ ✗ I have identified Process Z.[1]

✓ ✗ I have described the purpose of Process Z.[2]

c [During Process Y, introns are removed from the pre-mRNA strand and exons are spliced together to form an mRNA strand containing only coding segments.[1]][A poly-A tail is added to the 3′ end[2]][and a methyl-G cap is added to the 5′ end of the mRNA molecule.[3]]

✓ ✗ I have described the process of splicing.[1]

✓ ✗ I have identified the addition of a 3′ poly-A tail.[2]

✓ ✗ I have identified the addition of a 5′ methyl-G cap.[3]

d [Structure P is composed of structural and regulatory genes. Structural genes are responsible for producing proteins that form the structure or facilitate the functioning of an organism.[1]][Regulatory genes are responsible for producing proteins that influence the expression of other genes.[2]]

✓ ✗ I have described the role of structural genes.[1]

✓ ✗ I have described the role of regulatory genes.[2]

13 a [*lacZ*.[1]]

Other acceptable responses include:

- *lacY*.
- *lacA*.

✓ ✗ I have named a structural gene of the *lac* operon.[1]

b [The repressor protein binds to the operator region of the *lac* operon[1]][and prevents RNA polymerase from transcribing the structural genes of the *lac* operon.[2]]

✓ ✗ I have identified where the repressor protein binds.[1]

✓ ✗ I have outlined the role of the repressor protein.[2]

c [The promoter regions of the *lac* operon are the sites where RNA polymerase binds to in order to transcribe the DNA of its regulatory and structural genes.[1]]

✓ ✗ I have described the role of the promoter regions.[1]

d [No, the *lac* operon would likely not be active when lactose is absent. This is because if lactose is absent, the *lac* repressor protein will likely bind to the operator region of the *lac* operon[1]][preventing the unnecessary energy-intensive production of enzymes that catalyse the breakdown of lactose into glucose and galactose.[2]]

✓ ✗ I have identified that when lactose is absent, a repressor is likely bound to the operator region.[1]

✓ ✗ I have justified my response by referring to the unnecessary production of enzymes when lactose is absent.[2]

e [In order to survive, *E. coli* must conserve energy.[1]][By regulating expression of the *lac* operon, transcription and translation will only proceed when required, lowering the total energy expenditure of the bacterium.[2]]

✓ ✗ I have identified the importance of energy conservation.[1]

✓ ✗ I have explained the importance of regulating the *lac* operon.[2]

Key science skills and ethical understanding

14 a [An experimental control is the experimental group which does not receive the treatment or experience the influence of the independent variable (e.g. without the addition of tryptophan).[1]][This allows for the presence of a comparison group and can highlight the effects of uncontrolled variables on the dependent variable.[2]][Therefore, Sample 1 is the experimental control.[3]]

✓ ✗ I have defined an experimental control.[1]

✓ ✗ I have explained the purpose of an experimental control.[2]

✓ ✗ I have identified the experimental control in the experiment.[3]

b [When working with *E. coli* bacteria, the experiment should take place in an aseptic environment.[1]]

Other acceptable responses include:

- Incubating *E. coli* at a temperature that prevents excessive growth.
- Wearing personal protective equipment (e.g. lab coat, glasses).
- Practising appropriate hand hygiene.

✓ ✗ I have suggested a reasonable safety consideration.[1]

c [If there is an increase in tryptophan concentration, then there will be a decrease in concentration of tryptophan synthesising enzymes due to a reduced requirement for the synthesis of tryptophan.[1]]

✓ ✗ I have stated a possible hypothesis for the experiment.[1]

d i [Personal error.[1]]

✓ ✗ I have identified the correct type of error.[1]

ii [Integrity.[1]]

✓ ✗ I have identified the correct bioethical concept.[1]

iii [It is expected that Sample K represents Sample 3, Sample L represents Sample 1, Sample M represents Sample 4, and Sample N represents Sample 2.[1]][This can be explained by the fact that a greater amount of tryptophan activates more *trp* repressor proteins. This causes more *trp* repressor proteins to bind to the operator, resulting in greater inhibition of *trp* operon gene transcription and translation. Hence, the measured concentration of tryptophan synthesising enzymes decreases.[2]]

✓ ✗ I have identified which results match which samples.[1]

✓ ✗ I have briefly explained my response.[2]

2F The protein secretory pathway

Theory review questions

1 D-ribosome; E-Golgi apparatus; F-secretory vesicle

2 I-endoplasmic reticulum; II-secretory vesicle; III-mitochondrion; IV-Golgi apparatus; V-ribosome

3 out of; energy; secretory vesicle; extracellular

4 A

SAC skills questions

5 A **6** A **7** B **8** C

Exam-style questions

Within lesson

9 D **10** C **11** A **12** A

13 D **14** B

15 a [Ribosomes.[1]]

✓ ✗ I have identified the correct cellular organelle.[1]

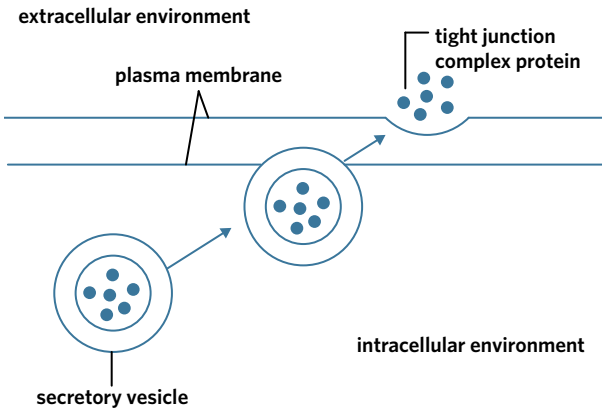
b [The tight junction complex proteins, which are synthesised at the ribosomes attached to the rough endoplasmic reticulum, are folded within the rough endoplasmic reticulum and transported to the Golgi apparatus in transport vesicles.[1]][At the Golgi apparatus, the proteins are modified before being packaged into secretory vesicles.[2]][The secretory vesicles fuse with the plasma membrane, releasing the tight junction complex proteins into the extracellular environment via the process of exocytosis.[3]]

✓ ✗ I have identified that the rough endoplasmic reticulum facilitates folding of the proteins.[1]

✓ ✗ I have identified that the Golgi apparatus is responsible for modifying and packaging of the proteins into secretory vesicles.[2]

✓ ✗ I have identified that secretory vesicles release the proteins via the process of exocytosis.[3]

c



I have correctly drawn the process of exocytosis.

I have labelled my diagram.

Multiple lessons

16 a [The process of translation would occur at the ribosome, which begins with the binding of an mRNA molecule to a ribosome, with the start codon initiating the process.[1]][tRNA molecules with anticodons complementary to the mRNA codons transport specific amino acids to the ribosome, which are added to the growing polypeptide chain via peptide bonds.[2]][Translation terminates once the stop codon is recognised and the polypeptide chain for the acetylcholine receptor is released.[3]]

I have described the initiation stage of translation.[1]

I have described the elongation stage of translation.[2]

I have described the termination stage of translation.[3]

I have used key biological terminology such as: translation, mRNA, codon, tRNA, complementary.

b [mRNA is responsible for carrying genetic information for the production of the acetylcholine receptor from the nucleus to the ribosome for protein synthesis.[1]]

I have described the role of mRNA in the synthesis of the acetylcholine receptor.[1]

c [Golgi apparatus.[1]]

I have identified the correct organelle.[1]

Key science skills and ethical understanding

17 a [The independent variable is the age of the mouse neurons.[1]][The dependent variable is the amount of APP endocytosis and level of beta-amyloid in the neurons.[2]]

I have identified the independent variable.[1]

I have identified the dependent variable.[2]

b i [The results support the scientists' hypothesis as the amount of beta-amyloid protein and the amount of APP endocytosis both increased as the age of the mouse neurons increased.[1]]

I have justified why the hypothesis was supported by the data.[1]

ii [Large vesicles may result in a greater number of APP molecules being taken up by the mouse neurons via endocytosis.[1]]

I have explained that large vesicles may indicate increased endocytosis of molecules.[1]

iii [The experiment was only undertaken *in vitro*,[1]][and therefore whether the experiment would yield similar results *in vivo* is undetermined.[2]]

Other acceptable responses include:

- The experiment was only performed using mouse neurons, so the effect in humans is unknown.
- The experiment was not replicated, increasing the chances of random errors being present and decreasing reliability.

I have identified one possible limitation.[1]

I have explained the effect of this limitation on the experiment.[2]

c [Non-maleficence involves the minimisation of preventable harms.[1]][Therefore, in this experiment, care should be taken in the treatment of the mice to prevent unnecessary suffering. For example, the mice should be humanely euthanised prior to the extraction of neurons.[2]]

I have described the bioethical concept of non-maleficence.[1]

I have described the relevance of the bioethical concept to the scenario.[2]

Chapter 2 SAC practice

1 [Hormones are cell signalling molecules that can be used to transmit signals from one part of the body to another.[1]]

I have described the function of a hormone.[1]

2 [Blood glucose levels[1]][and pH.[2]]

Other acceptable responses include:

- Temperature.
- Concentration of ions.

I have identified one factor that should be kept relatively constant.[1]

I have identified a second factor that should be kept relatively constant.[2]

3 [The shape of the hormone and receptor must be complementary in nature, so that they can bind with each other.[1]]

I have explained the significance of the shape of a hormone and its receptor.[1]

4 [Amino acid.[1]]

✓ ✗ I have named the correct monomer.[1]

5 [The process that occurs in the cytosol which directly leads to the production of insulin involves translation, which begins with the binding of an mRNA molecule to a ribosome, with the start codon initiating the process.[1]][tRNA molecules with anticodons complementary to the mRNA codons transport specific amino acids to the ribosome, which are added to the growing polypeptide chain via peptide bonds.[2]][Translation terminates once the stop codon is recognised and the polypeptide chain is released.[3]]

✓ ✗ I have described the initiation of translation when an mRNA molecule binds to a ribosome.[1]

✓ ✗ I have described the transportation of specific amino acids to a ribosome by tRNA molecules.[2]

✓ ✗ I have described the termination of translation.[3]

✓ ✗ I have used key biological terminology such as: translation, mRNA, codon, tRNA, complementary.

6 [After production, insulin will be transported from the rough endoplasmic reticulum, which is where it is made and folded,[1]] [to the Golgi apparatus, which is where it is packaged into secretory vesicles.[2]][From there, it will be exported from the cell through the process of exocytosis, which involves the fusion of the secretory vesicle with the plasma membrane and the release of insulin into the extracellular environment.[3]]

✓ ✗ I have identified that insulin is folded within the rough endoplasmic reticulum.[1]

✓ ✗ I have identified that insulin is packaged into secretory vesicles at the Golgi apparatus.[2]

✓ ✗ I have described the process of exocytosis.[3]

7 [A poly-A tail is added to the 3′ end and[1]][a methyl-G cap is added to the 5′ end of the pre-mRNA molecule.[2]][After that, introns are removed and exons are spliced together to form an mRNA strand.[3]]

✓ ✗ I have identified the addition of a 3′ poly-A tail.[1]

✓ ✗ I have identified the addition of a 5′ methyl-G cap.[2]

✓ ✗ I have described the process of splicing.[3]

8 [While structural genes code for proteins that are involved in the structure and function of a cell, regulatory genes code for proteins that are involved in controlling the expression of other genes.[1]]

✓ ✗ I have described the difference between structural and regulatory genes.[1]

9 [When blood glucose levels rise, insulin production increases and more insulin is secreted into the bloodstream by the pancreas.[1]] [Subsequently, the insulin binds to its receptors, causing the insertion of glucose channels which increase the cell's uptake of glucose, thereby decreasing blood glucose levels.[2]]

✓ ✗ I have described that insulin production and secretion are increased.[1]

✓ ✗ I have described the effect of insulin on blood glucose levels.[2]

10 [After eating a meal, blood glucose levels may increase.[1]]

✓ ✗ I have suggested a possible reason for increased blood glucose levels.[1]

11 [Quaternary structure,[1]][which occurs when a protein is composed of two or more polypeptide chains – insulin is composed of two polypeptide chains.[2]]

✓ ✗ I have identified the correct level of protein structure.[1]

✓ ✗ I have justified my response.[2]

12 [Bacteria, humans, pigs, and cows can all produce insulin through the same pathway due to the universal nature of the genetic code, which involves nearly all organisms using the same set of rules in the production of proteins.[1]]

✓ ✗ I have explained why it is possible for many different organisms to produce insulin through the same pathway.[1]

13 [4–6%.[1]]

✓ ✗ I have identified the correct percentage range.[1]

14 [Justice involves the equitable distribution of resources where possible.[1]][Therefore, pharmaceutical companies should decrease their pricing of insulin in order to make it accessible to everyone, regardless of their financial situation.[2]]

✓ ✗ I have described the bioethical concept of justice.[1]

✓ ✗ I have described the relevance of the bioethical concept to the scenario.[2]

Chapter 2 Exam practice

Section A

1 A	**2** B	**3** D	**4** C
5 A	**6** B	**7** D	**8** A
9 B	**10** D	**11** B	**12** C
13 A	**14** C	**15** B	**16** D

Section B

17 a [W represents quaternary protein structure, X represents secondary protein structure, Y represents primary protein structure, and Z represents tertiary protein structure.[1]]

✓ ✗ I have identified the correct level of protein structure represented by each diagram.[1]

b [As Z represents tertiary protein structure, it forms the functional 3D structure of the protein and is the minimum level of structure required for a protein to be functional.[1]]

✓ ✗ I have described the functional significance of Z.[1]

c [Production of Y involves translation, which begins with the binding of an mRNA molecule to a ribosome, with the start codon initiating the process.[1]][tRNA molecules with anticodons complementary to the mRNA codons transport specific amino acids to the ribosome, which are added to the growing polypeptide chain via peptide bonds.[2]][Translation terminates once the stop codon is recognised and the polypeptide chain is released.[3]]

✓ ✗ I have described the initiation of translation when an mRNA molecule binds to a ribosome.[1]

✓ ✗ I have described the transportation of specific amino acids to a ribosome by tRNA molecules.[2]

✓ ✗ I have described the termination of translation.[3]

✓ ✗ I have used key biological terminology such as: translation, mRNA, codon, tRNA, complementary.

18 a [Molecule X is mRNA.[1]][mRNA molecules carry genetic information from the nucleus to the ribosome by specifying the order of amino acids in the polypeptide chain.[2]]

✓ ✗ I have correctly identified Molecule X.[1]

✓ ✗ I have described the function of Molecule X.[2]

b [Molecule X is produced through the process of transcription, which involves the unwinding of the DNA helix which allows for the binding of RNA polymerase to the promoter region.[1]][RNA polymerase then synthesises a strand of pre-mRNA with the use of complementary RNA bases.[2]][Transcription is terminated when RNA polymerase reaches a termination sequence and the pre-mRNA strand undergoes post-transcriptional modifications to become an mRNA strand.[3]]

✓ ✗ I have described the unwinding of the DNA helix and binding of RNA polymerase.[1]

✓ ✗ I have described the synthesis of pre-mRNA.[2]

✓ ✗ I have described the termination of transcription and the modification of pre-mRNA.[3]

✓ ✗ I have used key biological terminology such as: transcription, RNA polymerase, promoter, pre-mRNA.

c [Translation.[1]]

✓ ✗ I have identified the correct cellular process.[1]

19 a [Amino acids.[1]]

✓ ✗ I have stated the monomer of insulin.[1]

b [Amino acids contain an amino group, a carboxyl group, and a variable R-group.[1]][On the other hand, nucleotides contain a phosphate group, a five-carbon sugar, and a nitrogen-containing base.[2]]

✓ ✗ I have described the structure of an amino acid.[1]

✓ ✗ I have described the structure of a nucleotide.[2]

c i [Sheep insulin is least similar, as it has the most differences in amino acids in both the alpha and beta chain when compared to humans.[1]]

✓ ✗ I have explained why sheelp insulin is the least similar.[1]

ii [Not necessarily. Due to the degenerate nature of the genetic code,[1]][where multiple different codons can code for the same amino acid, their nucleotide sequence may be different despite all resulting in the amino acid alanine.[2]]

✓ ✗ I have identified the degenerate nature of the genetic code.[1]

✓ ✗ I have described the degenerate nature of the genetic code.[2]

20 a [An operon is a group of structural genes that are controlled by a common promoter and operator.[1]]

✓ ✗ I have described what an operon is.[1]

b [Region X is a regulatory gene.[1]][Region Y is an operator region.[2]]

✓ ✗ I have identified Region X.[1]

✓ ✗ I have identified Region Y.[2]

c [RNA polymerase binds to the promoter region.[1]]

✓ ✗ I have identified the correct region.[1]

d [When tryptophan levels are low, there is insufficient tryptophan to bind to the repressor protein, thereby releasing it from the operator region.[1]][This allows the *trp* structural genes to be transcribed, leading to the production of enzymes that catalyse the formation of tryptophan.[2]]

✓ ✗ I have described the release of the repressor from the operator.[1]

✓ ✗ I have described the consequence of the release of the repressor from the operator.[2]

3A Introducing Enzymes

Theory review questions

1 B

2 V-substrate; W-active site; X-enzyme; Y-enzyme-substrate complex; Z-products

3 I; III; IV; VI

4 active site; decrease/lower; increase/raise; can/will

SAC skills questions

5 B **6** B **7** A **8** B

9 B

Exam-style questions

Within lesson

10 C **11** C **12** B **13** D

14 C

Multiple lessons

15 **a** [Peptide bond.[1]]

✓ ✗ I have identified the peptide bond.[1]

b **i** [Primary structure.[1]]

✓ ✗ I have identified the level of protein structure represented.[1]

ii [The secondary level of structure of an enzyme is determined by hydrogen bonds, and is the folding and coiling of a section of the polypeptide chain. Secondary structures include alpha helices and beta-pleated sheets.[1]][On the other hand, when the enzyme takes on a three-dimensional globular shape, it is said to have tertiary structure.[2]]

✓ ✗ I have described the secondary level of structure.[1]

✓ ✗ I have described the tertiary level of structure.[2]

✓ ✗ I have used key biological terminology such as: hydrogen bonds, folding, coiling, alpha helices, beta-pleated sheets, globular.

✓ ✗ I have used comparative language such as: on the other hand.

iii [At tertiary level, the protein is functional meaning not all proteins require a quaternary level of structure.[1]][Proteins have a quaternary structure if they require additional polypeptide chains or prosthetic groups to function.[2]]

✓ ✗ I have identified that proteins are functional at the tertiary level.[1]

✓ ✗ I have explained that proteins have a quaternary level when they need additional polypeptide chains or prosthetic groups to function.[2]

✓ ✗ I have used key biological terminology such as: functional, polypeptide chains, prosthetic groups.

16 **a** [pre-mRNA molecule.[1]]

✓ ✗ I have stated pre-mRNA.[1]

b [The mRNA binds to a ribosome in the cytoplasm. The start codon on the mRNA molecule initiates translation.[1]][tRNA molecules with anticodons complementary to mRNA deliver specific amino acids to the ribosome, and amino acids bind together with peptide bonds to form a polypeptide chain.[2]][Translation terminates once the stop codon is reached.[3]][The polypeptide is then released and undergoes folding to form amylase.[4]]

✓ ✗ I have described the initiation step of translation.[1]

✓ ✗ I have described the elongation step of translation.[2]

✓ ✗ I have described the termination step of translation.[3]

✓ ✗ I have related this to amylase.[4]

✓ ✗ I have used key biological terminology such as: mRNA, ribosome, start codon, tRNA, amino acids, anticodon, complementary, polypeptide chain, translation, stop codon.

c [Through alternative splicing, exons can be configured in different orders resulting in proteins with different functions.[1]]

Other acceptable responses include:

- Post-translational changes to a protein, such as alternative folding.

✓ ✗ I have explained how alternative splicing creates different polypeptides.[1]

✓ ✗ I have used key biological terminology such as: alternative splicing, exons.

Key science skills and ethical understanding

17 **a** [Water and oxygen.[1]]

✓ ✗ I have stated the products of the reaction.[1]

b [The shape of the active site of catalase is complementary and specific to hydrogen peroxide.[1]][This allows for catalase to catalyse the breakdown of hydrogen peroxide into water and oxygen.[2]]

✓ ✗ I have stated that their shapes are complementary.[1]

✓ ✗ I have stated the significance of their structures.[2]

✓ ✗ I have used key biological terminology such as: active site, complementary, catalyse, breakdown.

c [Flasks 2 and 3 serve as controls in the experiment. Flask 2 allows for a comparison to Flask 1 displaying the differences in reaction rate with the presence or absence of an enzyme.[1]][Flask 3 ensures that the enzyme catalase is responsible for the increased reaction rate, rather than any enzyme type.[2]]

✓ ✗ I have explained that the Flask 2 control allows for a comparison of enzyme to no enzyme.[1]

✓ ✗ I have explained that the Flask 3 control compares catalase to other enzymes.[2]

d [Integrity.[1]]

✓ ✗ I have stated they are ignoring the concept of integrity.[1]

3B Factors that affect enzymes

Theory review questions

1 B

2 III; IV; V; VI

3 W-substrate; X-active site; Y-coenzyme; Z-product

4 W-pH; X-enzyme/substrate concentration; Y-enzyme/substrate concentration; Z-temperature

5 II; V; VI; III; I; VII; IV

SAC skills questions

6 B **7** A **8** A **9** B

Exam-style questions

Within lesson

10 D **11** A **12** A **13** D

14 A **15** C **16** C **17** A

18 C **19** A

Multiple lessons

20 A

21 **a** [The substrate is glucose and the enzyme is hexokinase.[1]]

✓ ✗ I have identified the substrate and enzyme.[1]

b [The active site of hexokinase would have a complementary structure to glucose.[1]]

✓ ✗ I have stated that glucose has a complementary structure to the active site of hexokinase.[1]

c [ATP is the coenzyme which is used to provide energy for the enzyme-catalysed reaction converting glucose into glucose 6-phosphate to occur.[1]]

✓ ✗ I have described the role of ATP as a coenzyme in the reaction.[1]

Key science skills and ethical understanding

22 **a** [The graphs display blood testosterone before and after the ACTH test in normal individuals and those with CAH.[1]][Individuals with CAH had a slightly higher resting level of testosterone before the test.[2]][Following the ACTH test, CAH individuals had an increase in blood testosterone levels whereas the levels of normal individuals remained the same.[3]]

✓ ✗ I have described what the graph displays.[1]

✓ ✗ I have compared the results of the two groups before the test.[2]

✓ ✗ I have compared the results of the two groups after the test.[3]

✓ ✗ I have used comparative language such as: whereas.

b [The injection of ACTH in the test stimulates the biochemical pathway. In normal individuals, ACTH stimulation leads to the eventual production of cortisol, which in turn causes inhibition and the regulation of ACTH and therefore maintenance of testosterone levels.[1]][In CAH individuals the pathway is stimulated, however, the inhibited 21-hydroxylase does not produce cortisol and no inhibition occurs, leading to continued ACTH production and an increase in testosterone levels.[2]]

✓ ✗ I have explained the reasoning for the normal group's results.[1]

✓ ✗ I have explained the reasoning for the CAH group's results.[2]

c [This inhibition is likely to be competitive inhibition.[1]][Given the inhibitor shares extreme structural similarities with the substrate, it is likely that the inhibitor is competing to bind at the enzyme's active site.[2]]

✓ ✗ I have identified the type of inhibition as competitive.[1]

✓ ✗ I have justified my response by explaining that structural similarities between the substrate and inhibitor suggest that the inhibitor binds to the active site.[2]

Chapter 3 SAC practice

1 [Enzymes are critical for life as they lower the activation energy of virtually all chemical reactions in the body, speeding them up significantly. This includes reactions necessary for important functions such as digestion, respiration, and immunity.[1]][Without them, such reactions would take an extremely long time to occur, or never occur at all, meaning that the body would be unable to function altogether.[2]]

✓ ✗ I have described how enzymes speed up critical processes in the body.[1]

✓ ✗ I have explained that without them the reactions would take significantly longer or not occur.[2]

2 [Substrate is the name given to the reactant in an enzyme-catalysed reaction.[1]]

✓ ✗ I have stated a substrate is the name of the reactant in enzyme-catalysed reactions.[1]

3 [The enzyme-substrate complex is the name given to the structure formed when enzyme and substrate are bound together at the enzyme's active site.[1]]

✓ ✗ I have explained what the enzyme-substrate complex is.[1]

4 [Pancreatic lipase.[1]]

✓ ✗ I have stated pancreatic lipase.[1]

5 [The pepsin molecule would likely denature as a neutral pH of 7 is far greater than its optimum.[1]]

✓ ✗ I have identified that pepsin would most likely denature.[1]

6 [All of these enzymes would most likely not denature,[1]][given that temperatures below the optimal typically only slow enzyme functioning rather than denaturing them.[2]]

- I have stated that the enzymes would not denature.[1]
- I have described that temperatures below the optimal slow enzyme functioning rather than denaturing them.[2]

7 [Following the reaction, the coenzyme becomes unloaded and must be cycled back to a loaded form to facilitate further reactions.[1]]

- I have described coenzyme cycling.[1]

8 [As substrate concentration increases, the rate of reaction will increase up to a point where it will then remain constant and no longer increase.[1]]

- I have described that increasing substrate concentration will increase reaction rate up to a point where it remains constant.[1]

9 [For the drug to be a competitive inhibitor, its structure would have to be complementary to the structure of the enzyme's active site.[1]] [Additionally, as it is complementary to the active site, the structure of the drug would also share structural similarities with the substrate.[2]]

- I have explained that the structure of the drug would be complementary to the active site.[1]
- I have described how the structure of the drug would therefore be similar to the substrate.[2]

10 [In both graphs the overall trends were largely similar, with significantly more oxygen being produced at a high temperature in both trials.[1]] [The medium temperature generated the second-highest oxygen production and the low temperature produced the least. In the second trial, one value from the low temperature was significantly higher than the other values.[2]]

- I have stated that the high temperature produced the most oxygen in both trials.[1]
- I have explained the trends at medium and low temperatures.[2]

11 [In all three graphs very similar trends were observed. The neutral pH tests consistently produced significantly more oxygen than the other pH values.[1]][The acidic pH tests produced low levels of oxygen whereas the basic pH tests produced virtually no oxygen.[2]]

- I have stated that the neutral pH produced significantly more oxygen in all trials.[1]
- I have explained that the acidic pH produced some oxygen and the basic pH produced virtually no oxygen.[2]

12 [The error is a random error which impacts the precision of the results.[1]]

- I have stated that it is a random error and impacts precision.[1]

13 [The error occurred in Trial 3 in the basic pH value test, which impacts the precision of the results.[1]][Additionally, given that the results were consistently similar for the basic pH tests, this error could also impact on the accuracy of the results.[2]]

- I have identified the basic pH test in trial 3 as the error.[1]
- I have identified that this may also impact the accuracy of results.[2]

14 [Using Bridget's results, it can be concluded that the optimal temperature of Enzyme Q is closest to the high temperature used in the experiment, as it produced the most oxygen over the two minutes in both trials.[1]][Jarrod's results suggest that the optimal pH of Enzyme Q is closest to the neutral pH value used in the experiment, as it produced significantly more oxygen.[2]]

- I have identified the high temperature value as closest to the optimal of Enzyme Q.[1]
- I have identified the acidic pH value as closest to the optimal of Enzyme Q.[2]

Chapter 3 Exam practice

Section A

1 C	**2** D	**3** B	**4** A
5 D	**6** A	**7** B	**8** A
9 A	**10** A	**11** C	

Section B

12 a [Independent variable: pH of buffer.[1]][Dependent variable: % of oxygen in conical flask.[2]]

- I have identified the pH of buffer as the independent variable.[1]
- I have identified the percentage of oxygen in the flask as the dependent variable.[2]

b [The results do not support the students' hypothesis.[1]][The enzymes in flask 2 (high pH buffer) produced a flask filled with approximately 50% oxygen after five minutes. However the enzymes in flask 1 (neutral pH buffer) produced a flask filled with almost 75% oxygen after five minutes, disproving the students' hypothesis.[2]]

- I have stated the results do not support the hypothesis.[1]
- I have justified my answer.[2]
- I have used data from the experiment in my response.

c [A flask could be set up that contained only 50 mL of 3% hydrogen peroxide solution and 52 mL of distilled water. This control would determine the percentage of oxygen produced without the enzyme present.[1]][This would enable the students to determine the extent of oxygen production that is due to the enzyme's activity and not due to confounding variables.[2]]

Other acceptable responses include:

- A flask containing 50 mL of 3% hydrogen peroxide solution and 52 mL of distilled water (the 52 mL of distilled water is used to account for the 2 mL of enzyme solution used ensuring there is the same amount of room in the flask for oxygen. However, 50 mL would still produce a result of similar accuracy).

- I have described how a control could be implemented.[1]

✓ ✗ I have explained the function of this control flask.[2]

✓ ✗ I have used key biological terminology such as: hydrogen peroxide, distilled water, oxygen percentage, confounding variables.

d [The students' hypothesis was disproved as the highest oxygen production was seen in the neutral pH buffer.[1]][The students found that a neutral pH buffer solution resulted in a flask filled with 75% oxygen over 5 minutes due to the enzyme-catalyzed breakdown of hydrogen peroxide. The high pH buffer solution produced a 50% oxygen-filled flask, while the low pH flask produced no oxygen over 5 minutes.[2]][This suggests that the enzyme's optimal pH is closest to the neutral pH and that low pH values can denature the enzyme.[3]]

✓ ✗ I have stated that the hypothesis was not accurate.[1]

✓ ✗ I have stated how the enzyme performed in each flask, with reference to the variables identified in part a.[2]

✓ ✗ I have drawn a conclusion about the enzyme's activity.[3]

✓ ✗ I have used data in my response.

✓ ✗ I have used key biological terminology such as: enzyme-catalysed, breakdown, hydrogen peroxide, optimal, denature.

13 a [In PKU unaffected individuals, if PAH were to increase, fumarate and acetoacetate would also increase.[1]][This is because PAH catalyses the production of tyrosine, which is the substrate for the next reaction in the pathway, eventually leading to the production of fumarate and acetoacetate in the final reaction step.[2]]

✓ ✗ I have stated how the concentrations of PAH and fumarate and acetoacetate are linked.[1]

✓ ✗ I have explained how the production of PAH leads to the production of fumarate and acetoacetate.[2]

✓ ✗ I have used key biological terminology such as: PAH, fumarate, acetoacetate, catalyses, pathway.

b [Competitive inhibitors bind directly to an enzyme's active site, blocking it from any substrate trying to bind and undergo a reaction.[1]][On the other hand, non-competitive inhibitors bind to an enzyme at a site that is not the active site, causing a conformational change in the active site and the hindrance of enzyme-catalysed reactions.[2]]

✓ ✗ I have explained how competitive inhibitors impact an enzyme.[1]

✓ ✗ I have explained how non-competitive inhibitors impact an enzyme.[2]

✓ ✗ I have used comparative language such as: on the other hand.

c [The presence of a PAH inhibitor would mean the baby is more likely to suffer from the symptoms of PKU.[1]][If PAH were to be impacted by a competitive inhibitor, there would be decreased conversion, or no conversion, of phenylalanine to tyrosine, resulting in a buildup of phenylalanine and a greater risk of PKU symptoms.[2]]

✓ ✗ I have stated whether they would be more or less likely to suffer from PKU symptoms.[1]

✓ ✗ I have described how the inhibitor impacting PAH relates to the chance of suffering from PKU symptoms.[2]

✓ ✗ I have used key correct biological terminology such as: PAH, PKU, inhibitor, phenylalanine, symptoms.

14 a [The results show that Test 2 had an increased rate of oxygen production and finished with an overall higher oxygen volume when compared to Test 1.[1]][Tests 1 and 2 produced 0.5 cm^3 and 1.0 cm^3 of oxygen gas over 5 minutes, respectively, and both tests were still producing oxygen at the end of the 5 minute period.[2]]

✓ ✗ I have stated that Test 2 had greater oxygen production.[1]

✓ ✗ I have explained the trends of the results.[2]

✓ ✗ I have used data in my response.

b [The results support Student B's statement more than Student A's statement. This is because we know that the optimum pH of catalase is 7, which is the pH that both tests were conducted at.
This means that if the buffer's pH was changed and every other factor was kept constant, we would not see greater activity of the enzyme like we do in Test 2 on the graph.[1]][The optimum temperature of catalase is unknown, therefore the results of Test 2 could be from a change in temperature.[2]]

✓ ✗ I have explained why Student A's statement could not cause these results.[1]

✓ ✗ I have justified how Student B's statement could cause these results.[2]

✓ ✗ I have used key biological terminology such as: optimum, pH, activity, temperature.

c i [Approximately 37 °C.[1]]

Other acceptable responses include:

- Human body temperature

✓ ✗ I have stated what the optimum temperature of human catalase would be.[1]

ii [At catalase's optimum temperature, molecules move faster, increasing the number of collisions between molecules to form the enzyme-substrate complex. However, it is not hot enough to denature the enzyme.[1]]

✓ ✗ I have explained how temperature affects the number of enzymatic reactions.[1]

✓ ✗ I have used key biological terminology such as: optimum, catalyse, catalase, collisions, denature.

d [Test 3 would lie below Tests 1 and 2 on the graph, in both the steepness of the slope and final value after 5 minutes.[1]][This is because the 20 °C experimental temperature of Tests 1 and 2 is much closer to catalase's optimal temperature of 37 °C than the 5 °C temperature Test 3 was conducted at. Consequently, the lowest enzyme activity and line on the graph would correspond to Test 3.[2]]

✓ ✗ I have described the third line's slope and endpoint.[1]

✓ ✗ I have justified why the line would be here by referring to the lowered enzyme activity.[2]

4A Enzymes that manipulate DNA

Theory review questions

1 A

2 ligases; endonucleases; polymerases

3 Sticky end: I; III; V
Blunt end: II; IV

4 B

SAC skills questions

5 B **6** C **7** A **8** C

9 B

Exam-style questions

Within lesson

10 D **11** B **12** D **13** A

Multiple lessons

14 a [Transcription.[1]]

✓ ✗ I have named transcription as the process.[1]

b [The ribosome binds to and reads the mRNA of the GFP gene, initiating translation.[1]][The tRNA delivers specific amino acids to the ribosomes, as prescribed by the order of codons on the mRNA.[2]][Specific amino acids are joined until a stop codon is reached, the translation is terminated, and the GFP polypeptide chain is released.[3]]

✓ ✗ I have stated that the ribosome binds to and reads the mRNA of the GFP gene.[1]

✓ ✗ I have explained that tRNA delivers specific amino acids to the ribosome.[2]

✓ ✗ I have described the elongation and termination process.[3]

✓ ✗ I have referred to GFP in my response.

✓ ✗ I have used appropriate biological terminology such as: mRNA, tRNA, ribosome, codon, amino acid, termination.

c [Restriction endonuclease.[1]]

✓ ✗ I have identified endonuclease as the correct enzyme.[1]

d [To join fragments of DNA together.[1]]

✓ ✗ I have described the role of DNA ligase.[1]

Key science skills and ethical understanding

15 C

16 a [Polymerase.[1]]

Other acceptable responses include:

- DNA polymerase

✓ ✗ I have identified polymerase as the enzyme.[1]

b i [Sticky end restriction endonucleases cleave DNA with overhanging additional nucleotides.[1]][These overhanging sections are capable of joining with complementary fragments together in the correct orientation.[2]]

✓ ✗ I have explained the role of sticky end restriction endonucleases.[1]

✓ ✗ I have explained how overhanging nucleotides are useful.[2]

ii [SalI, HindIII, and ClaI.[1]]

✓ ✗ I have stated the correct sticky end restriction endonucleases.[1]

c i [The test tube without an enzyme acts as a control,[1]][serving as a comparison group and ensuring that the changes in the other test tubes are due to the independent variable (restriction endonuclease) rather than an uncontrolled variable to improve validity.[2]]

✓ ✗ I have labelled the test tube as a control.[1]

✓ ✗ I have explained the purpose of a control.[2]

✓ ✗ I have referred to the scenario in my response.

ii [The concentration[1]][and the volume of the restriction endonuclease in each sample.[2]]

Other acceptable responses include:

- The temperature of each test tube during incubation.

✓ ✗ I have identified one controlled variable.[1]

✓ ✗ I have identified a second controlled variable.[2]

iii [Sunitha is correct as one recognition site for HindIII is present in the DNA sample.[1]]

✓ ✗ I have explained why Sunitha is correct.[1]

d i [They cannot identify the enzyme as its recognition site does not match any of the known samples that they completed.[1]]

✓ ✗ I have explained that the recognition site is different to the other known enzymes.[1]

ii [The restriction endonuclease must produce sticky ends as there are overhanging nucleotides.[1]][The recognition site for this enzyme is 5′ G G A T C C 3′ and the cut occurs between the two guanine nucleotides.[2]]

✓ ✗ I have identified the type of restriction endonuclease.[1]

✓ ✗ I have stated the recognition site.[2]

4B CRISPR-Cas9

Theory review questions

1 B

2 CRISPR; two; blunt

3 A

4 X-viral DNA; Y-gRNA; Z-Cas9

5 I; II; III

6 C

SAC skills questions

7 B **8** D **9** A **10** B

Exam-style questions

Within lesson

11 D **12** B

Multiple lessons

13 D

14 a [Ribose sugar, phosphate, and nitrogenous bases.[1]]

✓ ✗ I have stated the three components of RNA.[1]

✓ ✗ I have used key biological terminology such as: ribose.

b [As Cas9 is an enzyme, the reaction rate is greatest at the optimum temperature.[1]][As temperature increases above the optimum, the enzyme will begin to denature, significantly decreasing the rate of reaction. If you decrease the temperature below its optimum, there will be less kinetic energy and thus, the rate of reaction will slow.[2]]

✓ ✗ I have stated that the reaction rate is greatest at the optimum temperature.[1]

✓ ✗ I have explained what will happen if the temperature increases and decreases from the optimum.[2]

c [CRISPR-Cas9 cuts target DNA, according to the complementary sequence provided by gRNA, to combat bacteriophage invasion.[1]]

✓ ✗ I have outlined the role of CRISPR-Cas9 in prokaryotes.[1]

d i [An endonuclease that cuts DNA at a specific recognition site.[1]]

✓ ✗ I have defined restriction endonuclease.[1]

ii [Cas9 is versatile in that it can be programmed to target any specific sequence dictated by a piece of gRNA,[1]][whereas restriction endonucleases will only act on their specific restriction site, which cannot be programmed.[2]]

✓ ✗ I have stated an advantage of Cas9.[1]

✓ ✗ I have compared this to restriction endonucleases.[2]

✓ ✗ I have used comparative language such as: whereas.

e i [Through alternative splicing, particular exons can be included or excluded from a final gene product.[1]][This can alter the translated sequence resulting in different functional proteins.[2]]

✓ ✗ I have explained how alternative splicing works.[1]

✓ ✗ I have explained how this changes protein function.[2]

ii [Through gene knockout, an intron could be disrupted[1]][and any changes can be observed to determine function.[2]]

Other acceptable responses include:

- Attaching fluorescent probes to Cas9 allows scientists to locate gene locations within a genome.

✓ ✗ I have described how CRISPR-Cas9 can assist in the scientific research of introns.[1]

✓ ✗ I have explained what findings can result from this.[2]

Key science skills and ethical understanding

15 a [To reduce the number of embryos destroyed.[1]]

✓ ✗ I have explained why a smaller sample size would be appropriate.[1]

b [As the technology is still being studied, it would be unethical to birth the embryo as there could be unforeseen harmful effects to the mother or the embryo.[1]]

✓ ✗ I have explained why the embryo should not be developed further.[1]

c [The experiment was completed *ex vivo* instead of *in vivo* to create a controlled environment and prevent the influence of other factors on the experiment and prevent potentially harmful side effects on the subject.[1]][The experiment was performed on single-celled embryos so the entire organism has the altered gene, instead of single cells, which would be the case if it was performed in adults.[2]]

✓ ✗ I have explained why *ex vivo* is more appropriate than *in vivo*.[1]

✓ ✗ I have explained why using embryos is more appropriate than adults.[2]

d [Quantitative data is obtained as each sample is given a binary score of successful or unsuccessful.[1]][This data provides objectivity and accuracy in the results of the experiment.[2]]

✓ ✗ I have justified why it is quantitative data.[1]

✓ ✗ I have explained the benefit of quantitative data.[2]

4C The polymerase chain reaction

Theory review questions

1 A

2 denaturation; 50–55; elongation; 72

3 D

4 C

5 II; IV; III; I

SAC skills questions

6 A **7** A **8** B **9** C

Exam-style questions

Within lesson

10 D **11** A

12 **a** [The polymerase chain reaction amplifies a sample of DNA to increase the quantity of DNA available.[1]]

✓ ✗ I have explained the purpose of the polymerase chain reaction.[1]

b [DNA is heated to approximately 90–95 °C to denature and separate double-stranded DNA into single-stranded DNA.[1]]

✓ ✗ I have outlined the process of Stage 1 and stated the temperature it occurs at.[1]

✓ ✗ I have used key biological terminology such as: denature.

c [DNA primers are added to the mixture to bind to complementary nucleotide sequences at the 5′ ends of each single-stranded piece of DNA.[1]][This provides *Taq* polymerase with a binding site to begin building a complementary strand.[2]]

✓ ✗ I have stated what primers do in the annealing stage.[1]

✓ ✗ I have explained why they are necessary.[2]

d [*Taq* polymerase has a very high optimal temperature, working optimally at 72 °C,[1]][whereas human DNA polymerase would denature and be incapable of synthesising a new strand at this temperature.[2]]

✓ ✗ I have stated why *Taq* polymerase is used.[1]

✓ ✗ I have stated why human DNA polymerase is not used.[2]

Multiple lessons

13 **a** [Restriction endonucleases cut DNA fragments at specific recognition sites.[1]]

✓ ✗ I have explained the role of restriction endonucleases.[1]

b [As temperature increases above optimal, the enzyme begins to denature, creating a conformational change in the active site and decreasing the rate of reaction.[1]][As temperature decreases below optimal, there is not enough kinetic energy for the enzyme to function efficiently, thereby decreasing the rate of reaction.[2]]

✓ ✗ I have explained what occurs when the temperature is too high.[1]

✓ ✗ I have explained what occurs when the temperature is too low.[2]

✓ ✗ I have used key biological terminology such as: optimal, denature, conformational change, active site, kinetic energy.

c [*Taq* polymerase.[1]]

✓ ✗ I have identified *Taq* polymerase as this enzyme.[1]

d [pH.[1]]

Other acceptable responses include:

- Sufficient concentration of substrate available.

✓ ✗ I have stated a factor that needs to be considered for optimal enzyme function.[1]

Key science skills and ethical understanding

14 **a** **i** [Polymerase chain reaction.[1]]

✓ ✗ I have named the process.[1]

ii [In the denaturation stage, the mixture is heated to approximately 94 °C to denature the DNA and separate the strands.[1]][In the annealing stage, DNA is cooled to approximately 55 °C to allow primers to attach to the single-stranded DNA.[2]][In the elongation stage, the DNA is heated to 72 °C and *Taq* polymerase copies the strands.[3]][The cycle is then repeated to produce more copies.[4]]

✓ ✗ I have explained the denaturation stage.[1]

✓ ✗ I have explained the annealing stage.[2]

✓ ✗ I have explained the elongation stage.[3]

✓ ✗ I have stated that the cycle is repeated.[4]

✓ ✗ I have used key biological terminology such as: denature, anneal, primers, elongation, *Taq* polymerase.

b **i** [Mohammad's method was unsuccessful, as the temperature he applied for the first step of the polymerase chain reaction was far too low and would not have denatured the double-stranded DNA.[1]]

✓ ✗ I have justified why Mohammad was unsuccessful.[1]

ii [Systematic error.[1]]

✓ ✗ I have identified the type of error that occurred.[1]

iii [They must calibrate their machine so their temperatures are accurate.[1]]

Other acceptable responses include:

- Account for heat loss.

✓ ✗ I have identified a possible factor that could influence the experiment.[1]

c [Primers attach to complementary DNA nucleotides.[1]][The addition of the primer and the joining to complementary nucleotides then allows the *Taq* polymerase to begin copying.[2]][Two primers are needed as the nucleotide sequence at the 5′ end of the coding and template strands are different[3]]

- I have stated what primers do.[1]
- I have explained why this is important for PCR.[2]
- I have explained why two primers are necessary.[3]
- I have used key biological terminology such as: complementary, *Taq* polymerase, nucleotide sequence, coding strand, template strand.

d [Integrity.[1]]

- I have identified the bioethical concept.[1]

4D Gel electrophoresis

Theory review questions

1 B

2 C

3 agarose; buffer; negative; negative; ethidium bromide; UV

4 P-well; Q-lane; R-agarose; S-band

SAC skills questions

5 A **6** A **7** C **8** B

9 B

Exam-style questions

Within lesson

10 D **11** A **12** D **13** C

14 a [Differing molecular size[1]][and a negative charge.[2]]

- I have stated that different molecular size allows DNA to be separated.[1]
- I have stated that having a negative charge allows DNA to be separated.[2]
- I have used comparative language such as: differing.

b [The viscosity of agarose in the gel.[1]]

Other acceptable responses include:

- Voltage or power applied to the gel.
- Buffer concentration.

- I have identified a factor influencing the rate of movement of DNA in a gel.[1]

c i [The individuals corresponding to lanes 2 and 4 are homozygous for the D18S51 STR and so contain twice the amount of DNA for that allele as all their fragments are of equal size, resulting in a thicker band.[1]][The lanes with two bands contain DNA samples from heterozygous individuals and so contain fragments of two different sizes.[2]]

- I have explained why there is only one band in some lanes.[1]
- I have explained why there are two bands in some lanes.[2]
- I have used key biological terminology such as: heterozygous, homozygous.

ii [Four.[1]]

- I have stated the correct number of alleles.[1]

iii [Fragment A.[1]]

- I have stated the correct fragment.[1]

iv [The suspect whose DNA sample is found in lane 6.[1]][The bands in this lane are identical to the bands in the blood sample found on the victim in lane 3, and are therefore likely the same DNA, identifying this suspect as the perpetrator.[2]]

- I have identified the correct suspect.[1]
- I have explained my reasoning.[2]

Multiple lessons

15 C **16** C **17** D **18** B

Key science skills and ethical understanding

19 a [The sample could be contaminated.[1]]

- I have suggested a possible reason why the results for Sample 3 are different.[1]

b i [Sample B is Riku's mother. Sample C is his maternal grandfather. Sample D is his father.[1]]

- I have correctly identified the three samples in the gel.[1]

ii [A standard ladder allows for the measurement of fragment length by comparing the distance it has moved through the gel to a series of fragments with a known size.[1]]

- I have stated the purpose of standard ladders.[1]

iii [A standard ladder would not help this experiment as the purpose is to compare the relatedness between individuals by assessing the band present in the same gel rather than calculating the size of the fragments.[1]]

- I have stated why a standard ladder is not necessary.[1]

c [Riku must make sure the electrophoresis equipment is turned off to avoid electrocution[1]][and wear safety glasses to protect the eyes.[2]]

Other acceptable responses include:

- Wearing gloves to avoid contamination by DNA or the chemicals being used.
- Correct disposal of materials.

- I have outlined one safety measure Riku should follow.[1]
- I have outlined a second safety measure Riku should follow.[2]

d [Respect.[1]]

✓ ✗ I have identified the correct bioethical concept.[1]

4E Recombination and Transformation

Theory review questions

1 A

2 M-recombinant plasmid; N-transformed bacteria; O-untransformed bacteria

3 II, IV, V, I, III

4 A

SAC skills questions

5 A **6** A **7** B **8** A

9 B **10** B

Exam-style questions

Within lesson

11 D **12** B **13** D **14** C

15 D

Multiple lessons

16 **a** ['Transform' refers to the uptake of plasmids by bacteria.[1]]

✓ ✗ I have stated that transformation is the uptake of plasmids by bacteria.[1]

✓ ✗ I have used appropriate biological terminology such as: plasmid, bacteria.

b **i** [Restriction endonucleases are used to cut the plasmid vector and the gene of interest, producing complementary 'sticky ends' that allow the gene to be inserted into the plasmid.[1]]

✓ ✗ I have described the use of restriction endonucleases in making recombinant plasmids.[1]

✓ ✗ I have used appropriate biological terminology such as: plasmid, vector.

ii [DNA ligase joins the gene of interest and plasmid vector together by forming phosphodiester bonds between each DNA sugar-phosphate backbone.[1]]

✓ ✗ I have stated that DNA ligase joins the gene of interest to the plasmid vector.[1]

✓ ✗ I have used appropriate biological terminology such as: sugar-phosphate backbone, plasmid, vector.

c [The scientists should only observe colonies of transformed bacteria on the nutrient agar.[1]][Transformed bacteria should form colonies because they contain the *tcl* gene, conferring resistance to tetracycline in the culture.[2]][The untransformed bacteria do not contain the *tcl* gene, which means they would be unable to form colonies and would instead die.[3]]

✓ ✗ I have stated that transformed bacteria would form colonies.[1]

✓ ✗ I have explained why transformed bacteria would form colonies.[2]

✓ ✗ I have explained that untransformed bacteria would not form colonies.[3]

✓ ✗ I have used appropriate biological terminology such as: transformed, culture, resistance.

d [The genetic code is universal, meaning that a human gene can be expressed by bacteria and its downstream protein can be produced, despite the gene coming from a different species.[1]]

✓ ✗ I have explained the meaning of the term 'universal' with respect to the genetic code.[1]

Key science skills and ethical understanding

17 B **18** C **19** D

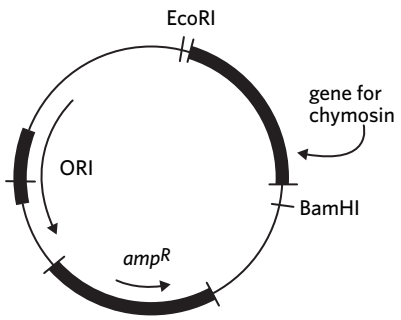
20 **a** [The *E. coli* strain is unable to cause disease and contains foreign DNA.[1]]

✓ ✗ I have explained what is meant by the terms non-pathogenic and recombinant.[1]

b [Polymerase chain reaction.[1]]

✓ ✗ I have stated the polymerase chain reaction.[1]

c



✓ ✗ I have drawn and labelled the chymosin gene where the HindIII recognition site should be located.

d [The vector in this experiment is the plasmid[1]][since it is needed to transport the foreign chymosin gene into the bacteria.[2]]

✓ ✗ I have identified the plasmid as the vector.[1]

✓ ✗ I have justified my response by referring to a vector as a means of transferring a foreign gene into a cell.[2]

e i [The bacterial growth on plate A would form a lawn as the bacteria on this plate are untransformed bacteria, which readily grow on nutrient agar.[1]][There would be no growth on plate B as these bacteria have not been transformed and are not ampicillin-resistant. Therefore, they would be unable to grow on ampicillin-containing nutrient agar.[2]][The bacterial growth on plate C would form a lawn. Both transformed and untransformed bacteria would be able to grow since this plate contains only nutrient agar.[3]] [The bacteria on plate D would form colonies. Bacteria in these colonies have been transformed and contain the ampicillin-resistance gene and so will still be able to grow on ampicillin-containing agar. Untransformed bacteria would not survive since they do not contain the ampicillin-resistance gene.[4]]

- ✓ ✗ I have explained that all bacteria are able to grow on nutrient agar.[1]
- ✓ ✗ I have explained that untransformed bacteria are not able to grow on ampicillin-containing agar.[2]
- ✓ ✗ I have explained that all bacteria are able to grow on nutrient agar.[3]
- ✓ ✗ I have explained that only transformed bacteria are able to grow on ampicillin-containing agar.[4]
- ✓ ✗ I have used appropriate biological terminology such as: lawn, colony, transformed.

ii [Plate D contains *E. coli* that have been exposed to recombinant plasmids and have been heat-shocked, meaning that it will contain only transformed bacteria[1]][since the ampicillin will kill off any untransformed bacteria.[2]]

- ✓ ✗ I have identified plate D.[1]
- ✓ ✗ I have justified my answer by referring to antibiotic selection.[2]

iii [Plate A and B are controls that act as comparison groups.[1]] [Plate A shows that untransformed bacteria are able to grow on nutrient agar. Plate B shows that untransformed bacteria are unable to survive on ampicillin-containing media. Based on the results of the controls, we can reasonably conclude that the bacterial colonies on plate D must contain transformed bacteria.[2]]

- ✓ ✗ I have identified plates A and B as controls.[1]
- ✓ ✗ I have explained the purposes of plate A and B.[2]

f [Non-maleficence.[1]]

- ✓ ✗ I have identified the supported bioethical concept.[1]

4F Genetic engineering

Theory review questions

1 C

2 I-cisgenic organism; II-genetic engineering; III-transgene; IV-host organism

3 III; IV; II; I

4 C

5 Non-GMO: I; III
GMO: II; IV; V; VI
TGO: V; VI

SAC skills questions

6 B **7** B **8** A **9** C

Exam-style questions

Within lesson

10 D **11** A **12** B **13** B

14 A **15** D

16 a [A genetically modified organism is any organism that has had its DNA altered using genetic engineering technology. The cotton plants have been genetically modified via *Agrobacterium* to include a gene that was not originally part of their genome.[1]][A transgenic organism is a specific type of GMO that contains foreign genetic material from a separate species. All TGOs are GMOs. The cotton plant is therefore also transgenic given that the introduced gene was taken from a strain of bacteria.[2]]

- ✓ ✗ I have explained why the cotton plant is a GMO.[1]
- ✓ ✗ I have explained why the cotton plant is a TGO.[2]

b [One economic advantage of using Roundup Ready™ Cotton is that it reduces the time and costs associated with crop loss via exposure to Roundup™ herbicide.[1]]

- ✓ ✗ I have identified one economic advantage of using Roundup Ready™ Cotton.[1]

Multiple lessons

17 a [It is assumed that there will be a decrease in the number of *A. aegypti* mosquitoes,[1]][given that the offspring of introduced GM males will die before reproductive age.[2]]

- ✓ ✗ I have explained that there will be a decrease in the *A. aegypti* mosquito population size.[1]
- ✓ ✗ I have linked this to the information provided.[2]

b [The genetic code is universal, which means that the codons in mRNA code for the same amino acids and produce the same protein irrespective of the species.[1]]

- ✓ ✗ I have explained that the genetic code is universal.[1]

18 a [Inserting the gene from the bacteria may give algae the ability to tolerate warm and highly acidic conditions.[1]][Such algae will be less likely to leave the coral tissues due to environmental changes such as increased temperature and water acidity, thus reducing susceptibility to bleaching.[2]]

- I have explained the effect of the gene insertion on algae.[1]
- I have explained how such algae will reverse coral bleaching.[2]
- I have referred to the scenario in my response.

b [Less coral bleaching would result in increased tourism to the Great Barrier Reef.[1]]

Other acceptable responses include:

- Public outcry due to introduced GM algae may cause people to boycott the Great Barrier Reef and reduce tourism.
- Reducing coral bleaching will improve public morale.

- I have suggested a social implication of creating GM coral to reverse coral bleaching.[1]

c [This is not considered genetic modification since there is no alteration to any organism's DNA using genetic engineering technologies.[1]][This is simply just introducing a distinct algae population from one environment to another.[2]]

- I have correctly explained why this approach is not genetic modification.[1]
- I have justified my answer by referring to the scenario.[2]

Key science skills and ethical understanding

19 a [The independent variable is treating citrus trees with the viral vector,[1]][whilst the dependent variable is the presence/absence of citrus greening.[2]]

- I have identified the independent variable.[1]
- I have identified the dependent variable.[2]

b [Citrus trees containing the viral vector will be less affected by citrus greening than trees without the viral vector.[1]]

- I have stated a plausible hypothesis.[1]
- I have referred to both the independent and dependent variable.

c [The genetic engineers need to use a sufficiently large number of trees for both the treatment and control groups.[1]][They should also ensure that as many variables are controlled for as possible, such as the trees in each group being kept in the same environmental conditions (e.g. light and water).[2]]

Other acceptable responses include:

- The same number of incisions are made in each tree.
- The same amount of viral vector is delivered into each tree in the treatment group.
- All trees are treated at the same time.

- I have outlined one measure that would increase the reliability of this experiment.[1]
- I have outlined a second measure that would increase the reliability of this experiment.[2]
- I have referred to the scenario in each of my answers.

d [Non-maleficence prioritises the minimisation of any unnecessary harm resulting from a course of action.[1]][In this case, extensive field trials help minimise unnecessary harm in that they help assess the effect of the GM trees on an ecosystem, such as on pollinators and soil, and these results can inform harm minimisation strategies.[2]]

- I have outlined the concept of non-maleficence.[1]
- I have used the concept of non-maleficence to demonstrate the importance of extensive field trials.[2]

Chapter 4 SAC practice

1 [Human growth hormone is responsible for growth and development, cellular repair, and metabolism.[1]]

- I have outlined the function of hGH.[1]

2 [A blood test could be obtained to measure levels of hGH in the blood of the patient under investigation and compare it to the normal range observed in patients with otherwise similar characteristics (e.g. age, sex).[1]]

- I have outlined a test that would distinguish hGH deficiency from other growth disorders.[1]

3 [The bioethical concept of justice involves ensuring that there is no unfair treatment or burden on a particular group from an action.[1]][hGH consumption leads to unfair treatment as athletes who have the money to enhance their performance this way, and the motivation to break the rules, will perform better than their rule-following counterparts.[2]]

- I have defined justice.[1]
- I have explained how the use of hGH in sport does not follow the bioethical concept of justice.[2]

4 [In the denaturing stage, the mixture is heated to approximately 94 °C to break hydrogen bonds and denature the double-stranded DNA, separating the strands in the sample.[1]][In the annealing stage, the DNA sample is cooled to approximately 55 °C to allow primers that flank the target sequence to form hydrogen bonds with, and attach to, the single-stranded DNA.[2]][In the elongation stage, the DNA sample is heated to 72 °C and *Taq* polymerase copies the strands by adding complementary nucleotides, extending the primers.[3]][The cycle is then repeated to produce more copies of the target DNA sequence.[4]]

- I have outlined the denaturing stage of the polymerase chain reaction.[1]
- I have outlined the annealing stage of the polymerase chain reaction.[2]
- I have outlined the elongation stage of the polymerase chain reaction.[3]
- I have explained that the process must be repeated.[4]

5 [The bacterium that takes up this plasmid will be resistant to ampicillin due to the presence of the ampicillin resistance gene *amp*^{R}.[1]][However, the bacterium will not be resistant to tetracycline as the *GH1* gene has been inserted inside the *tcI* gene, disrupting its complete transcription and preventing the production of the *tcI* gene product that would otherwise confer tetracycline resistance.[2]]

I have stated that the bacterium will be resistant to ampicillin.[1]

I have explained why the bacterium will not be resistant to tetracycline with reference to the diagram.[2]

6 [The BamHI restriction endonuclease is used to cut the *GH1* gene and plasmid vector to create complementary sticky ends,[1]][which can then be joined together using DNA ligase to repair the double-stranded DNA sugar-phosphate backbone.[2]]

I have outlined how restriction endonucleases are used to make a recombinant plasmid.[1]

I have outlined how DNA ligase is used to make a recombinant plasmid.[2]

I have used key biological terminology such as: restriction endonuclease, plasmid, vector, sticky end, ligase.

7 [If the restriction site is already known, time can be saved using a restriction endonuclease instead of producing a gRNA and Cas9.[1]]

Other acceptable responses include:

- If the process is well understood and has been safely replicated multiple times, then it may be safer and less risky than new technologies like CRISPR-Cas9.

I have explained why restriction endonucleases may be more advantageous than CRISPR-Cas9.[1]

8 [Bands C and D.[1]]

I have identified which bands have not been cut by the restriction endonuclease.[1]

9 [Bands A and C both represent fragments that include the gene of interest, with A being those cut with a restriction endonuclease, and C being those that are super coiled.[1]][Ultimately, both bands A and C are significantly smaller than bands B and D, indicating that the majority of plasmids did not take up the gene of interest.[2]]

I have explained why bands A and C represent plasmids with the gene of interest.[1]

I have explained that the majority of plasmids have not taken up the gene of interest.[2]

10 [Heat shocking bacteria aims to increase the uptake of plasmids by bacteria.[1]][The rapid change in temperature is responsible for increasing the permeability of the plasma membrane to DNA, promoting transformation.[2]]

I have explained the purpose of heat shocking.[1]

I have explained how heat shocking increases bacterial transformation.[2]

11 [A virtues-based approach to bioethics focuses on doing what is 'good' according to values such as kindness and compassion.[1]][It would be more compassionate, and therefore more appropriate, to use recombinant hGH produced by bacteria rather than hGH sourced from animals, as this method avoids the killing of complex animals such as pigs.[2]]

I have defined a virtues-based approach.[1]

I have explained why recombinant hGH is more appropriate.[2]

12 [It is possible that the pig GH is not identical to hGH and therefore would be less effective than naturally-produced hGH.[1]]

Other acceptable responses include:

- There could be unknown side-effects from human consumption of pig GH.

I have outlined a biological implication of pig growth hormone.[1]

Chapter 4 Exam practice

Section A

1 C	**2** A	**3** B	**4** C
5 D	**6** B	**7** B	**8** C
9 A	**10** C	**11** D	**12** B
13 C	**14** A	**15** C	

Section B

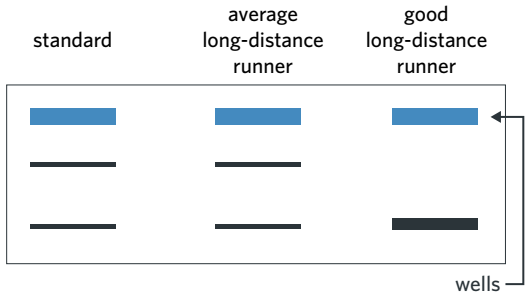
16 a [The temperature must be reduced in the annealing stage to allow for hydrogen bonds to form between the primer and the complementary region of the single strand of DNA.[1]][The temperature is then increased to 72 °C during the elongation stage as this is the optimal temperature for *Taq* polymerase[2]][to extend the primer and create a new strand of DNA.[3]]

I have outlined why temperature is decreased for the annealing stage of the polymerase chain reaction.[1]

I have explained that 72 °C is the optimal temperature for *Taq* polymerase.[2]

I have outlined the function of *Taq* polymerase.[3]

b



✓ ✗ I have drawn a gel with wells at the top.

✓ ✗ I have labelled the three lanes.

✓ ✗ I have included the standard bands.

✓ ✗ I have drawn all bands in the correct place

c [The average long-distance runner is heterozygous at the locus of the *ACTN3* gene and should have one band corresponding to the protein products of each of the *577R* and *577X* alleles in the standard.[1]][The good long-distance runner is homozygous for the shorter *577X* allele and should therefore only show one thicker band corresponding to the shorter truncated protein product from the stop codon mutation, which is positioned further away from the well as smaller proteins will travel faster through an agarose gel.[2]]

✓ ✗ I have explained what a heterozygous lane looks like.[1]

✓ ✗ I have explained what a homozygous lane looks like.[2]

✓ ✗ I have referred to the standard.

✓ ✗ I have used appropriate biological terminology such as: homozygous, heterozygous, protein, allele.

d [The standard ladder contains proteins of the same length as the protein products of the known alleles (*577R* and *577X*).[1]][It is used to confirm which band from the athletes' samples corresponds to which allele's protein product.[2]]

✓ ✗ I have identified that the standard ladder shows the placement of proteins of the same length as the *ACTN3* protein products in the gel.[1]

✓ ✗ I have explained that the standard is necessary to identify proteins encoded by specific alleles.[2]

17 a [Drought-tolerant corn would have increased survival during droughts. This increases crop productivity since there would be fewer crops dying from dry weather conditions.[1]]

✓ ✗ I have explained the effect drought-tolerant corn would have on crop productivity.[1]

b i [Scientists could insert the three drought tolerance genes (*NOT*, *TOO*, and *HOT*) into the genome of *Z. mays* to create a transgenic crop with the drought tolerance trait.[1]]

Other acceptable responses include:

- Using genetic engineering technologies, such as CRISPR-Cas9, to insert the three drought tolerance genes.

✓ ✗ I have suggested inserting the three genes (*NOT*, *TOO*, and *HOT*) into *Z. mays*.[1]

ii [It is unknown if the three genes will be properly taken up by *Z. mays*. This risk may not make it worthwhile for farmers to buy the genetically modified crop.[1]]

Other acceptable responses include:

- Without further research, it is unknown if these three genes alone give *Z. mays* drought tolerance.
- Inserting these genes may result in off-target effects on *Z. mays*.

✓ ✗ I have explained a potential consequence of using genetically modified *Z. mays*.[1]

c i [The Cas9 target site can be altered by changing the gRNA, whereas endonucleases can only recognise one site.[1]]

Other acceptable responses include:

- Cas9's target site of approximately 20 bp makes it more specific than the 6 bp from restriction endonucleases.
- CRISPR is a cheap and fast way to genetically modify organisms.

✓ ✗ I have stated one advantage of using CRISPR-Cas9 over restriction endonucleases.[1]

ii [CRISPR-Cas9 can knock out a gene by deleting nucleotides.[1]][Alternatively, if additional nucleotides are also introduced to the cells, they can be added into the genome at the location where Cas9 cuts.[2]]

✓ ✗ I have stated that nucleotides can be deleted.[1]

✓ ✗ I have stated that nucleotides can be added in.[2]

18 a [The gene for dystrophin is cut from human DNA and inserted into plasmids that have been digested with the same restriction endonucleases, and the sugar-phosphate backbone is repaired by DNA ligase.[1]][The recombinant plasmids are then introduced to bacteria via a method such as heat shock therapy or electroporation. Only some bacteria will take up the recombinant plasmids, and these are selected for using antibiotics.[2]][Transformed bacteria are cultured, and will then transcribe and translate the new gene to produce human dystrophin, which scientists can extract for use.[3]]

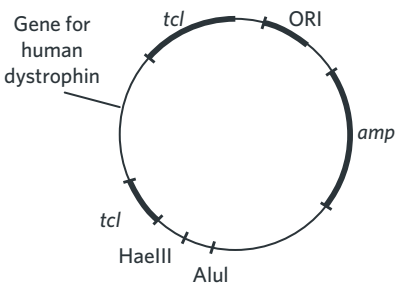
✓ ✗ I have explained that genes are inserted into plasmids.[1]

✓ ✗ I have stated that the recombinant plasmids are introduced into the bacteria.[2]

✓ ✗ I have explained the processes that transformed bacteria will undergo to produce the new proteins.[3]

✓ ✗ I have used appropriate biological terminology such as: restriction endonuclease, DNA ligase, recombinant plasmid, transformed bacteria.

b i



✓ ✗ I have drawn a circular plasmid.

✓ ✗ I have labelled the recognition sites for HaeIII and AluI.

✓ ✗ I have indicated the location of the other genes on the plasmid.

✓ ✗ I have labelled the gene for human dystrophin.

ii [The culture of bacteria is grown on a nutrient agar plate containing ampicillin to kill the untransformed bacteria.[1]] [However, bacteria that have taken up the recombinant plasmid with the human dystrophin gene have also acquired the *amp* gene, and therefore have resistance to ampicillin. This screening method enables scientists to select for these transformed bacteria.[2]]

- ✓ ✗ I have explained what should be included on the agar plate.[1]
- ✓ ✗ I have explained that transformed bacteria contain both the human dystrophin gene and the *amp* gene which confers ampicillin resistance.[2]
- ✓ ✗ I have not stated that the bacteria are selected for using tetracycline.

c i [EcoRI is a sticky end restriction endonuclease, which means that after each cut there will be overhanging nucleotides.[1]] [This is different to the blunt end restriction endonucleases, which leave DNA fragments with no overhanging nucleotides.[2]]

- ✓ ✗ I have described the features of this restriction endonuclease.[1]
- ✓ ✗ I have compared EcoRI to the other restriction endonucleases.[2]

ii [The use of EcoRI to cut the plasmid results in complementary sets of overhanging nucleotides, which means that other fragments of DNA that have also been cut with EcoRI can form hydrogen bonds between the complementary base pairs of each strand.[1]][Additionally, the blunt-end cuts provided by AluI and HaeIII can result in target fragments being inserted back-to-front. Both of these factors mean that using EcoRI makes successful recombination more likely than using AluI or HaeIII.[2]]

- ✓ ✗ I have outlined how sticky ends increase specificity with target DNA.[1]
- ✓ ✗ I have outlined why blunt ends are less effective.[2]
- ✓ ✗ I have signposted my response using terms such as: additionally, both.

5A The process of C3 photosynthesis

Theory review questions

1 B

2 A

3 X-granum; Y-thylakoid; Z-stroma

4 $NADP^+$; CO_2; NADPH

5 oxygen; glucose; water

6 Light-dependent: I; II; IV; VII
Light-independent: III; V; VI

7 III; V; VI; IV; II; I

SAC skills questions

8 B **9** A **10** B **11** A

12 C

Exam-style questions

Within lesson

13 C **14** A **15** D **16** D

17 C **18** D

19 **a** [H_2O.[1]]

✓ ✗ I have identified input X as H_2O.[1]

b [CO_2.[1]]

✓ ✗ I have identified input Y as CO_2.[1]

20

Name of the stage of photosynthesis that occurs at the stroma	Light-independent stage
Two input molecules that are required for reactions at the stroma	Any two of the following: • Carbon dioxide (CO_2) • ATP • NADPH
Two output molecules from the reactions at the stroma	Any two of the following: • Glucose ($C_6H_{12}O_6$) • ADP + P_i • $NADP^+$ • Water (H_2O)

Name of the stage of photosynthesis that occurs at the grana	Light-dependent stage
Two input molecules that are required for reactions at the grana	Any two of the following: • Water (H_2O) • $NADP^+$ • ADP + P_i
Two output molecules from the reactions at the grana	Any two of the following: • Oxygen (O_2) • NADPH • ATP

✓ ✗ I have stated the name of each stage at the correct location.

✓ ✗ I have identified two inputs of each stage.

✓ ✗ I have identified two outputs of each stage.

Multiple lessons

21 B

22 **a** [Glucose.[1]]

✓ ✗ I have identified glucose as the product.[1]

b [Chloroplasts.[1]]

✓ ✗ I have stated chloroplast.[1]

c [Gene expression is the process where information stored within genes is used to produce a functional product, typically a protein, via transcription and translation.[1]][On the other hand, gene regulation involves controlling gene expression to increase or decrease the production of specific products.[2]]

✓ ✗ I have described gene expression.[1]

✓ ✗ I have contrasted gene expression to gene regulation.[2]

d [*E. chlorotica* would eat less than the black sea slug[1]][as they are able to undergo photosynthesis to produce their own food source, making them less dependent upon eating other organisms to produce energy.[2]]

✓ ✗ I have stated that *E. chlorotica* would eat less.[1]

✓ ✗ I have linked the ability of *E. chlorotica* to undergo photosynthesis with why they do not require as much food.[2]

Key science skills and ethical understanding

23 **a** [The independent variable in this experiment is the concentration of $NADP^+$.[1]][The dependent variable is the concentration of oxygen gas produced.[2]]

✓ ✗ I have stated the independent variable.[1]

✓ ✗ I have stated the dependent variable.[2]

b [Students must ensure that the amount of light that each thylakoid sample is exposed to is consistent in all trials of the experiment.[1]]

Other acceptable responses include:

- Temperature.
- pH of solution.
- Amount of grana/thylakoids.
- Time exposed to the independent variable.

✓ ✗ I have stated a variable that must be controlled.[1]

c **i** [The results could differ if Student A had not properly sealed the sample.[1]][This personal error would mean oxygen could escape into the environment and the detected concentration was inaccurate.[2]]

Other acceptable responses include:

- A systematic error such as Student A's class was later in the day than Student B's, meaning that the prepared thylakoid membranes in their sample had become inactive, resulting in a lower reaction rate compared to the fresher samples used by Student B.
- A random error such as Student A's grana were less efficient than Student B's by chance.

✓ ✗ I have identified why the students' results are different.[1]

✓ ✗ I have explained how this may have impacted their results.[2]

ii [This action violates the bioethical concept of respect,[1]] [as Student A has not acknowledged that Student B has the right and capacity to make their own decisions.[2]]

✓ ✗ I have stated that the bioethical concept of respect was violated.[1]

✓ ✗ I have explained that ignoring Student B's concerns is not an ethical decision.[2]

5B Rubisco in C3, C4, and CAM photosynthesis

Theory review questions

1 C

2 B

3 B

4 A

5 X-carbon fixation; Y-reduction; Z-regeneration

6 B

SAC skills questions

7 A **8** B **9** B **10** C

Exam-style questions

Within lesson

11 A

12 **a** [C4 plants separate the initial carbon fixation from the remainder of the Calvin cycle in photosynthesis between cells. Initial carbon fixation occurs in a mesophyll cell whilst the remainder of the Calvin cycle takes place in a bundle-sheath cell.[1]] [This separation allows for a high concentration of CO_2 around Rubisco, encouraging it to bind CO_2 rather than O_2, which decreases photorespiration and increases photosynthesis.[2]]

✓ ✗ I have described how C4 plants separate the light-independent stage spatially between cells.[1]

✓ ✗ I have explained how this separation allows for a constant supply of CO_2 to Rubisco, thereby increasing photosynthesis.[2]

✓ ✗ I have used key biological terminology such as: C4, Calvin cycle, mesophyll cell, bundle-sheath cell, concentration, photorespiration, photosynthesis.

b [C4 photosynthesis separates initial carbon fixation from the remainder of the Calvin cycle over space, between a mesophyll cell and a bundle-sheath cell.[1]] [Conversely, CAM photosynthesis separates these two steps over time, between day and night with the use of a vacuole.[2]]

✓ ✗ I have described how C4 plants separate steps of the light-independent stage over space.[1]

✓ ✗ I have compared this to CAM plants that separate these steps over time.[2]

✓ ✗ I have used key biological terminology such as: C4, Calvin cycle, mesophyll cell, bundle-sheath cell, CAM, vacuole.

c [As sugarcane undertakes C4 photosynthesis to cope with hot environments, it expends more energy compared to the standard C3 photosynthesis.[1]] [Whilst this payoff is advantageous in hot environments, in colder environments such as Victoria, the sugarcane is now expending significantly more energy than other C3 plants to undertake photosynthesis.[2]] [This likely means the other cereals would outcompete sugarcane as it costs them less energy to photosynthesise.[3]]

✓ ✗ I have described that the C4 plant sugarcane has to expend more energy than regular plants to cope with hot environments.[1]

✓ ✗ I have discussed how the expenditure of energy is worth it in hot environments but in colder environments it may not be.[2]

✓ ✗ I have concluded that other crops could likely outcompete sugarcane as they spend less energy to photosynthesise.[3]

✓ ✗ I have used key biological terminology such as: C4, hot environments, energy, C3, advantageous, outcompete.

Multiple lessons

13 D

14 **a** [Thylakoid membranes.[1]]

Other acceptable responses include:

- Grana.

✓ ✗ I have stated the location of the light-dependent reactions.[1]

b [Oxygen[1]] [and ATP.[2]]

Other acceptable responses include:

- NADPH.

✓ ✗ I have identified an output of the light-dependent reactions.[1]

✓ ✗ I have identified a second output of the light-dependent reactions.[2]

✓ ✗ I have not listed more than two outputs.

c [For photosynthesis to occur, water molecules are required as inputs.[1]][Because photosynthesis is so important to plant growth and survival, CAM plants cannot sacrifice photosynthesis rates to save a few molecules of water.[2]]

I have stated that water molecules are required as an input of photosynthesis.[1]

I have described that photosynthesis is critically important for plant survival and can't be sacrificed to conserve water.[2]

Key science skills and ethical understanding

15 a [The independent variable is species of grass and type of photosynthesis[1]][and the dependent variable is the growth in grass height.[2]]

I have identified the independent variable.[1]

I have identified the dependent variable.[2]

b [The C3 grass will experience greater growth than the CAM grass.[1]]

I have stated a reasonable hypothesis LaMarcus could be testing.[1]

c i [This approach is not reproducible as under different conditions and with different researchers the same method of measurement could not be carried out.[1]][The approach is also not repeatable as without proper measurement there is no way to confirm that LaMarcus is pouring accurate volumes each time.[2]]

I have explained why this approach is not reproducible.[1]

I have explained why this approach is not repeatable.[2]

ii [Using the larger cm scale rather than mm negatively impacts the accuracy of the result as it does not allow the measurement to get as close to the true value as the smaller scale could.[1]]

I have explained that accuracy is negatively affected.[1]

iii [Taking two measurements per species at each sampling point is a limitation as their values may not be representative of the entire grass populations.[1]][The low level of replication also means that the results could be heavily influenced by statistical outliers or chance.[2]][To improve the experiment, greater replication in terms of blades measured would limit the influence of outliers or chance in the results, improving validity.[3]]

Other acceptable responses include:

- The low amounts of sampling could be influenced by sampling bias (i.e. LaMarcus may have chosen the tallest blades of grass as they are the easiest to reach). Increasing replication or the use of random sampling could improve the experimental design.

I have stated that the measurements may not be a representative sample.[1]

I have described how low replication makes results susceptible to outliers and chance.[2]

I have explained that experimental validity can be improved by increasing replication of measurements.[3]

I have used key biological terminology such as: representative sample, populations, replication, outliers, chance, validity.

iv [The results of the experiment, where the C3 grass had an average growth of 0.5 cm and the CAM grass had an average growth of 2 cm, are unexpected[1]][as you would predict the C3 grass to grow more than the CAM grass in the experimental environment described.[2]][This would be expected as the classroom environment of 20°C aligns more closely with the conditions that C3 plants are adapted to (environments not exposed to extreme heat or dryness), as opposed to CAM plants, which have adaptations for extremely hot and dry environments.[3]]

I have stated that the results are unexpected.[1]

I have described the results that were expected to occur.[2]

I have justified the expected results by relating the classroom environment to the preferred conditions of C3 and CAM plants.[3]

I have used comparative language such as: as opposed to.

I have used data in my response.

v [Integrity.[1]]

I have identified the bioethical concept.[1]

5C Factors affecting the rate of photosynthesis

Theory review questions

1 A

2 C

3 increases; C3; photorespiration; C4

4 B

5 Light: II, III,
Temperature: V, VII
CO_2 concentration: I, VI
Water: IV

SAC skills questions

6 A **7** C **8** A **9** A

10 B

Exam-style questions

Within lesson

11 B **12** D **13** D **14** D

15 C

Multiple lessons

16 a i [Grana.[1]]

Other acceptable responses include:

- Granum.
- Thylakoid.

✓ ✗ I have identified structure X.[1]

ii [Light-dependent stage.[1]]

✓ ✗ I have stated the light-dependent stage of photosynthesis.[1]

b [$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$.[1]]

✓ ✗ I have identified the balanced and simplified chemical equation of photosynthesis.[1]

✓ ✗ I have not included H_2O on the products side of the equation.

c [Increasing the light intensity increases photosynthesis rate, until the enzyme-catalysed reactions cannot operate any faster and the maximum rate of photosynthesis is reached.[1]][An increase in CO_2 concentration also increases the reaction rate until the maximum rate is reached.[2]][As photosynthesis rate is greatest when the pH is at the optimal, increasing the pH value beyond this will cause the rate to decrease[3]][as the enzymes within the cells begin to denature.[4]]

✓ ✗ I have described that increasing light increases the rate of photosynthesis up to the maximum rate when other factors are unlimited.[1]

✓ ✗ I have described that increasing CO_2 increases the rate of photosynthesis up to the maximum rate when other factors are unlimited.[2]

✓ ✗ I have described how increasing pH above the optimal will decrease the rate of photosynthesis.[3]

✓ ✗ I have outlined that this decrease occurs because of denaturation.[4]

✓ ✗ I have used key biological terminology such as: enzyme-catalysed, maximum, rate, optimal, enzymes, denature.

d [Changes in water availability can cause a plant to close its stomata to conserve water. This means that CO_2 cannot enter the plant and its concentration decreases, whilst O_2 cannot leave the plant and its concentration increases.[1]][In C3 plants, this imbalance of gases can severely disrupt photosynthesis and lower the overall photosynthesis rate due to the function of the enzyme Rubisco and initiation of wasteful photorespiration.[2]][C4 and CAM plants comparatively are far less effected, as they have mechanisms to combat photorespiration and maintain stable photosynthetic rates, meaning they are only impacted under extreme water stress conditions.[3]]

✓ ✗ I have explained how water stress leads to decreased CO_2 levels due to stomata closing.[1]

✓ ✗ I have described how C3 plants are significantly impacted as they cannot combat photorespiration.[2]

✓ ✗ I have contrasted this to C4 and CAM plants that are less affected as they have mechanisms to combat photorespiration.[3]

✓ ✗ I have used key biological terminology such as: stomata, conserve, CO_2, O_2, gases, Rubisco, photorespiration.

Key science skills and ethical understanding

17 a [The oxygen meter records the oxygen produced by the plant, which corresponds to the rate of photosynthesis as oxygen is an output of the light-dependent stage.[1]]

✓ ✗ I have identified that the meter measures the rate of photosynthesis.[1]

b [Flask 1 acted as a control, as the absence of light on this control flask allows us to compare the experimental flasks exposed to varying light.[1]]

✓ ✗ I have stated the purpose of Flask 1.[1]

c [To ensure that oxygen was not released into the atmosphere, or introduced from the atmosphere, so that the meter recorded only the total amount of dissolved oxygen produced by photosynthesis.[1]]

✓ ✗ I have identified why the flasks were sealed.[1]

d [It is expected that Flask 4 would have the fastest photosynthesis rate,[1]][as light is required for photosynthesis and the light intensity on Flask 4 is the greatest.[2]]

✓ ✗ I have identified which flask is predicted to show the fastest rate.[1]

✓ ✗ I have explained that this is due to the high light intensity.[2]

e [The temperature of the flasks,[1]][the length of time that the oxygen levels were measured,[2]][and the size of the plants.[3]]

Other acceptable responses include:

- The health of plants, background light intensity, the time the plants spend in the flasks, CO_2 levels in flasks at the beginning, water levels in the flasks, the cleanliness of the flasks.

✓ ✗ I have identified a first variable that needs to be controlled.[1]

✓ ✗ I have identified a second variable that needs to be controlled.[2]

✓ ✗ I have identified a third variable that needs to be controlled.[3]

f i [George is most likely correct. The greater the light intensity, the more quickly photosynthesis proceeds.[1]][The greatest light intensity was on Flask 4 which resulted in the fastest rate, and the lowest intensity light was on Flask 1 which resulted in the slowest rate.[2]]

✓ ✗ I have explained why George is correct.[1]

✓ ✗ I have related this to the graphs.[2]

ii [Integrity.[1]]

✓ ✗ I have stated integrity.[1]

5D Agricultural applications of CRISPR-Cas9

Theory review questions

1 B

2 B

3 genes; genome; drought tolerance; herbicide resistance; disease resistance

4 III; II; IV; VI; I; V

SAC skills questions

5 B **6** B **7** C **8** B

Exam-style questions

Within lesson

9 C **10** C **11** A

Multiple lessons

12 B

13 **a** [Pineapples are CAM plants making them well-suited to their hot location.[1]][CAM plants are well-suited to hot environments as they keep photosynthesis rates high by separating initial carbon fixation and the remainder of the Calvin cycle over time. This is achieved with the help of a vacuole and stomata which open during the night and close during the day.[2]]

- I have identified that CAM plants are suited to hot environments.[1]
- I have described how CAM plants separate initial carbon fixation from the remainder of the Calvin cycle over time.[2]
- I have used key biological terminology such as: photosynthesis, carbon fixation, Calvin cycle, vacuole, stomata.

b [CAM plants separate initial carbon fixation from the remainder of the Calvin cycle over time, between night and day with the help of a vacuole.[1]][On the other hand, C4 plants separate these steps over space, between a mesophyll cell and a bundle-sheath cell.[2]]

- I have described CAM plants as separating steps over time.[1]
- I have compared this to C4 plants that separate steps over space.[2]
- I have used key biological terminology such as: carbon fixation, Calvin cycle, vacuole, mesophyll cell, bundle-sheath cell.
- I have used comparative language such as: on the other hand.

c **i** [To alter Rubisco's affinity towards CO_2 or O_2 so that it binds to CO_2 more frequently.[1]]

- have stated that the intended change would be to have Rubisco bind to CO_2 more often.[1]

ii [As the concentration of CO_2 increases, the rate of photosynthesis increases and the rate of photorespiration decreases.[1]][The increased rate of photosynthesis is because the enzyme Rubisco is more likely to bind CO_2 rather than O_2.[2]]

- I have stated that increasing CO_2 increases the rate of photosynthesis and decreases photorespiration.[1]
- I have explained that this is because Rubisco more likely to bind to CO_2 rather than O_2.[2]
- I have used key biological terminology such as: photosynthesis, photorespiration, Rubisco, O_2.

14 **a** [CRISPR-Cas9 could edit the plant's genome to increase the capture of light within chloroplasts, thereby increasing the rate of photosynthesis.[1]]

- I have stated that editing the genome to increase light capture would increase photosynthesis rate.[1]

b [CRISPR-Cas9 could edit the plant's genome to improve the efficiency of CO_2 uptake in the plant or improve the efficiency of CO_2 in the role of Rubisco's function, both of which would improve the rate of photosynthesis.[1]][Similarly, increasing water capture would increase the rate of photosynthesis and ensure the plant does not lose CO_2 and suffer the consequences of reduced CO_2 levels.[2]]

- I have described how editing the genome to increase CO_2 uptake or CO_2 use would increase the rate of photosynthesis.[1]
- I have also explained how increasing water capture would increase the rate of photosynthesis.[2]

Key science skills and ethical understanding

15 **a** [The average weight of the control potatoes was 173.4 grams, which was greater than edited sample two (171.0 g) but less than the edited samples one (179.7 g) and three (178.4 g).[1]][The average shelf life of the control potato was 38.4 days, which was longer than all three edited samples (36.1, 34.1 and 36.7 days).[2]]

- I have described the results of potato weight.[1]
- I have described the results of potato shelf life.[2]
- I have used data in my response.

b [This method of measurement is repeatable as the researcher knows how to repeat their measurements, even if they are not entirely scientifically sound due to their subjective nature and thus potentially are not as accurate as they could be.[1]]

- I have stated that the method is repeatable but may not be accurate.[1]

c [The experiment successfully increased potato size as two of the three edited samples produced a greater average weight than the control sample, and the average of the three edited samples exceeded the control.[1]][However, it was unsuccessful in increasing the shelf life as all three edited samples resulted in a shorter shelf life than the control sample.[2]]

✓ ✗ I have stated that the experiment was successful in increasing the size of the potatoes.[1]

✓ ✗ I have stated that the experiment was unsuccessful in increasing the shelf life of the potatoes.[2]

d [Integrity.[1]]

✓ ✗ I have identified the relevant bioethical concept.[1]

Chapter 5 SAC practice

1 [Week 9.[1]]

✓ ✗ I have stated Week 9.[1]

2 [Week 1.[1]]

✓ ✗ I have stated Week 1.[1]

3 [Week 7.[1]]

✓ ✗ I have stated Week 7.[1]

4 [Weeks 7 and 8.[1]]

✓ ✗ I have identified Weeks 7 and 8.[1]

5 [Sammy in Week 5.[1]]

✓ ✗ I have identified Sammy in Week 5.[1]

6 [Lara's plant experienced more consistent growth in height, given that the weekly percentage growth stayed somewhat similar and ranged between 4.71% and 8.97%, with the exception of a 17.89% value.[1]] [Conversely, Sammy's plant had less consistent growth in height with a wide range of weekly percentage growth ranging from 3.7% to 20.13%. Additionally, Sammy's plant experienced a high degree of variance over a three week period, with 14.5%, 6%, and then 20.13% growth, respectively.[2]]

✓ ✗ I have explained that Lara's plant experienced more consistent growth in height.[1]

✓ ✗ I have described how Sammy's plant was far less consistent and more variable.[2]

✓ ✗ I have used comparative language such as: conversely.

7 [After 10 weeks, the height of Sammy's plant had only marginally eclipsed the expected height of a full-grown plant whilst the width was still slightly lower than the expected full-grown width.[1]] [Both the height and width of Lara's full-grown plant were slightly lower than expected after 10 weeks.[2]]

✓ ✗ I have described how Sammy's plant was taller and slightly narrower than the expected full-grown measurements.[1]

✓ ✗ I have described how Lara's plant was shorter and narrower than the expected full-grown measurements.[2]

8 [15.38% growth in width for Sammy's plant,[1]] [8.19% growth in height for Lara's plant,[2]] [and 5.71% growth in width for Lara's plant.[3]]

✓ ✗ I have calculated the growth in width of Sammy's plant to be 15.38%.[1]

✓ ✗ I have calculated the growth in height of Lara's plant to be 8.19%.[2]

✓ ✗ I have calculated the growth in width of Lara's plant to be 5.71%.[3]

9 [No it is not likely[1]] [as using a straight ruler to measure plant height or width could be undertaken using several different methods depending on the measurer. Without detailing how they measured the lengths in terms of the ruler placement and start and endpoints, the method would not be reproducible by another group trying to replicate the experiment.[2]]

✓ ✗ I have stated no.[1]

✓ ✗ I have explained that depending on the measurer the measurement could be taken using several different methods.[2]

10 [Water is an input in photosynthesis. Increasing its levels can increase the rate of photosynthesis.[1]] [When a plant experiences water stress, it closes its stomata, which limits CO_2 intake and O_2 output. This imbalance of gases can lead to lower rates of photosynthesis as the plant cells undertake photorespiration rather than photosynthesis.[2]]

✓ ✗ I have described that water is an input and therefore increasing it can increase the rate of reaction.[1]

✓ ✗ I have explained how water stress leads to the closing of stomata and stops gaseous exchange leading to a lower photosynthesis rate.[2]

✓ ✗ I have used key biological terminology such as: input, stomata, CO_2, O_2, gases, photorespiration

11 [C3 plants do not separate initial carbon fixation with the remainder of the Calvin cycle in the light-independent stage of photosynthesis.[1]] [C4 plants separate these two steps spatially between two cells,[2]] [while CAM plants separate these two steps over time between night and day with the use of a vacuole.[3]]

✓ ✗ I have explained that C3 plants do not separate initial carbon fixation and the remainder of the Calvin cycle.[1]

✓ ✗ I have compared this to C4 plants that separate the two steps between cells.[2]

✓ ✗ I have compared this to CAM plants that separate the two steps between night and day.[3]

✓ ✗ I have used key biological terminology such as: carbon fixation, vacuole.

12 [CRISPR-Cas9 technology could engineer genes to modify the activity of Rubisco or other Calvin cycle enzymes to improve their function.[1]] [Additionally, alternative pathways to photorespiration could be encouraged by making C3 plants more like C4 or CAM plants.[2]]

✓ ✗ I have described how CRISPR-Cas9 could modify genes to alter the function of Rubisco or other enzymes in photosynthesis.[1]

I have explained how CRISPR-Cas9 could modify genes to introduce alternatives to photorespiration and make C3 plants more like C4 and CAM plants.[2]

I have used key biological terminology such as: engineer, activity, Rubisco, enzymes, pathways, photorespiration.

Chapter 5 Exam practice

Section A

1	B	**2**	B	**3**	A	**4**	B
5	C	**6**	A	**7**	B	**8**	D
9	A	**10**	D	**11**	A	**12**	B
13	C						

Section B

14 a [Structure Z, which is known as the stroma.[1]]

I have identified and named structure Z as the location of the light-independent stage.[1]

b [Plant A is from a roadside habitat as it has fewer thylakoid membranes, indicating that there is a large amount of light available to this cell.[1]][This is unlike the cell from the shaded rainforest habitat which is exposed to limited light and requires many thylakoids to capture more light and meet photosynthetic needs.[2]]

I have explained why this plant has fewer thylakoid membranes.[1]

I have compared this to the other environment.[2]

I have used key biological terminology such as: thylakoids.

c [The concentration of carbon dioxide affects the rate of photosynthesis.[1]][As the concentration of carbon dioxide increases, the rate of photosynthesis increases until it plateaus due to other limiting factors such as light.[2]]

Other acceptable responses include:

- Temperature affects the rate of photosynthesis. As temperature increases or decreases from the optimum, the rate of photosynthesis decreases.
- Water affects the rate mainly by influencing carbon dioxide intake.

I have stated a factor.[1]

I have explained the relationship between the factor and the rate of photosynthesis.[2]

I have not discussed light as a factor.

15 a [NADPH.[1]]

I have identified the loaded form of the proton carrier in photosynthesis.[1]

b [The net production of NADPH is 0[1]][as all NADPH is cycled to $NADP^+$ in the light-independent reactions.[2]]

I have stated that the net production of NADPH as 0.[1]

I have justified my answer by referring to coenzyme cycling.[2]

I have used key biological terminology such as: NADPH, $NADP^+$, light-independent reactions.

16 a [The rate of photosynthesis increases as temperature increases due to more frequent enzyme-substrate collisions, until the optimal temperature of the enzymes within cells is reached.[1]][Above this temperature, the rate begins to drop as the enzymes start to denature due to the high temperatures.[2]]

I have explained that the initial increase in the rate of photosynthesis is due to more frequent enzyme-substrate collisions.[1]

I have outlined that the reaction rate decreases past the optimal temperature due to denaturation.[2]

I have used key biological terminology such as: enzyme-substrate collisions, optimal, denature.

b [CAM plants are well suited to hot and dry environments due to their photosynthetic adaptations,[1]][whilst C3 plants are better suited to cool, moderate environments where water is adequate.[2]]

I have explained that CAM plants are suited to hot and dry environments.[1]

I have compared this to C3 plants that are suited to cooler and less dry environments.[2]

I have used comparative language such as: whilst.

c [ATP[1]][and NADPH.[2]]

I have identified ATP.[1]

I have identified NADPH.[2]

I have not stated ADP or $NADP^+$ as they are not high in energy.

17 a [Carbon dioxide is an input of the light-independent stage,[1]][which occurs in the stroma.[2]]

I have identified the light-independent stage of photosynthesis.[1]

I have identified the stroma.[2]

b [Glucose.[1]]

I have identified glucose.[1]

c i [An increased resistance to herbicide would allow the crop to better survive periods when propanil is used to kill the weeds.[1]][As such, the overall production of the crop would increase due to less competition with the weeds.[2]]

✓ ✗ I have explained that greater herbicide resistance would allow the crop to survive better when the weeds are targeted with propanil.[1]

✓ ✗ I have stated that the overall production would increase.[2]

ii [Non-maleficence.[1]]

✓ ✗ I have identified non-maleficence.[1]

18 a [Two inputs are carbon dioxide and water.[1]][Two outputs are glucose and oxygen.[2]]

Other acceptable responses include:

- Output: water.

✓ ✗ I have stated two inputs of photosynthesis.[1]

✓ ✗ I have stated two outputs of photosynthesis.[2]

b [Oxygen gas.[1]]

✓ ✗ I have stated what forms the bubbles.[1]

c [Grana.[1]]

Other acceptable responses include:

- Thylakoid membrane.

✓ ✗ I have stated where oxygen is produced.[1]

d [Chlorophyll.[1]]

✓ ✗ I have identified chlorophyll.[1]

e i [If the light source is removed, then the rate of the light-dependent reactions will decrease[1]][as light is required to energise chlorophyll and split water molecules in the light-dependent stage.[2]][Therefore, the overall rate of photosynthesis will decrease.[3]]

✓ ✗ I have stated that removing the light will decrease the rate of light-dependent reactions.[1]

✓ ✗ I have outlined the role of light in the light-dependent reactions.[2]

✓ ✗ I have stated that the overall rate of photosynthesis will decrease.[3]

✓ ✗ I have used key biological terminology such as: light-dependent reactions, energise, chlorophyll, water.

ii [No, the expected drop in overall photosynthesis rate would still be seen[1]][as C3 and C4 plants both undertake the same type of light-dependent stage of photosynthesis that requires light energy to energise chlorophyll and split water.[2]]

✓ ✗ I have stated that the results would not differ.[1]

✓ ✗ I have justified my response by explaining that C3 and C4 plants undertake the same mode of action in the light-dependent stage of photosynthesis.[2]

✓ ✗ I have used key biological terminology such as: rate, C3, C4, light-dependent stage, energise, chlorophyll, water.

f [If the light source increases water temperature too much, the rate of photosynthesis will decrease.[1]][This is because the temperature could shift above the plant's optimal, causing enzymes involved in photosynthesis to denature.[2]]

✓ ✗ I have stated that the rate of photosynthesis will decrease.[1]

✓ ✗ I have explained that the decrease is due to enzymes not functioning at their optimal temperature.[2]

✓ ✗ I have used key biological terminology such as: optimal, enzymes, denature.

6A Aerobic cellular respiration

Theory review questions

1 glucose; oxygen; ATP

2 R-cytosol; S-intermembrane space; T-inner mitochondrial membrane; U-mitochondrial matrix

3 pyruvate; anaerobic; NADH

4 C

5 C

6 enzyme; coenzyme

7 I-cytosol; II-mitochondrial matrix; III-mitochondrial matrix; IV-cristae; V-cytosol; VI-mitochondrial matrix

8 C

SAC skills questions

9 B **10** B **11** B **12** A

13 A

Exam-style questions

Within lesson

14 D **15** A **16** B **17** A

18 D

Multiple lessons

19 B **20** B **21** D

22 a [Structure Y represents the mitochondria.[1]][The mitochondria are the site of aerobic cellular respiration, which produces the majority of energy/ATP for the cell.[2]]

- ✓ ✗ I have identified the mitochondria.[1]
- ✓ ✗ I have described the role of the mitochondria.[2]
- ✓ ✗ I have not described mitochondria as the 'powerhouse of the cell'.

b [An oxygen molecule crosses the plasma membrane (structure X)[1]][via simple diffusion down a concentration gradient[2]][as it is a small, hydrophobic molecule.[3]]

- ✓ ✗ I have identified the plasma membrane.[1]
- ✓ ✗ I have stated the process to cross the membrane.[2]
- ✓ ✗ I have justified my answer by referring to the chemical nature of oxygen.[3]

c [The heart requires a large supply of ATP from mitochondria so that it can contract consistently and effectively.[1]][Therefore, the heart's cardiac muscle cells require a large number of mitochondria (structure Y) to function efficiently.[2]]

- ✓ ✗ I have stated why the heart needs a large amount of energy.[1]
- ✓ ✗ I have explained how this affects the number of mitochondria.[2]

23 a [Output X is carbon dioxide[1]][and molecule Y is oxygen.[2]]

- ✓ ✗ I have identified output X as carbon dioxide.[1]
- ✓ ✗ I have identified molecule Y as oxygen.[2]

b

Process	Name of process(es)	Site of process
M	light-dependent reactions	**grana/thylakoid membranes of chloroplast**
O	**glycolysis**	cytosol
P	Krebs cycle and electron transport chain	**mitochondria/matrix and cristae**

- ✓ ✗ I have identified the site of the light-dependent reactions.
- ✓ ✗ I have identified the process O.
- ✓ ✗ I have identified the site of the Krebs cycle and electron transport chain.

24 a [Cellular respiration.[1]]

- ✓ ✗ I have identified the metabolic process that produces energy.[1]

b [Glucose + Oxygen → Carbon dioxide + Water + ATP.[1]]

- ✓ ✗ I have written the correct equation.[1]
- ✓ ✗ I have not written the equation for photosynthesis.
- ✓ ✗ I have not written chemical formulas.

c i [This process is glycolysis, which occurs in the cytosol of the cell.[1]]

- ✓ ✗ I have stated the process and its location.[1]

ii [This process is the electron transport chain,[1]][which occurs on the inner mitochondrial membrane, or cristae, of the mitochondria.[2]]

- ✓ ✗ I have stated the process.[1]
- ✓ ✗ I have stated the correct location in the cell.[2]

d i [The O_2 produced is equal to the O_2 used as the rate of photosynthesis is equal to the rate of aerobic cellular respiration.[1]]

- ✓ ✗ I have explained how overall oxygen produced can equal zero.[1]

ii [At point M, O_2 production is greater than O_2 consumption[1]][which means more photosynthesis is occurring than aerobic cellular respiration,[2]][as oxygen is an output for photosynthesis, but an input for aerobic cellular respiration.[3]]

I have compared O_2 consumed to O_2 produced at point M.[1]

I have stated which metabolic process is occurring to a greater extent.[2]

I have described the relevance of O_2 with reference to the appropriate metabolic processes.[3]

iii [At point N, the graph plateaus because factors such as carbon dioxide availability (rather than light intensity) become limiting factors.[1]][At this point, the rate of O_2 production from photosynthesis has reached its maximum under these environmental conditions.[2]]

I have explained why the graph plateaus with reference to carbon dioxide availability.[1]

I have explained why the graph plateaus with reference to oxygen production.[2]

I have used appropriate biological terminology such as: limiting factor.

Key science skills and ethical understanding

25 a [The independent variable is temperature[1]][and the dependent variables are oxygen and carbon dioxide concentrations.[2]]

I have identified the independent variable.[1]

I have identified the dependent variables.[2]

b [In aerobic cellular respiration, oxygen is required as an input,[1]][whereas carbon dioxide is produced as an output.[2]][Therefore, the experimental design should measure levels of both oxygen and carbon dioxide to determine if temperature affects how quickly oxygen is consumed, or carbon dioxide is produced, and thereby measure the rate of aerobic cellular respiration in the spiny stick insect.[3]]

I have stated that oxygen is an input of aerobic cellular respiration.[1]

I have stated that carbon dioxide is an output of aerobic cellular respiration.[2]

I have explained how this will influence the experimental design and the purpose of these changes.[3]

c [A systematic error.[1]]

I have identified the type of error.[1]

6B Anaerobic fermentation

Theory review questions

1 anaerobic fermentation; glucose; glycolysis; lactic acid

2 C

3 B

4 A

SAC skills questions

5 A **6** B **7** B **8** A

9 A

Exam-style questions

Within lesson

10 A **11** D **12** A **13** C

14 A **15** A

Multiple lessons

16 A **17** C **18** B

19 a [Anaerobic fermentation, which produces two ATP and two NADH per glucose molecule.[1]][Glucose is broken down via glycolysis into pyruvate, which will then react further to recycle the NADH and form lactic acid in human cells.[2]]

I have named and described the process that produces ATP in the absence of oxygen.[1]

I have described the process with reference to its final product.[2]

b i [Carbon monoxide is a competitive inhibitor.[1]][As the pure oxygen is inhaled and oxygen concentration increases, carbon monoxide is displaced from the haemoglobin protein and its poisoning effects are mitigated.[2]][This means that carbon monoxide must bind to the same active site as oxygen and therefore, it must be a competitive inhibitor of haemoglobin.[3]]

I have stated carbon dioxide's mode of inhibition.[1]

I have identified a relationship between oxygen concentration and carbon monoxide binding.[2]

I have explained where oxygen and carbon monoxide must bind to haemoglobin.[3]

ii [Glycolysis, which occurs in the cytosol, involves glucose being sequentially broken down into two pyruvate molecules, producing two ATP in the process.[1]][The Krebs cycle, which occurs in the mitochondrial matrix, involves acetyl-CoA (produced from pyruvate) being converted to a number of intermediates, releasing carbon dioxide, and generating NADH, $FADH_2$, and two ATP.[2]][Finally, the electron transport chain (on the mitochondrial cristae) involves NADH and $FADH_2$ being unloaded, with this energy harnessed to produce 32 or 34 ATP, and oxygen acting as the final electron acceptor as it binds with hydrogen ions to produce stable water.[3]]

I have named and described the first stage of aerobic cellular respiration.[1]

I have named and described the second stage of aerobic cellular respiration.[2]

I have named and described the third stage of aerobic cellular respiration.[3]

I have used key biological terminology such as: glycolysis, the Krebs cycle, the electron transport chain, NADH, $FADH_2$, ATP, pyruvate, carbon dioxide.

Key science skills and ethical understanding

20 a [Ethanol, carbon dioxide, and ATP.[1]]

I have identified all of the products of anaerobic fermentation in yeast.[1]

I have not given lactic acid as a product.

b [Fermentation enables yeast cells to rapidly produce ATP in the absence of oxygen.[1]]

Other acceptable responses include:

- Anaerobic fermentation regenerates NAD^+ to allow glycolysis and ATP production to continue in the absence of oxygen.

I have explained the importance of fermentation in yeast cells.[1]

c [Both aerobic fermentation and anaerobic cellular respiration involve glycolysis.[1]] [Anaerobic fermentation only produces two ATP per glucose molecule, whereas aerobic cellular respiration produces 36 or 38 ATP per glucose molecule.[2]]

Other acceptable similarities include:

- Both use glucose as an input.
- Both involve reactions that occur in the cytosol.
- Both use NAD^+ as an electron and proton carrier.

Other acceptable differences include:

- Anaerobic fermentation produces ATP faster than aerobic cellular respiration.
- Anaerobic fermentation cannot be sustained indefinitely due to a build-up of toxins (such as lactic acid), whereas aerobic cellular respiration can continue indefinitely.
- Anaerobic fermentation produces lactic acid whilst aerobic cellular respiration produces carbon dioxide and water.

I have stated a similarity between aerobic cellular respiration and anaerobic fermentation.[1]

I have stated a difference between aerobic cellular respiration and anaerobic fermentation.[2]

I have used comparative language such as: whereas, both.

d i [The independent variable is the concentration of oxygen surrounding the agar plate.[1]] [The dependent variable is the amount of *K. xylinus* growth upon each agar plate.[2]]

I have identified the independent variable.[1]

I have identified the dependent variable.[2]

ii [An environment with little to no oxygen available.[1]]

I have described the environment where the bacteria can survive.[1]

iii [Personal error.[1]]

I have identified the type of error.[1]

iv [The scientists would observe no growth of *K. xylinus* on agar plates exposed to an oxygenated environment, whereas there would be lots of growth of *K. xylinus* on agar plates under deoxygenated conditions.[1]]

I have stated results that would support the hypothesis that *K. xylinus* bacteria are obligate anaerobes.[1]

6C Factors affecting the rate of cellular respiration

Theory review questions

1 temperature/enzyme inhibitors; temperature/enzyme inhibitors; oxygen concentration; optimal; glucose; increase

2 A

3 D

4 Temperature: I, III, VII, VIII
Glucose availability: I, II, IV, V, VII, VIII, IX
Oxygen concentration: I, V, VI, VIII, IX

5 D

SAC skills questions

6 D **7** B **8** C **9** C

10 A **11** D

Exam-style questions

Within lesson

12 B **13** D **14** D

Multiple lessons

15 D **16** D

17 a [Danny is incorrect. When there is no environmental O_2, there is no oxygen for aerobic respiration to occur.[1]] [The CO_2 is produced from anaerobic fermentation.[2]]

I have explained the significance of no environmental O_2.[1]

I have explained the process in which CO_2 is produced.[2]

b [Deprivation of O_2 means the root cells will undergo only anaerobic fermentation[1]] [to produce energy, which produces less ATP for growth or metabolism. Respiring anaerobically results in a large amount of ethanol being produced, which in large amounts can be toxic and may result in death of the root cells.[2]]

I have described the consequence for the plant root cells.[1]

I have outlined what is produced when this happens.[2]

c i [The rate of aerobic respiration has reached its maximum as the enzyme-catalysed systems are fully saturated with substrate and are working at full capacity, so no further intake of O_2 will increase the rate.[1]]

Other acceptable responses include:

- There is another limiting factor, such as glucose, that is limiting the maximum rate of respiration.

I have explained why the graph plateaus.[1]

ii [Altering the glucose (or sucrose) availability could result in a higher plateau on the graph.[1]][If more glucose is present, more reactant is available to undergo the reaction and be turned into CO_2, raising the maximal rate of respiration.[2]]

Other acceptable responses include:

- The temperature or pH may not be optimal for the plant. These could be altered to increase the maximal rate of respiration.

I have stated the variable that could raise the graph's plateau.[1]

I have described how this change could change the graph.[2]

Key science skills and ethical understanding

18 a [The independent variable is the species of yeast in the container[1]][and the dependent variables are the percentages of oxygen and ethanol at the start and end of the experiment.[2]]

I have identified the independent variable.[1]

I have identified the dependent variables.[2]

b i [Aerobic cellular respiration.[1]]

I have identified which type of respiration can be measured using O_2 levels.[1]

ii [This process is anaerobic fermentation.[1]][Without the use of oxygen, yeast cells anaerobically turn glucose into ethanol and carbon dioxide in a reaction that produces two ATP.[2]]

I have identified anaerobic respiration.[1]

I have described anaerobic respiration in yeast.[2]

I have used key biological terminology such as: anaerobic, oxygen, ethanol, ATP.

c i [It is expected that the ethanol levels will be lower,[1]][as the high temperature is likely to denature the yeast enzymes responsible for both aerobic respiration and anaerobic fermentation, resulting in decreased ethanol production.[2]]

I have identified the expected change in ethanol levels.[1]

I have described why this change is expected.[2]

I have used key biological terminology such as: denature, enzyme.

ii [Integrity,[1]][which involves the accurate representation of the facts, whether favourable or unfavourable. This is because the student deliberately changed their recorded results.[2]]

I have identified the bioethical concept that should be followed.[1]

I have explained my answer with reference to the bioethical concept.[2]

d i [Personal error.[1]]

I have identified the error type.[1]

ii [The error lowers the class mean of percentage of oxygen at the start of the experiment.[1]]

I have identified which class mean has been impacted.[1]

iii [If the temperature is changed to be further away from the optimal temperature for enzyme functioning, the rate of both reactions will decrease.[1]][This is because high temperatures denature enzymes and low temperatures reduce the amount of kinetic energy in molecules.[2]]

Other acceptable responses include:

- Increasing or decreasing the pH levels away from the optimal pH for the enzymes involved in respiration will denature enzymes and therefore will decrease the overall respiration rate within yeast.

I have identified a factor other than sucrose that could lower respiration rates.[1]

I have explained why changing this factor could lower respiration rates.[2]

I have used key biological terminology such as: optimal, enzyme, denature.

6D Biofuel from fermentation

Theory review questions

1 A

2 renewable; non-renewable; higher; biomass; ethanol; fermentation

3 B

4 B

SAC skills questions

5 A **6** A **7** A **8** B

Exam-style questions

Within lesson

9 B **10** B **11** C **12** B

13 a [Around 22 months.[1]]

I have identified anywhere between 20–25 months.[1]

b [Bacteria break down and digest organic material.[1]]

I have stated that the role of bacteria is to help break down the organic material.[1]

c [Biogas is created by the process of anaerobic digestion, which occurs in the absence of oxygen.[1]]

I have mentioned anaerobic digestion and explained that this occurs in the absence of oxygen.[1]

d [According to the information, the biogas will be more efficient when combusting as its methane concentration increases. Therefore, biogas is more efficient at 120 months than at 60 months.[1]] [This is because the higher the proportion of methane, the less heat is wasted during combustion, and given that methane concentration is around 60% at 120 months compared with under 30% at 60 months, we would expect the biogas to be more efficient at 120 months.[2]]

I have stated that biogas is more efficient at 120 months than at 60 months.[1]

I have justified my response with direct reference to the data in the graph.[2]

14 a [The food vs fuel debate is concerned with using arable agricultural land for the production of fuel over food and the impact this has on food security in the decades to come.[1]] [Microalgae do not need high-quality agricultural land for biomass production, which satisfies this concern by minimising the use of arable land for fuel production.[2]]

I have briefly described the food vs fuel debate.[1]

I have explained that microalgae do not need land to produce biomass.[2]

b [Bioethanol is produced from the fermentation of plant sugars,[1]] [while biodiesel is produced via the chemical breakdown of lipids.[2]]

I have explained that bioethanol is produced from the fermentation of plant sugars.[1]

I have explained that biodiesel is produced from the breakdown of lipids.[2]

c [The relative ease of microalgae biomass production may serve to make biofuel production more evenly distributed across different areas, given that the growth and harvesting of microalgae can take place in many places and requires less high-quality agricultural land.[1]] [Microalgae-based biofuels may be grown and harvested domestically with relative ease, creating community-based industry and potentially reducing the reliance of many countries on the import and export of traditional fossil fuels.[2]]

I have explained that the relative ease of micro algae biomass may make its production more evenly distributed.[1]

I have explained how this may reduce reliance on international exchange of fossil fuels.[2]

Multiple lessons

15 a [$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$.[1]]

I have provided the correct chemical equation for photosynthesis.[1]

I have not provided the worded equation.

b [glucose → carbon dioxide + ethanol.[1]]

I have correctly provided the worded equation for anaerobic fermentation in yeasts.[1]

I have not provided the chemical equation.

c [Biofuels are considered carbon neutral due to the fact that the carbon dioxide that is released when the fuels are burned is approximately equal to the amount of carbon dioxide originally absorbed by the plants during photosynthesis.[1]] [This means that the combustion of the biofuel does not release any extra carbon dioxide into the atmosphere than what was originally consumed by the biomass to photosynthesise.[2]]

I have explained that the CO_2 that is released during combustion is approximately equal to the CO_2 absorbed during photosynthesis.[1]

I have explained that this means there is no net increase in the CO_2 released.[2]

Key science skills and ethical understanding

16 a [A model is a constructed representation that approximates an object or event and can be used to describe systems or make predictions.[1]]

I have defined the purpose of a model as helping to describe systems and make predictions.[1]

b [A potential aim of the researchers could be to better understand the water footprint involved in cultivating biofuels from GM algae.[1]]

I have stated a potential aim of the researchers.[1]

c [A genetically modified organism is an organism with genetic material that has been altered using genetic engineering technology.[1]]

I have defined a GMO as an organism with genetic material that has been altered using genetic engineering technology.[1]

d [Open-pond cultivation might result in greater water loss via evaporation into the surrounding environment, therefore yielding a higher WF than closed system cultivation which limits the exposure of the algae to its external environment.[1]]

Other acceptable responses include:

- Open-pond cultivation may increase water loss via leakage.

I have stated one reason as to why open-pond cultivation might yield a higher WF.[1]

e [A potential bioethical issue facing this country is whether to commit to the production of microalgal biodiesel on a large scale and risk further exacerbating their water restrictions.[1]][A consequences-based approach to bioethics aims to maximise positive outcomes for a party while minimising negative outcomes.[2]][In this hypothetical situation, the country may be disadvantaged if they continue with the production of microalgal biodiesel, and may experience greater harm due to water loss than the gains associated with a higher degree of biofuel use, such as reduced carbon dioxide emissions.[3]]

- I have identified a potential bioethical issue.[1]
- I have briefly explained a consequences-based approach.[2]
- I have used a consequences-based approach to justify the discontinuing of microalgal biodiesel production in this hypothetical country.[3]

Chapter 6 SAC practice

1 [A ketogenic diet is a diet that is high in fat, moderate in protein and low in carbohydrates.[1]]

- I have defined a ketogenic diet.[1]

2 [Glycolysis, the Krebs cycle, and the electron transport chain.[1]]

- I have listed the three stages of cellular respiration where glucose is used as a source of energy.[1]

3 [Glycolysis,[1]][which occurs in the cytosol of a cell.[2]]

- I have identified the stage that rarely occurs in a ketogenic diet.[1]
- I have identified the location of that stage.[2]

4 [The net inputs of glycolysis are one glucose molecule, 2 ADP + 2 P_i, and 2 NAD^+ + 2 H^+.[1]][The outputs are 2 pyruvate molecules, 2 ATP molecules, and 2 NADH molecules.[2]]

- I have listed the inputs of glycolysis.[1]
- I have listed the outputs of glycolysis.[2]

5 [Ketogenesis occurs at the mitochondrial matrix.[1]][The products of ketogenesis are ketone bodies including ACA, BHB, and acetone.[2]][They are taken up by cells in the body and converted into acetyl-CoA molecules, which are then used to produce ATP molecules.[3]]

- I have identified the location of ketogenesis.[1]
- I have identified the products of ketogenesis.[2]
- I have explained the function of the products of ketogenesis in providing energy.[3]

6 [Acetyl-CoA will enter the Krebs cycle instead of undergoing ketogenesis when the levels of both glucose and fat in the body are low.[1]]

- I have determined the condition in which acetyl-CoA molecules will enter the Krebs cycle instead of undergoing ketogenesis.[1]

7 [The Krebs cycle occurs in the mitochondrial matrix.[1]][The inputs of the Krebs cycle are 2 acetyl-CoA molecules, 2 ADP + 2 P_i, 6 NAD^+ + 6 H^+, and 2 FAD + 4 H^+.[2]][The outputs of the Krebs cycle are 4 CO_2 molecules, 2 ATP molecules, 6 NADH molecules, and 2 $FADH_2$ molecules.[3]]

- I have determined the location of the Krebs cycle.[1]
- I have listed the inputs of the Krebs cycle.[2]
- I have listed the outputs of the Krebs cycle.[3]

8 [A competitive inhibitor binds directly to the active site of an enzyme, which prevents the enzyme binding to its substrate(s).[1]][A non-competitive inhibitor binds to the allosteric site of an enzyme, which causes conformational changes to the enzyme and prevents it from freely binding to its substrate(s).[2]]

- I have explained how a competitive inhibitor works.[1]
- I have explained how a non-competitive inhibitor works.[2]

9 [ATP is a non-competitive inhibitor of isocitrate dehydrogenase[1]][because it binds to the allosteric site of the enzyme, which prevents the enzyme from binding to its substrate.[2]]

- I have identified that ATP is a non-competitive inhibitor of isocitrate dehydrogenase.[1]
- I have justified my answer.[2]

10 [NADH is a competitive inhibitor of isocitrate dehydrogenase[1]][because it binds to the active site of the enzyme and displaces NAD^+, which is the enzyme's subtrate.[2]]

- I have identified that NADH is a competitive inhibitor of isocitrate dehydrogenase.[1]
- I have justified my answer.[2]

11 [Increased levels of ATP, NADH, and succinyl CoA will reduce the rate of the Krebs cycle.[1]][This is because they inhibit the activity of α-ketoglutarate dehydrogenase, which catalyses the conversion of isocitrate to α-ketoglutarate in the cycle.[2]]

- I have described the effect of increased levels of ATP, NADH, and succinyl CoA on the rate of the Krebs cycle.[1]
- I have explained my answer.[2]

Chapter 6 Exam practice

Section A

1 D	**2** A	**3** C	**4** A
5 B	**6** B	**7** B	**8** C
9 C	**10** C	**11** D	**12** C
13 D	**14** A	**15** D	

Section B

16 a [Aerobic cellular respiration.[1]]

- I have correctly identified the metabolic process.[1]
- I have not just stated cellular respiration.
- I have used appropriate biological terminology such as: aerobic.

b [As there is a lack of oxygen, cells will rely more on anaerobic respiration, which produces ATP and lactic acid.[1]][As lactic acid is an acid, it will decrease the overall pH within cells.[2]]

- I have explained that there is a greater reliance on the anaerobic process which produces more lactic acid.[1]
- I have explained the effect on pH of cells.[2]

c [At high altitudes, the amount of oxygen available is often too low to support aerobic respiration, meaning that the tree relies more on anaerobic respiration.[1]][This process produces ethanol and carbon dioxide as products, which cannot be metabolised.[2]][Ethanol is toxic and eventually accumulates enough to kill the plant.[3]]

- I have explained that there is a greater reliance on fermentation.[1]
- I have stated the products of fermentation.[2]
- I have stated that ethanol is toxic for the plant.[3]
- I have used appropriate biological terminology such as: fermentation, ethanol, metabolised, toxic.

17 a [Anaerobic respiration allows a cell to still produce ATP despite an absence of oxygen.[1]]

- I have described a useful characteristic of anaerobic respiration.[1]

b [Lactic acid and ATP.[1]]

- I have identified all products of anaerobic respiration in mammalian cells.[1]
- I have not stated: carbon dioxide, water, ethanol.

c [The rate of cellular respiration increases when the temperature increases, and is greatest at the optimal temperature of the enzymes involved in cellular respiration, but above this optimal temperature the rate decreases.[1]][At temperatures lower than the optimal, enzymes and substrates have less kinetic energy and move slower, resulting in fewer enzyme–substrate interactions and a lower respiration rate. At temperatures higher than optimal, enzymes can denature, also lowering the respiration rate.[2]]

- I have described the relationship between temperature and respiration rate.[1]
- I have explained the effect of lower and higher temperatures on enzyme activity.[2]

d [Anaerobic respiration and aerobic respiration both involve glycolysis.[1]][Anaerobic respiration only produces 2 ATP per glucose molecule, whereas aerobic respiration produces 36 or 38 ATP per glucose molecule.[2]]

Other acceptable similarities include:

- Both involve reactions that occur in the cytosol.
- Both use glucose as an input.
- Both use NAD^+ as an electron and proton carrier.

Other acceptable differences include:

- Anaerobic respiration produces ATP faster than aerobic respiration.
- Anaerobic respiration cannot be sustained indefinitely due to a build-up of toxins, whereas aerobic respiration can be sustained
- Anaerobic respiration produces lactic acid whilst aerobic respiration produces carbon dioxide and water.

- I have stated a similarity between anaerobic respiration and aerobic respiration.[1]
- I have stated a difference between anaerobic respiration and aerobic respiration.[2]
- I have used comparative language such as: whereas, both.

18 a [L-The Krebs cycle; M-Cytosol; N-Glucose; O-Oxygen; P-CO_2; Q-Water.[1]]

- I have correctly filled in each of the blanks in the table.[1]

b [As hydrogen cyanide is a non-competitive inhibitor, it binds to an allosteric site on the enzyme[1]][and alters the structure of the active site. Consequently, the substrate can no longer bind to the active site.[2]]

- I have stated that hydrogen cyanide binds to an allosteric site and not the active site preventing the substrate from binding.[1]
- I have stated that this prevents the substrate from binding to the active site.[2]
- I have used appropriate biological terminology such as: allosteric site, active site, substrate, enzyme.
- I have referred to the scenario in my response.

c [Hydrogen cyanide prevents the electron transport chain from operating which stops aerobic cellular respiration.[1]][Aerobic cellular respiration is required to produce sufficient ATP to sustain life. Therefore, inhibition by hydrogen cyanide is lethal.[2]]

- I have explained that hydrogen cyanide stops the electron transport chain.[1]
- I have linked the lack of ATP produced with the survival of the cell.[2]
- I have referred to the scenario in my response.

19 a [The cytosol.[1]]

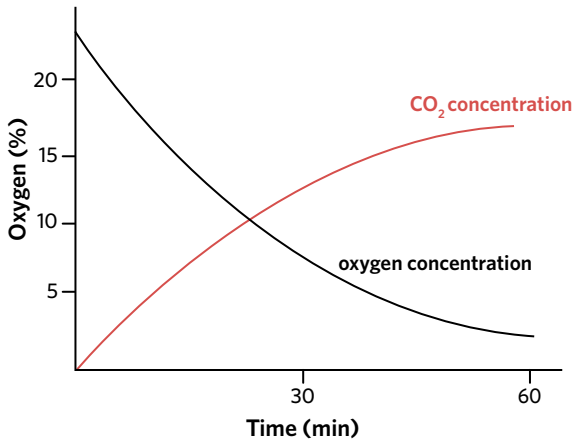
- I have identified where ethanol would be produced in a yeast cell.[1]

b [There will be an increase in ethanol concentration as the oxygen concentration decreases.[1]][This is because the rate of aerobic cellular respiration will decrease and therefore the rate of anaerobic fermentation will increase, producing more ethanol.[2]]

✓ ✗ I have predicted what will happen to the ethanol concentration.[1]

✓ ✗ I have explained my response.[2]

c



✓ ✗ I have identified that the concentration of carbon dioxide increases.

✓ ✗ I have identified that the carbon dioxide concentration rate of increase is similar (but opposite) to the rate of change in oxygen concentration.

✓ ✗ I have started my graph from a concentration of 0.

d [The Krebs cycle which occurs in the mitochondrial matrix.[1]]

✓ ✗ I have identified the stage of cellular respiration and its location.[1]

7A Detecting pathogens

Theory review questions

1 A

2 antigens; MHC proteins; high; non-self; autoimmune disease

3 Cellular: I; II; IV; VI
Non-cellular: III; V

4 I-virus; II-protozoa; III-fungus; IV-bacterium; V-prion

5 I-bacterium; II-protozoa; III-prion; IV-fungus; V-virus

SAC skills questions

6 B **7** A **8** A **9** B

10 C **11** B

Exam-style questions

Within lesson

12 A **13** C **14** C **15** B

16 C **17** B **18** D **19** A

20 a [The Rhesus protein is considered a non-self antigen[1]][because it is a molecule that is recognised by the mother's immune system as non-self, initiating an immune response.[2]]

- I have stated that the Rhesus protein is a non-self antigen.[1]
- I have identified that the Rhesus protein would initiate an immune response.[2]
- I have used key biological terminology such as: antigen, immune system, non-self.

b [No, because the foetal blood cells cannot cause disease in the mother.[1]]

- I have identified that foetal blood cells cannot cause disease.[1]
- I have used key biological terminology such as: disease.

Multiple lessons

21 a [Disease refers to the impaired functioning of cells or processes within the body.[1]]

- I have defined what a disease is.[1]

b [Post-transcriptional modifications to the transcribed pre-mRNA through alternative splicing can produce many different strands of mature mRNA, leading to the production of many different proteins.[1]]

Other acceptable responses include:

- Post-translational changes to the protein such as alternative folding may produce a different functional 3D protein.

- I have identified that post-transcriptional modifications can lead to the production of different strands of mature mRNA.[1]
- I have used key biological terminology such as: post- transcriptional modifications, pre-mRNA, alternative splicing.

c i [Rifampicin inhibits the process of transcription.[1]][Transcription involves the unwinding of the DNA helix which allows for the binding of RNA polymerase to the promoter region.[2]][RNA polymerase then synthesises a strand of pre-mRNA with the use of complementary RNA molecules.[3]][Transcription is terminated when RNA polymerase reaches a termination sequence.[4]]

- I have identified the process of transcription.[1]
- I have described the unwinding of the DNA helix and binding of RNA polymerase.[2]
- I have described the synthesis of pre-mRNA.[3]
- I have described the termination of transcription.[4]
- I have used key biological terminology such as: transcription, RNA polymerase, promoter, pre-mRNA.

ii [These antibiotics inhibit the process of translation.[1]][Translation begins with the binding of a mRNA molecule to a ribosome, with the start codon initiating the process.[2]][tRNA molecules with anticodons complementary to the mRNA strand transport specific amino acids to the ribosome, which are added to the growing polypeptide chain via peptide bonds.[3]][Translation terminates once the stop codon is recognised and the polypeptide chain is released.[4]]

- I have identified the process of translation.[1]
- I have described the initiation stage of translation.[2]
- I have described the elongation stage of translation.[3]
- I have described the termination stage of translation.[4]
- I have used key biological terminology such as: translation, mRNA, codon, tRNA, complementary.

Key science skills and ethical understanding

22 a [Bacteria and fungi.[1]]

- I have correctly stated the type of pathogen present based on the presence of a cell wall.[1]

b [A control would have been an agar plate containing the pathogen without the presence of any medication.[1]]

- I have stated what a control would be in this experiment.[1]

c [A control group would have allowed Sharon to ensure that it was the medication producing the effects and not another uncontrolled variable.[1]][It would have also allowed her to compare how much the bacteria grew without the medication present to how much they grew under different concentrations of the medication.[2]]

- I have identified the role of a control in eliminating uncontrolled variables.[1]
- I have identified the role of a control as a comparison group.[2]
- I have referred to the scenario in my response.

d [Contamination from the outside environment.[1]]

- I have suggested a possible origin.[1]

23 a [The RNA may be in the form of mRNA, which can be used to directly produce viral proteins via the process of translation.[1]]

Other acceptable responses include:

- Reverse transcriptase may convert the RNA into DNA so that the cell can produce mRNA.

✓ ✗ I have suggested a possible method of producing viral particles from RNA.[1]

b [After the accumulation of viruses inside a cell and the weakening of the cell's cytoskeleton, the cell bursts, releasing the viral particles into the extracellular environment. Therefore, every time viruses burst from cells, there is a sudden increase in the number of extracellular viruses.[1]][Conversely, bacteria are able to continuously replicate in the extracellular environment, resulting in a smooth exponential curve.[2]]

✓ ✗ I have explained the graph of viral replication.[1]

✓ ✗ I have explained the graph of bacterial replication.[2]

✓ ✗ I have used key biological terminology such as: cytoskeleton, extracellular environment, exponential.

c [Non-maleficence involves the minimisation of preventable harm.[1]] [In this scenario, non-maleficence would involve taking all necessary precautions (e.g. wearing safety gear) and ensuring that during the development of vaccines, individuals are not harmed and risks are minimised.[2]]

✓ ✗ I have described the bioethical concept of non-maleficence.[1]

✓ ✗ I have described the relevance of non-maleficence to the scenario.[2]

7B The first line of defence

Theory review questions

1 first line of defence; innate immune system; non-specific/immediate; non-specific/immediate

2 B

3 Physical: II; III; IV
Chemical: I; V
Microbiological: VI

4 I-normal flora; II-cilia; III-galls; IV-waxy cuticle; V-lysozymes

SAC skills questions

5 B **6** B **7** A **8** A

9 D

Exam-style questions

Within lesson

10 D **11** B **12** A **13** B

14 C **15** B

Multiple lessons

16 C

17 a [Presence of normal flora such as non-pathogenic bacteria in the gut.[1]]

Other acceptable responses include:

- Non-pathogenic organisms in the vagina.
- Bacteria on the surface of the skin.

✓ ✗ I have identified one microbiological barrier in humans.[1]

b [Mucus traps inhaled bacteria[1]][and cilia beat the trapped bacteria up from the airways into the throat, where they are swallowed and destroyed by the gastrointestinal tract.[2]]

✓ ✗ I have explained how mucus traps bacteria.[1]

✓ ✗ I have explained the role of cilia in preventing infection.[2]

18 a [Normal flora compete with pathogenic bacteria such as *Salmonella* for space and resources, potentially challenging their survival and preventing them from thriving and causing infection.[1]]

✓ ✗ I have explained how normal gut flora can prevent infection.[1]

b i [The acid contained in the stomach.[1]]

✓ ✗ I have identified the chemical barrier in the stomach.[1]

ii [The low pH in the stomach environment is outside of the optimal pH range for *Salmonella* ATPase.[1]][Denaturation of ATPase occurs outside of its optimal pH, and the shape of the active site changes. ATPase can no longer bind to ATP, and the reaction releasing energy is no longer catalysed.[2]][Consequently, there is insufficient energy for *Salmonella* metabolic reactions, resulting in death of the bacteria.[3]]

✓ ✗ I have compared stomach pH to the optimal pH of ATPase.[1]

✓ ✗ I have stated the effect of pH on the structure and function of ATPase.[2]

✓ ✗ I have stated the consequence for *Salmonella* bacteria.[3]

19 a [Viruses are composed of genetic material (DNA or RNA) housed inside a protein coat known as a capsid. A lipid envelope may also surround the protein coat.[1]]

✓ ✗ I have described the structure of a virus.[1]

b [No, viruses cannot be cultured on standard nutrient agar because they are non-cellular pathogens and require the presence of a host cell to replicate.[1]]

✓ ✗ I have explained why viruses cannot be cultured on standard nutrient agar.[1]

c [Intact skin/surfaces.[1]]

Other acceptable responses include:

- Mucous secretions.
- Cilia.

✓ ✗ I have identified one physical barrier preventing the invasion of a virus.[1]

d [The use of radioactive primers complementary to viral DNA in the polymerase chain reaction can help detect the presence of a viral infection.[1]][By annealing to the viral DNA, when the replicated DNA is viewed under UV light, the detection of the radioactive dye would indicate a viral infection.[2]]

✓ ✗ I have suggested how radioactive primers could be used to detect a viral infection.[1]

✓ ✗ I have described the results that would indicate a viral infection.[2]

Key science skills and ethical understanding

20 a [Chemical barrier.[1]]

✓ ✗ I have identified the correct type of barrier.[1]

b [Have two large groups of plants (e.g. 50) that do not produce jasmonic acid naturally.[1]][The plants are of the same species, age, size, health, and are given the same amount of water, nutrients, and sunlight.[2]][Both groups are infested with the same number of caterpillars and the pathogen that infects them, and placed in separate enclosures.[3]][The experimental group gets sprayed with jasmonic acid;[4]][the control group is not exposed to jasmonic acid.[5]][After a week, count the number of caterpillars infected with the pathogen.[6]]

Other acceptable responses include:

- Other controlled variables include the health status of the caterpillars, the amount of spray used, the duration of the groups' exposure to the spray.

✓ ✗ I have specified two groups of specimens and stated that they must be large in number.[1]

✓ ✗ I have identified three controlled variables.[2]

✓ ✗ I have outlined the experimental procedure.[3]

✓ ✗ I have described the experimental group.[4]

✓ ✗ I have described the control group.[5]

✓ ✗ I have stated how results are collected.[6]

c [The experimental plant group with jasmonic acid will have a larger number of infected and/or dead caterpillars compared to the control group.[1]]

✓ ✗ I have described results that support the hypothesis.[1]

✓ ✗ I have used comparative language such as: compared to.

d [Integrity involves the accurate reporting of all results.[1]][Therefore, the researchers will need to publish all their results concerning their experiment even if their results do not support their hypothesis that jasmonic acid inhibits caterpillar innate immunity.[2]]

✓ ✗ I have described the bioethical concept of integrity.[1]

✓ ✗ I have described the relevance of integrity to the scenario.[2]

7C The second line of defence

Theory review questions

1 B

2 I-dendritic cell; II-natural killer cell; III-neutrophil; IV-eosinophil; V-mast cell

3 I-cytokine; II-interferon; III-complement protein; IV-histamine

4 A

5 mast cells; phagocytes; complement proteins; opsonisation

SAC skills questions

6 A **7** B **8** C **9** A

10 C

Exam-style questions

Within lesson

11 C **12** B **13** C **14** C

15 B **16** A

17 a [An increase in mast cell activation would result in the release of histamine.[1]][This causes blood vessels to dilate allowing more blood to flow near the affected area.[2]][Migration of phagocytes to the area will then destroy the pathogen.[3]]

Other acceptable responses include:

- Blood vessels become more permeable, allowing more leukocytes to enter the site of infection.
- Release of cytokines by dying cells.

✓ ✗ I have described one change that would occur during the inflammatory response.[1]

✓ ✗ I have described a second change that would occur during the inflammatory response.[2]

✓ ✗ I have described a third change that would occur during the inflammatory response.[3]

b [Fevers, which increase core body temperature, can affect the functioning of bacterial enzymes through the elevation of temperature beyond their optimal temperature.[1]][Therefore, the increased temperature may affect the 3D structure of those enzymes,[2]][preventing their substrate from binding to the active site, helping limit infection by inhibiting reactions necessary for the survival of the pathogen from occurring.[3]]

Other acceptable responses include:

- Increased temperature can help increase the efficacy of specific immune cells.

✓ ✗ I have identified that fevers can elevate temperature beyond the optimal temperature for enzymes.[1]

✓ ✗ I have identified the consequence of elevated temperatures on the functioning of enzymes.[2]

✓ ✗ I have described the consequence of elevated temperatures on the functioning of enzymes on a cellular level.[3]

c i [Dendritic cells[1]][and macrophages.[2]]

✓ ✗ I have identified one innate antigen-presenting cell.[1]

✓ ✗ I have identified a second innate antigen-presenting cell.[2]

ii [Antigen-presenting cells phagocytose pathogens/foreign material and[1]][present antigens on their surface to specific cells of the adaptive immune system.[2]]

✓ ✗ I have stated that antigen-presenting cells phagocytose.[1]

✓ ✗ I have stated that antigen-presenting cells present antigens to the adaptive immune system.[2]

✓ ✗ I have used key biological terminology such as: phagocytose, pathogens, antigen, adaptive immune system.

d [Natural killer cells.[1]]

✓ ✗ I have identified the correct cell type.[1]

Multiple lessons

18 D **19** A

20 a [Complement proteins, which are involved in the chemotaxis and opsonisation of pathogens, and[1]][cytokines, which are involved in communication between cells of the immune system.[2]]

Other acceptable responses include:

- Lysozymes, which can degrade pathogens.
- Interferons, which increase resistance to viral infection.

✓ ✗ I have described the role of one chemical from the innate immune system.[1]

✓ ✗ I have described the role of a second chemical from the innate immune system.[2]

b [They are non-specific and respond to all pathogens in the same manner.[1]]

Other acceptable responses include:

- Respond immediately/rapidly to pathogens.
- Provide no immunological memory.

✓ ✗ I have stated a defining property of the innate immune system.[1]

Key science skills and ethical understanding

21 a [If a greater concentration of pathogenic bacteria is applied to the skin of the mice, then they will have greater levels of neutrophils and histamine present as part of the second line of defence.[1]]

✓ ✗ I have stated the hypothesis of the scientists.[1]

b [Yes, a control group was used in this experiment. The control group for this experiment was the mouse that wasn't infected with pathogenic bacterial.[1]]

✓ ✗ I have stated which mouse served as the control.[1]

c [No, this experiment doesn't allow scientists to draw a sound conclusion, as the scientists would have had to conduct their experiment on a larger population of mice.[1]]

Other acceptable responses include:

- The scientists would have had to replicate their experiment multiple times.
- More leukocytes and molecules of the innate immune system need to be measured.

✓ ✗ I have explained why the experiment would not have allowed scientists to make a sound scientific conclusion.[1]

d [Neutrophils would phagocytose and destroy the pathogenic bacteria.[1]][Histamine would initiate an inflammatory response which causes vasodilation, allowing more blood and leukocytes to reach the site of infection.[2]]

✓ ✗ I have explained the role of neutrophils.[1]

✓ ✗ I have explained the role of histamine.[2]

✓ ✗ I have used key biological terminology such as: phagocytose, inflammatory response, vasodilation.

7D The third line of defence

Theory review questions

1 adaptive immune system; T helper cell; cytokines

2 I; V; II; III; IV

3 W-antigen-binding site; X-light chain; Y-disulphide bridge; Z-heavy chain

4 I-agglutination; II-opsonisation; III-neutralisation; IV-immobilisation

5 I-plasma cell; II-memory cell; III-cytotoxic T cell; IV-T helper cell

SAC skills questions

6 A **7** D **8** C **9** A

10 B

Exam-style questions

Within lesson

11 B **12** A **13** B **14** C

15 a [Rotavirus infected cells would be eliminated via cell-mediated immunity.[1]][Cell-mediated immunity involves the complementary binding of a pathogenic antigen between a T helper cell and an antigen-presenting cell, thereby selecting it.[2]][Upon selection, the T helper cell will release cytokines to stimulate a similarly selected naive T cell to undergo clonal expansion and differentiation into cytotoxic T cells and T memory cells.[3]][Cytotoxic T cells then destroy the cells infected with the rotavirus via the release of chemicals that induce apoptosis.[4]]

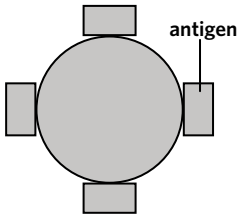
- I have identified the use of the cell-mediated immune response.[1]
- I have described antigen-presentation to T helper cells.[2]
- I have described the expansion and differentiation of T cells.[3]
- I have described the function of cytotoxic T cells.[4]

b [Antibodies are a component of the humoral immune response against extracellular pathogens.[1]][Because viruses can exist as free-floating viral particles outside of cells in the extracellular environment, antibodies could be produced.[2]]

- I have identified that antibodies target extracellular pathogens.[1]
- I have identified that viruses can exist as viral particles in the extracellular environment.[2]

Multiple lessons

16 a



- I have correctly drawn a pathogen with surface antigens that are complementary to the antigen-binding sites of the antibody.
- I have correctly labelled an antigen.

b [Antibodies can neutralise pathogens,[1]][agglutinate pathogens,[2]][and allow phagocytes to engulf pathogens more easily.[3]]

Other acceptable responses include:

- Antibodies can opsonise pathogens.
- Antibodies can immobilise pathogens.
- Antibodies can activate complement proteins.

- I have stated one way antibodies provide protection.[1]
- I have stated a second way antibodies provide protection.[2]
- I have stated a third way antibodies provide protection.[3]

c [In response to an extracellular pathogen, the humoral immune response would be initiated.[1]][After antigen-presentation, activated T helper cells release cytokines to stimulate selected B cells[2]][to undergo clonal expansion and differentiation into plasma cells and B memory cells.[3]][These plasma cells secrete antibodies into the bloodstream to help neutralise the pathogen.[4]]

- I have identified that the humoral immune response would be used.[1]
- I have described that T helper cells produce cytokines to stimulate B cells.[2]
- I have described the expansion and differentiation of B cells.[3]
- I have described the function of plasma cells.[4]

d [The tertiary structure refers to the overall functional 3D structure of a protein.[1]][It is determined by the folding and interactions between the components of the secondary structure such as alpha helices and beta-pleated sheets.[2]]

- I have stated that the tertiary structure is the overall 3D shape of a protein.[1]
- I have identified what causes tertiary protein structure.[2]

17 a [When comparing the innate and adaptive immune responses, the innate immune response provides the body with a form of non-specific and immediate protection,[1]][whereas the adaptive immune response provides the body with a form of pathogen-specific but slow protection.[2]]

- I have identified the distinguishing features of the innate immune response.[1]
- I have identified the distinguishing features of the adaptive immune response.[2]
- I have used comparative language such as: whereas.

b [This cell is an antigen-presenting cell.[1]][MHC II allows the cell to present digested foreign antigens on its surface and interact with T and B cells to stimulate the adaptive immune response.[2]]

Other acceptable responses include:

- Dendritic cell.
- Macrophage.
- B cell.

- I have correctly identified the type of cell described.[1]
- I have explained the role of MHC II.[2]

c [Neutrophils are a phagocytic cell which are not involved in the initiation of the adaptive immune response.[1]][Because they are not antigen-presenting cells and do not express MHC II, they cannot interact with T and B cells.[2]]

- I have identified neutrophils.[1]
- I have explained why neutrophils are not involved in the initiation of the adaptive immune response.[2]

Key science skills and ethical understanding

18 a [That antibodies taken from infected mice will be able to recognise influenza *in vitro* and prevent red blood cells from clumping.[1]]

- I have identified a reasonable hypothesis of the experiment.[1]

b [Well A is a control[1]][since it has no independent variable applied to it, meaning it shows what happens to red blood cells that are unaffected by influenza.[2]]

Other acceptable responses include:

- Well B is a positive control since it shows what happens to red blood cells that are affected by influenza (and without antibodies present).
- Well A is a negative control since it has no independent variable applied and shows what happens to red blood cells that are unaffected by influenza or antibodies.

✓ ✗ I have identified one of the control groups.[1]

✓ ✗ I have explained my reasoning.[2]

c [The independent variable is the source of the antibodies used.[1]] [The dependent variable is the presence of clumping of red blood cells.[2]]

✓ ✗ I have identified the independent variable.[1]

✓ ✗ I have identified the dependent variable.[2]

d [The antibodies form antigen-antibody complexes, immobilising the virus particles.[1]]

Other acceptable responses include:

- Antibodies block viral interaction with the host cell, preventing viral attachment and entry into the cell

✓ ✗ I have identified a method by which antibodies prevent clumping.[1]

e [Non-maleficence involves the minimising of harms.[1]][Therefore, in this experiment, harm should be minimised when infecting mice with influenza and during the extraction of blood (e.g. through the use of a local anaesthetic).[2]]

Other acceptable responses include:

- Monitoring the health of mice and terminating the experiment or treating the mice if they are suffering unduly.

✓ ✗ I have described the bioethical concept of non-maleficence.[1]

✓ ✗ I have described the relevance of non-maleficence to the scenario.[2]

7E The lymphatic system

Theory review questions

1 antigen-presenting cells; lymph nodes; bone marrow; bone marrow; thymus

2 Primary lymphatic tissues: I; III
Secondary lymphatic tissues: II; IV; V

3 B

4 afferent; clusters of immune cells; clonal selection; efferent

SAC skills questions

5 A **6** A **7** A **8** C

9 D

Exam-style questions

Within lesson

10 B

11 **a** [Lymph nodes contain mature lymphocytes[1]][and are the sites for antigen recognition by T and B lymphocytes, which leads to the initiation of the adaptive immune response via clonal selection and lymphocyte differentiation.[2]]

✓ ✗ I have stated mature lymphocytes are present in lymph nodes.[1]

✓ ✗ I have stated that lymph nodes are the site for antigen presentation and initiation of the adaptive immune response.[2]

b [Afferent lymphatic vessels carry lymph fluid to the lymph nodes.[1]]

✓ ✗ I have explained the role of afferent lymphatic vessels.[1]

Multiple lessons

12 D **13** A

14 **a** [Due to the lengthy transportation of the antigen-presenting cell to the lymph nodes, where it must interact with the complementary receptor on a helper T cell, there is a delay in the activation of the adaptive immune system.[1]]

✓ ✗ I have explained the delay in initiating the adaptive immune response.[1]

✓ ✗ I have used key biological terminology such as: helper T cell, antigen-presenting cell, lymph nodes.

b [In lymph nodes, clonal selection and differentiation will occur,[1]] [where antigen-presenting cells present the pathogenic antigens located on their MHC II proteins to T helper cells. Subsequently, these T helper cells will secrete cytokines to stimulate selected T and B cells.[2]]

✓ ✗ I have identified that clonal selection and differentiation occurs in lymph nodes.[1]

✓ ✗ I have described clonal selection and differentiation in the lymph nodes.[2]

✓ ✗ I have used key biological terminology such as: clonal selection, differentiation, antigen-presenting cell, antigen, MHC II, cytokine, T helper cell.

c [The microbe will not be able to cause disease due to the presence of memory B cells,[1]][which upon activation can rapidly differentiate into plasma cells. These plasma cells will then eliminate the microbe through the rapid production of antibodies before it is able to sufficiently replicate and disrupt the normal functioning of cells.[2]]

✓ ✗ I have identified that memory B cells will be present.[1]

✓ ✗ I have identified that the microbe will be eliminated and will not disrupt the functioning of cells.[2]

✓ ✗ I have used key biological terminology such as: plasma cell, antibodies.

15 a [Macrophages are antigen-presenting cells.[1]] [This means that after engulfing and destroying a pathogen, they present antigens on MHC II proteins to T cells within the lymph nodes.[2]]

- I have stated that macrophages are antigen-presenting cells.[1]
- I have stated the role of antigen-presenting cells.[2]
- I have used key biological terminology such as: antigen-presenting cell, pathogen, MHC II, lymph node.

b i [Due to the inability to form mature T cells, patient's with ALL will have decreased levels of T helper and cytotoxic T cells.[1]] [Therefore, their immune system cannot easily combat viruses due to the inability to undergo clonal selection and expansion.[2]]

Other acceptable responses include:

- Patients with ALL are unable to form mature B cells, which means that B cell clonal selection and differentiation into antibody-producing plasma cells does not occur. Therefore, antibodies against the virus are not able to be produced in significant numbers.

- I have identified that patients will have decreased levels of mature T cells.[1]
- I have explained the impact of decreased numbers of mature T cells on the immune system.[2]

ii [Patients with ALL are unable to mount an effective humoral response due to the inability to produce mature B cells.[1]] [Therefore, the immune response against bacteria would be impaired as the process of B clonal selection and differentiation would not occur in the lymph node, preventing the formation of antibody-secreting plasma cells.[2]]

- I have identified that patients will have an impaired humoral response.[1]
- I have identified that B cell clonal selection and differentiation would not occur.[2]
- I have not suggested that T cell differentiation would not occur, because the pathogen is not intracellular.

Key science skills and ethical understanding

16 a [Dendritic cells and macrophages.[1]]

Other acceptable responses include:

- B cells.

- I have named two types of antigen-presenting cell.[1]

b i [The independent variable is whether the mice were infected with *W. bancrofti* or not.[1]] [The dependent variables are the levels of antibodies, T helper cells, cytotoxic T cells, and antigen-presenting cells in the mice.[2]]

- I have correctly identified the independent variable.[1]
- I have correctly identified the dependent variables.[2]

ii [Group 2 served as a positive control, which was exposed to a pathogen with a known immune response.[1]] [Group 3 served as a negative control, which was not exposed to a pathogen and therefore expected to yield no changes in immune function.[2]] [Both served as points of comparison for Group 1 in order to observe and compare the immune response generated by *W. bancrofti* and to ensure that the results were not influenced by an uncontrolled variable.[3]]

- I have identified Group 2 as a control.[1]
- I have identified Group 3 as a control.[2]
- I have explained the role of the controls.[3]

iii [Helper T cells serve as the bridge between the innate and adaptive immune systems through their interaction with antigen-presenting cells and their role in clonal selection and expansion.[1]] [Therefore, a decreased level of helper T cells would prevent the activation of the adaptive immune response against *W. bancrofti*.[2]]

- I have described the role of helper T cells in the immune response.[1]
- I have identified the consequence of a decreased level of helper T cells.[2]
- I have used key biological terminology such as: innate, adaptive, antigen-presenting cell, clonal selection and expansion.

iv [Firstly, the lymphatic system serves as the means of transport for the pathogen and antigen-presenting cells to the lymph nodes.[1]] [Secondly, once lymph arrives at the lymph node, the lymphatic system is the site of selection, cloning, and differentiation.[2]]

Other acceptable responses include:

- The lymphatic system drains fluid from the tissues of the mice.

- I have stated one function of the lymphatic system.[1]
- I have stated a second function of the lymphatic system.[2]

Chapter 7 SAC practice

1 [Immunodeficiency can make individuals more susceptible to infection.[1]]

- I have stated one consequence of immunodeficiency.[1]

2 [The first line of defence, which includes physical, chemical, and microbiological barriers, prevents pathogenic agents from entering the body.[1]]

- I have described the first line of defence.[1]

3 [No, normal flora is not typically considered pathogenic because under normal circumstances it does not cause disease within the body.[1]]

- I have described why normal flora is not typically pathogenic.[1]

4 [Lysozymes.[1]]

✓ ✗ I have identified one chemical mediator released by phagocytes.[1]

5 [Secondary immunodeficiency, because it is caused by a factor that is external to the immune system itself.[1]]

✓ ✗ I have described why immunodeficiency caused by malnutrition is a secondary immunodeficiency.[1]

6 [During the first year of HIV infection, the T cell concentration slightly increases and the HIV concentration significantly increases, before decreasing rapidly.[1]]

✓ ✗ I have described the trends occuring in the first year of infection.[1]

7 [Because viruses are non-cellular and non-living agents[1]][they cannot replicate independently but require a host to replicate. Instead, they must enter cells and use the machinery of that cell to replicate.[2]]

✓ ✗ I have identified that viruses are non-cellular.[1]

✓ ✗ I have described why viruses must enter cells to replicate.[2]

✓ ✗ I have used key biological terminology such as: non-cellular.

8 [Macrophages are responsible for phagocytosis, which involves the engulfing and destruction of pathogens,[1]][as well as antigen-presentation, which involves the presentation of pathogenic antigens on their cell surface.[2]]

✓ ✗ I have described the process of phagocytosis.[1]

✓ ✗ I have described the process of antigen-presentation.[2]

9 [Because HIV destroys T helper cells, which form the bridge between the innate and adaptive immune responses by interacting with antigen-presenting cells, individuals infected with HIV cannot activate the adaptive immune response.[1]][Without the adaptive immune response, individuals cannot activate B cells, which typically differentiate into antibody producing plasma cells,[2]][and cytotoxic T cells, which target virally infected cells.[3]]

✓ ✗ I have recognised that the adaptive immune response cannot be initiated.[1]

✓ ✗ I have stated the humoral immune response will not be activated.[2]

✓ ✗ I have stated the cell-mediated immune response will not be activated.[3]

10 [During the processes of clonal expansion and differentiation, which occur in the lymph nodes, large numbers of lymphocytes will be produced, thereby producing the appearance of swollen lymph nodes.[1]]

✓ ✗ I have explained why lymph nodes would appear swollen.[1]

11 [Poor health education/literacy.[1]]

Other acceptable responses include:

- Poor access to health care and medication.

✓ ✗ I have suggested one reason why the prevalence of HIV/AIDS is significantly higher in Eswatini.[1]

12 [One potential reason for the high prevalence of HIV in Eswatini children could be the transmission of HIV from affected mothers to their children via breast milk or during pregnancy.[1]]

✓ ✗ I have suggested one potential reason for the high rates of HIV in Eswatini children.[1]

13 [Beneficence prioritises the maximising of benefits for the parties involved in an action or decision.[1]][Therefore, due to the considerable pain that Mark is likely experiencing, as well as the indication that he does not wish for any further medical intervention, it would benefit him the most to remove that pain and be taken off the ventilator.[2]]

Other acceptable responses include:

- To certain individuals, prolonging life may be considered beneficial in this scenario regardless of his state of health, and therefore he should be kept on the ventilator.

✓ ✗ I have described the bioethical concept of beneficence.[1]

✓ ✗ I have described the relevance of the bioethical concept to the scenario.[2]

14 [Non-maleficence prioritises the minimising of unnecessary harm.[1]][Because doctors have assessed that Mark is unlikely to ever regain consciousness, any further medical intervention might be seen as futile and may only cause further harm to Mark.[2]]

✓ ✗ I have described the bioethical concept of non-maleficence.[1]

✓ ✗ I have described the relevance of the bioethical concept to the scenario.[2]

15 [Justice involves a commitment to fairness and prioritises the fair distribution of resources as well as equal access to the benefits of an action.[1]][In this scenario, there is no indication that Mark has not had equitable access to treatments nor any indication that providing Mark with further treatment will prevent another patient's access to medical equipment or staff.[2]]

✓ ✗ I have described the bioethical concept of justice.[1]

✓ ✗ I have described the relevance of the bioethical concept to the scenario.[2]

Chapter 7 Exam practice

Section A

1 B	**2** C	**3** D	**4** C
5 B	**6** D	**7** A	**8** C
9 C	**10** D	**11** A	**12** D

Section B

13 a [Pathogens are causative agents of disease.[1]]

✓ ✗ I have correctly defined the term 'pathogen'.[1]

✓ ✗ I have not made reference to pathogens being cellular or organisms that cause disease.

b i [Cellular, because *Haemophilus meningitis* is a bacteria which has a cellular structure, exhibits the processes of a living organism, and can replicate independently.[1]]

I have justified why *Haemophilus meningitis* is a cellular organism.[1]

ii [Complement proteins could combat *Haemophilus meningitis* through either opsonisation, where they would make it easier for phagocytes to detect the bacteria as foreign,[1]][or through the formation of membrane attack complexes, which would cause lysis of the bacteria.[2]]

I have described one mechanism of complement proteins.[1]

I have described another mechanism of complement proteins.[2]

iii [Pathogenic antigens from *Haemophilus meningitis* will be displayed on the MHC II markers of antigen-presenting cells following phagocytosis.[1]][Antigen-presenting cells will then present these pathogenic antigens to T helper cells, which initiate the adaptive immune response through the release of cytokines, activating complementary B cells.[2]]

I have described the mechanism of antigen-presenting cells.[1]

I have described the interaction between antigen-presenting cells and T helper cells in activating the adaptive immune response.[2]

iv [No. Upon re-exposure to *Haemophilus meningitis*, memory B cells that are present from the previous infection[1]][will be able to rapidly differentiate and proliferate into plasma cells, which are capable of secreting vast quantities of antibodies to prevent the formation of disease.[2]]

I have identified the presence of existing memory B cells.[1]

I have described how these memory B cells can prevent disease.[2]

c i [By raising the core body temperature above its normal level, fevers can raise the temperature above the optimum temperature for pathogenic enzymes.[1]][These enzymes may become denatured and no longer be capable of carrying out metabolic processes necessary for the pathogen's survival, preventing them from causing disease within the body.[2]]

I have described the mechanism of fever.[1]

I have described the effect of increasing temperature on the function of pathogenic enzymes.[2]

ii [One change involves blood vessel dilation and increased permeability, which allows for an increased number of immune cells to reach the site of injury.[1]][Another change involves the attraction of phagocytes to the site of injury/infection through the release of cytokines, increasing the phagocytosis of any pathogens present.[2]]

Other acceptable responses include:

- Increased activation of mast cells and the release of histamine, which helps potentiate the inflammatory response.
- Recruitment of healthy T helper cells to facilitate the activation of the adaptive immune response.
- Increased swelling, which indicates the increase in blood flow and migration of immune cells to the site of injury.

I have described one change and its significance.[1]

I have described another change and its significance.[2]

14 a [Waxy cuticles can help create a barrier against pathogens.[1]]

Other acceptable responses include:

- Presence of thick bark, which creates a barrier to pathogens.
- Closing of stomata, which prevents further infection from pathogens.
- Formation of galls, which limits the spread of pathogens beyond the infected tissue.

I have described one physical defence present in plants.[1]

b [Firstly, plants can secrete toxins such as antimicrobials that are harmful to pathogens, potentially killing them.[1]][Secondly, plants can produce enzymes that affect the functioning of pathogens and/or inhibit their development.[2]]

Other acceptable responses include:

- Plants can produce chemicals that repel insects.

I have stated one way chemical barriers help plants fight pathogens.[1]

I have stated a second way chemical barriers help plants fight pathogens.[2]

c [Plants do not have an adaptive immune system.[1]][Therefore, their innate immune system is important because it is the only protection they have against pathogens.[2]]

I have stated that plants do not have an adaptive immune system.[1]

I have related this back to the importance of the innate immune system in plants.[2]

15 a [The independent variable is the strain of varicella zoster applied to each group of mice.[1]][The dependent variables include the levels of antigen-presenting cells and cytotoxic T cells.[2]]

I have identified the independent variable.[1]

I have identified the dependent variables.[2]

b [Group 7 is the control group.[1]][This group ensures that the changes in immune response were caused by the infection with varicella zoster and not another uncontrolled variable. It also serves as a point of comparison with the other experimental groups.[2]]

I have identified the control group.[1]

I have described the purpose of the control group in this experiment.[2]

c [Cytotoxic T cells recognise abnormal/infected cells and kill them via the release of cytotoxic chemicals such as perforins.[1]]

✓ ✗ I have described the role of cytotoxic T cells in the immune response.[1]

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d **i** [MHC I markers are responsible for marking a cell as 'self' to prevent attack from immune cells.[1]][MHC II markers are responsible for presenting foreign antigens to helper T cells.[2]]

✓ ✗ I have described the purpose of MHC I markers.[1]

✓ ✗ I have described the purpose of MHC II markers.[2]

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ii [The lymphatic system is responsible for transporting antigen-presenting cells and foreign antigens to lymph nodes, where they can interact with complementary T helper cells or B cells.[1]] [Additionally, the lymphatic system is responsible for the production and maturation of lymphocytes.[2]]

✓ ✗ I have described one function of the lymphatic system.[1]

✓ ✗ I have described another function of the lymphatic system.[2]

8A Acquiring immunity

Theory review questions

1 A

2 B

3 Passive strategy: II; IV
Active strategy: I; III

4 herd immunity; booster vaccine/shot; memory cells; artificial passive immunity

SAC skills questions

5 B **6** C **7** B **8** B

9 B **10** A

Exam-style questions

Within lesson

11 B

12 **a** [Artificial, active immunity. This immunity is artificial because the vaccine injection is a medical intervention[1]][and it is active as the vaccination contains antigens from the *Bordetella pertussis* bacteria which stimulates an adaptive immune response.[2]]

- ✓ ✗ I have explained why this immunity is artificial.[1]
- ✓ ✗ I have explained why this immunity is active.[2]

b **i** [Booster vaccine.[1]][Booster vaccines work by introducing antigens that trigger the production of more memory B cells[2]] [and are necessary because memory B cells die over time. As they die, a person's immunity to a disease decreases, so their population must be replenished to maintain immunity.[3]]

- ✓ ✗ I have stated the correct type of vaccine.[1]
- ✓ ✗ I have stated how booster vaccines work.[2]
- ✓ ✗ I have explained why booster vaccines are necessary.[3]
- ✓ ✗ I have used appropriate biological terminology such as: memory B cell.

ii [Herd immunity, which aims to protect unvaccinated individuals by having a high rate of vaccination within a population.[1]] [By doing so, the number of people who become ill with pertussis and could infect the unvaccinated population is lowered, meaning it is unlikely the disease will spread to babies who have not been vaccinated.[2]]

- ✓ ✗ I have explained what herd immunity aims to achieve.[1]
- ✓ ✗ I have explained the benefit of herd immunity.[2]
- ✓ ✗ I have referred to the scenario in my response.

Multiple lessons

13 D

14 **a** [If a child does not receive a full vaccination schedule their immune system will not be stimulated to produce sufficient memory cells and antibodies to confer long-lasting immunity.[1]][During infection, therefore, the pathogen will not be destroyed quickly and it will be capable of causing disease.[2]]

- ✓ ✗ I have stated the impact of an incomplete vaccination schedule on the child's immunity.[1]
- ✓ ✗ I have stated the pathogen will not be destroyed and therefore will cause disease.[2]

b [Macrophages.[1]]

Other acceptable responses include:

- Dendritic cells.

- ✓ ✗ I have stated one type of antigen-presenting cell.[1]

c [The parents who are supportive of vaccination but fear potential negative outcomes and therefore do not vaccinate their children fully.[1]][Given that widespread vaccination is crucial for successful herd immunity, this group should be targeted because they make up 6% of parents in Australia, as opposed to parents who completely reject vaccines who only comprise 2% of the population.[2]]

- ✓ ✗ I have chosen the correct group of parents.[1]
- ✓ ✗ I have explained why this group should be targeted.[2]
- ✓ ✗ I have used evidence from the article to justify my response.

d [Improving access to vaccines,[1]][addressing concerns of hesitant parents,[2]][and educating anti-vaccination parents about the benefits and safety of vaccination.[3]]

Other acceptable responses include:

- Decrease the amount of coverage media outlets give to non-vaccinating parents.

- ✓ ✗ I have stated one possible strategy.[1]
- ✓ ✗ I have stated a second possible strategy.[2]
- ✓ ✗ I have stated a third possible strategy.[3]

Key science skills and ethical understanding

15 **a** [A vaccine is a medical intervention that contains non-disease causing pathogen antigens used to trigger an immune response.[1]] [Antigen-presenting cells present antigens of the inactivated or fragmented pathogen to T and B cells, binding to those which have complementary receptors to the antigen.[2]][These lymphocytes then undergo clonal selection and differentiation, resulting in the formation of antibody-secreting plasma cells, cytotoxic T cells, and memory B and T cells.[3]][The memory cells reside in the body for extended periods of time, and will enable the body to mount a larger, faster response to the pathogen if it is re-encountered, providing long-lasting immunity.[4]]

✓ ✗ I have stated the definition of a vaccine.[1]

✓ ✗ I have described the initiation of the adaptive immune system.[2]

✓ ✗ I have described the process of clonal selection and differentiation.[3]

✓ ✗ I have explained how immunological memory results in lifelong immunity.[4]

✓ ✗ I have used correct biological terminology such as: antigen-presenting cell, complementary, lymphocyte, B cell, T cell, memory cell, pathogen.

b i [Intervention A is an injection of VZV antibodies.[1]][This is because the concentration of antibodies in Patient 1's blood immediately increases on day 1 and then begins to steadily decrease.[2]][Intervention B is an injection with the VZV vaccine.[3]] [This is because the concentration of antibodies increases gradually in Patient 2 as their adaptive immune system takes a few days to mount a specific response to the inactivated pathogen's antigens.[4]]

✓ ✗ I have correctly identified intervention A.[1]

✓ ✗ I have referred to the graph of Patient A in my response.[2]

✓ ✗ I have correctly identified intervention B.[3]

✓ ✗ I have referred to the graph of Patient B in my response.[4]

ii [A second injection with the VZV vaccine.[1]][This is needed because typically more than one dose of a vaccine is required to induce the production of enough B memory cells and antibodies to confer lifelong immunity.[2]]

✓ ✗ I have stated what occurred on day 22 of the experiment.[1]

✓ ✗ I have explained why this is necessary.[2]

✓ ✗ I have not stated that a booster vaccine was given.

iii [Artificial, passive immunity.[1]]

✓ ✗ I have stated the correct type of immunity.[1]

c [A control was not used in this experiment.[1]][A control could have involved injecting a third patient with a placebo known to have no effect on the immune system.[2]]

Other acceptable responses include:

- A control could have involved not injecting a third patient with anything.

✓ ✗ I have stated a control was not used.[1]

✓ ✗ I have explained what a control could have been.[2]

d [The original experimental design had a large potential to cause harm to the participants by deliberately giving them chickenpox, and as such did not adhere to the bioethical concept of non-maleficence.[1]]

✓ ✗ I have stated that because the original experimental design could have caused harm it did not adhere to the bioethical concept of non-maleficence.[1]

8B Emergence of pathogens

Theory review questions

1 A

2 B

3 epidemics; infectious; pandemics; pandemics; epidemics

4 I; II; IV

SAC skills questions

5 A **6** B **7** B **8** C

9 B

Exam-style questions

Within lesson

10 A **11** D

Multiple lessons

12 a [An infectious disease caused by a pathogen that has transferred from an animal to a human.[1]]

✓ ✗ I have defined the term zoonosis.[1]

b [A disease outbreak that has spread globally or across a large geographical area in different regions of the world.[1]]

✓ ✗ I have defined the term pandemic.[1]

c [Interferons are released by cells infected by viruses and warn nearby cells, increasing their ability to resist viral infection.[1]]

Other acceptable responses include:

- Interferons cause the production of antiviral proteins.

✓ ✗ I have correctly described the role of interferons.[1]

d [T helper cells. In the humoral response, T helper cells that have been selected through antigen presentation recognise selected B cells and secrete cytokines that cause the B cells to undergo clonal expansion and differentiation into plasma cells and B memory cells.[1]] [In the cell-mediated response, T helper cells secrete cytokines that stimulate selected naive T cells to undergo expansion and differentiation into cytotoxic T cells and T memory cells.[2]]

✓ ✗ I have explained the role of T helper cells in the humoral response.[1]

✓ ✗ I have explained the role of T helper cells in the cell-mediated response.[2]

13 a [Because the disease has not spread to multiple countries in different regions around the world.[1]]

✓ ✗ I have explained why the disease is an epidemic.[1]

b [This suggests that yellow fever was once a disease of public health concern but was brought under control,[1]][however from 2017 is once again posing a large threat to public health.[2]]

✓ ✗ I have stated yellow fever was once a disease of concern but was controlled.[1]

✓ ✗ I have stated that it is no longer under control and threatens public health.[2]

c i [Artificial active immunity.[1]]

✓ ✗ I have stated the correct type of immunity.[1]

ii [A yellow fever vaccine would contain antigens of the virus that causes yellow fever[1]][and, when injected, would facilitate the production of specific antibodies against the yellow fever virus.[2]] [This would also result in the production of memory cells,[3]] [meaning that when the body encounters the yellow fever virus in the future the immune response will be faster and greater than normal.[4]]

✓ ✗ I have explained a vaccine would contain antigens specific to the virus.[1]

✓ ✗ I have explained specific antibodies would be created.[2]

✓ ✗ I have explained memory cells would be created.[3]

✓ ✗ I have stated the future immune response would be faster and greater.[4]

✓ ✗ I have referred to the scenario throughout my response.

14 a [Natural active immunity.[1]]

✓ ✗ I have stated the correct type of immunity.[1]

b [A lack of prior immunity to European diseases[1]][and a forced change in lifestyle that decreased their general health status.[2]]

Other acceptable responses include:

- A lack of knowledge and experience with European diseases.
- Being forced to live in densely populated areas.

✓ ✗ I have stated one reason for the Indigenous population's susceptibility to European diseases.[1]

✓ ✗ I have stated a second reason for the Indigenous population's susceptibility to European diseases.[2]

Key science skills and ethical understanding

15 C **16** C

8C Controlling pathogen spread

Theory review questions

1 D

2 I-direct physical contact; II-faecal-oral/indirect physical contact; III-droplet; IV-indirect physical contact; V-airborne

3 B

4 disinfectants; antiseptics; antibiotics; antivirals; antimicrobial resistance

SAC skills questions

5 A **6** B **7** C **8** C

9 D **10** B **11** B

Exam-style questions

Within lesson

12 B **13** B

14 a [Polio is spread via the faecal-oral route. Therefore, an effective method to prevent its spread would be to improve hygiene via washing hands.[1]]

Other acceptable responses include:

- Specific examples of improving sanitation/hygiene. This could include improving access to clean, fresh water; and/or separating wastewater from drinking water.

✓ ✗ I have stated a method for preventing the spread of polio that is based on its mode of transmission.[1]

✓ ✗ I have not stated a method that could not be identified using the information provided such as: vaccination.

b [Ebola.[1]]

✓ ✗ I have correctly identified the least contagious disease.[1]

c i [Scientists could test for the presence of the Ebola virus in patient serum by using an enzyme-linked immunosorbent assay.[1]][This test identifies the presence of antibodies in the blood by using antigens from Ebola pathogen material.[2]]

Other acceptable responses include:

- Visualising the pathogen.
- Use of biochemical testing.
- Use of molecular techniques, including hybridisation-based detection, amplification-based detection, or whole-genome sequencing.

✓ ✗ I have identified one method of pathogen identification that could be used in this case.[1]

✓ ✗ I have described this method.[2]

ii [Authorities would quarantine the traveller until they showed no signs of disease or it was confirmed they did not carry the pathogen.[1]][This would prevent the disease from entering and being transmitted to the rest of the Australian population.[2]]

Other acceptable responses include:

- Testing for the presence of the Ebola virus.

✓ ✗ I have identified an appropriate course of action.[1]

✓ ✗ I have stated why this course of action would be taken.[2]

✓ ✗ I have used appropriate biological terminology such as: quarantine.

Multiple lessons

15 **a** [1958.[1]]

✓ ✗ I have identified the correct year.[1]

b [No, antibiotics would not be effective in treating this person[1]] [because measles is a disease caused by a virus, and antibiotics only work against bacteria.[2]]

✓ ✗ I have correctly stated that antibiotics would not be effective.[1]

✓ ✗ I have explained why antibiotics would not be effective in this situation.[2]

c **i** [Herd immunity.[1]]

✓ ✗ I have correctly identified the type of immunity the government is trying to achieve.[1]

ii [By vaccinating a large proportion of the population and achieving herd immunity, there are fewer people who could carry the disease.[1]] [This means that the capacity for the disease to spread is limited, protecting those who haven't been vaccinated.[2]]

✓ ✗ I have stated how herd immunity works at a population level.[1]

✓ ✗ I have stated how this protects people who are unvaccinated.[2]

d [Firstly, mucus production in the airways creates a physical barrier that helps to protect against inhaled airborne droplets containing the viral pathogens.[1]] [Secondly, if the virus did invade the body, interferons would be released to combat infection by disrupting viral replication.[2]]

Other acceptable responses include:

- Cilia in the airways would sweep trapped viral pathogens from the respiratory tract.
- Release of pro-inflammatory cytokines stimulating the immune response to destroy the virus.
- White blood cells (e.g. NK cells, macrophages) would be recruited to destroy viral particles or remove cells that have been infected by the virus.

✓ ✗ I have identified one innate immune mechanism.[1]

✓ ✗ I have identified a second innate immune mechanism.[2]

✓ ✗ I have signposted my response using terms such as: firstly, secondly.

✓ ✗ I have not referred to other pathogens such as bacteria in my response.

Key science skills and ethical understanding

16 **a** [The higher the concentration of antibiotics, the lower the amount of observed bacterial growth.[1]]

✓ ✗ I have identified the expected difference.[1]

b [This is an example of a random error[1]] [because it affects the precision of a measurement and is unpredictable and inconsistent between repeated measurements.[2]] [This uncertainty could be reduced by using a measuring device that has smaller, more precise units of measurement such as a ruler with 1 mm markings instead of 1 cm markings.[3]]

✓ ✗ I have stated what type of error could occur.[1]

✓ ✗ I have justified why this is a type of random error.[2]

✓ ✗ I have provided a way to reduce this error.[3]

c [The dependent variable is the size of the zone of inhibition of bacterial growth around the antibiotic.[1]] [This is because the amount of bacterial growth changes depending on the concentration of antibiotic, the independent variable.[2]]

✓ ✗ I have correctly identified the dependent variable in this experiment.[1]

✓ ✗ I have justified my response by referring to the change in the independent variable.[2]

d [Sharon would expect that the antifungal drug would have no effect on bacterial growth.[1]] [This is because antifungals are not effective against bacteria and only work against fungi.[2]]

✓ ✗ I have stated the result Sharon would expect to obtain.[1]

✓ ✗ I have explained why Sharon would expect this result by referring to the limitations of antifungals.[2]

e [Sharon's actions are not adhering to the bioethical concept of integrity, which prioritises the honest and accurate reporting of information.[1]] [By changing her results post-experiment in order to suit her hypothesis, Sharon is misrepresenting her findings and failing to publish unfavourable results.[2]]

✓ ✗ I have identified Sharon's actions as failing to adhere to the bioethical concept of integrity.[1]

✓ ✗ I have explained my response with reference to the information provided.[2]

8D Immunotherapy

Theory review questions

1 A

2 III; V; VI; I; II; IV

3 D

4 autoimmune diseases; autoantibodies; autoreactive; suppression immunotherapy

SAC skills questions

5 A **6** A **7** B **8** B

9 A

Exam-style questions

Within lesson

10 C **11** A

12 a [Activation immunotherapies enhance the functioning of the immune system, whereas suppression immunotherapies reduce it.[1]]

- I have compared how activation immunotherapies and suppression work.[1]
- I have used comparative terminology such as: whereas.

b i [Traditional chemotherapy typically works by targeting and killing rapidly dividing cells.[1]][This leads to many rapidly dividing, but non-cancerous cells, such as hair follicles and cells lining the gut, being killed, causing widespread side effects.[2]] [Immunotherapy, on the other hand, can use antibodies to target cancerous cells more specifically, killing only cancerous cells and not damaging other cells of the body. As a result, it typically causes fewer side effects.[3]]

- I have explained how chemotherapy works.[1]
- I have linked how chemotherapy works to the side effects it typically causes.[2]
- I have explained how immunotherapy works.[3]

ii [Ipilimumab monoclonal antibodies would be artificially made to bond/attach to CTLA-4.[1]][Given this protein inhibits the immune system, ipilimumab would act as a checkpoint inhibitor, reducing inhibition of the immune system and increasing its ability to recognise and destroy the cancerous melanoma cells.[2]]

- I have stated that ipilimumab would attach to CTLA-4.[1]
- I have explained how ipilimumab would act as a checkpoint inhibitor to treat melanoma.[2]

Multiple lessons

13 a [An autoimmune disorder is when the body's immune system initiates an immune response that targets healthy self cells.[1]]

- I have defined autoimmune disorder.[1]

b [Yes, as mAbs specifically 'target cancerous cells while avoiding healthy cells',[1]][unlike chemotherapy which has a low cell-specificity and causes unwanted side effects.[2]]

- I have explained the extent of damage of monoclonal antibodies using evidence from the article.[1]
- I have explained the extent of damage of chemotherapy.[2]

c [Passive artificial immunity.[1]]

- I have identified the type of immunity.[1]

d [mAbs can block signals by binding to signalling molecules that initiate cell division in cancerous cells[1]][which prevents the growth of cancer in an organism.[2]]

Other acceptable responses include:

- Signal for immune cells to attack cancerous cells.
- Induces apoptosis.
- Induces formation of the membrane attack complex (MAC).

- I have described a mode of action for mAbs that affects cancerous cells.[1]
- I have explained how this can treat cancer.[2]

e i [The mAbs would be different, as antibodies have a binding site that is highly specific to the targeted antigen.[1]][These future treatments would therefore treat different cells using unique mAbs, as the target antigens expressed on these cells would be different.[2]]

- I have explained why the mAbs are different.[1]
- I have outlined that different treatments target different cells in different ways.[2]
- I have used appropriate biological terminology such as: mAbs, antibody, active site.

ii [mAbs can deliver a drug directly to the target site due to the specificity of an antibody's active site for the target antigen,[1]] [which can likely increase the efficiency and efficacy of a drug's mode of action.[2]]

- I have explained the role of mAbs in drug delivery.[1]
- I have outlined the overall implication of mAbs being used in drug delivery.[2]
- I have used appropriate biological terminology such as: mAbs, target site, specificity, antibody, active site, efficiency, efficacy.

Key science skills and ethical understanding

14 a [The amount of sample extracted from each patient would affect the results,[1]][as extra sample would mean there were likely more antigens present to bind to the surface of the tube, which would result in a darker colour following antibody binding.[2]]

Other acceptable responses include:

- The amount of antibodies added.
- Human error in detecting the colour as it is an observation and not a quantitative result.
- CA19-9 antigen may not have properly attached to the surface of the container.

- I have identified a variable that may have affected the results.[1]
- I have explained how the variable could affect the results.[2]

b [A positive control sample would have a large concentration of the CA19-9 antigen and would produce a brown colour.[1]] [A negative control sample would contain no CA19-9 antigen and would produce a transparent/white colour.[2]]

✓ ✗ I have stated the contents and colour of the positive control sample.[1]

✓ ✗ I have stated the contents and colour of the negative control sample.[2]

c [mAbs can be used as activation immunotherapies[1]] [to stimulate the immune system and increase its ability to recognise and destroy cancer cells.[2]]

✓ ✗ I have stated that mAbs can be used as activation immunotherapy.[1]

✓ ✗ I have explained what is meant by the term activation immunotherapy.[2]

d [According to the bioethical concept of non-maleficence, harm should be avoided when conducting research.[1]] [Deliberately inducing tumours in mice involves harming them, and as such this method of antibody creation needs to be ethically evaluated to ensure whether or not it should be used, or how any harms caused can be minimised.[2]]

✓ ✗ I have explained the relevant bioethical concept.[1]

✓ ✗ I have applied this bioethical concept to the bioethical issue and stated a relevant conclusion.[2]

Chapter 8 SAC practice

1 [Penicillin inhibits bacterial growth by preventing cell wall synthesis.[1]]

✓ ✗ I have described how penicillin inhibits bacterial growth.[1]

2 [Penicillin cannot be used to treat viral infections, as viruses are non-cellular and do not contain the cellular structure (the cell wall) that penicillin targets.[1]]

✓ ✗ I have explained why penicillin cannot treat viral infections.[1]

3 [Prevention of bacterial transmission can include washing hands thoroughly with soap[1]] [and wearing personal protective equipment such as gloves.[2]]

Other acceptable responses include:

- practising social distancing.
- antibiotics specific to the given bacterium.
- quarantine of infectious cases, or suspected cases.

✓ ✗ I have identified one method that could prevent bacterial transmission.[1]

✓ ✗ I have identified a second method that could prevent bacterial transmission.[2]

4 [Microscopy, which involves viewing bacterial cultures under a microscope so that they can be identified.[1]]

Other acceptable responses include:

- Gram staining.
- blood tests.

✓ ✗ I have described one technique that could be used to identify bacteria.[1]

5 [Due to the rapidly evolving nature of the influenza virus, it may develop resistance to Relenza through the changing of its neuraminidase active site.[1]]

✓ ✗ I have suggested how resistance against Relenza may arise.[1]

6 [A high-density population, where lots of people live in close proximity to each other,[1]] [and a highly infectious pathogen with little immunity in the community may cause a pathogen to rapidly spread through a local community.[2]]

✓ ✗ I have described one mechanism causing rapid pathogenic spread.[1]

✓ ✗ I have described a second mechanism causing rapid pathogenic spread.[2]

7 [Vaccines contain pathogenic antigens, such as an inactivated component or a weakened form of a pathogen,[1]] [that trigger the production of antibodies and memory cells but do not cause disease.[2]]

✓ ✗ I have described what vaccines contain.[1]

✓ ✗ I have described how vaccines work.[2]

8 [By maintaining large proportions of vaccinated individuals, herd immunity can be created.[1]] [Herd immunity involves the reduction of susceptible hosts, thereby preventing diseases from being spread around the community as easily.[2]]

✓ ✗ I have identified the creation of herd immunity.[1]

✓ ✗ I have described the purpose of herd immunity.[2]

9 [July.[1]]

✓ ✗ I have identified the correct month.[1]

10 [Decreased vaccination rates against influenza may have occurred in 2017.[1]]

Other acceptable responses include:

- Formation of a new influenza strain with minimal immunity in the community.

✓ ✗ I have suggested a possible reason for the significant increase.[1]

11 [Production would commence with the stimulation of a mouse's immune system against the molecule of interest – the bacterial toxin – and the extraction of its plasma B cells.[1]] [Then, the mouse's plasma B cells would be fused with cancerous cells to create a hybridoma, which produces large amounts of monoclonal antibodies specific to the bacterial toxin.[2]]

I have described the extraction of plasma B cells from mice.[1]

I have described the fusion of plasma B cells with cancerous cells.[2]

12 [The use of monoclonal antibodies to treat a bacterial infection would be a form of artificial passive immunity.[1]][This is because the antibodies are introduced to the body through medical intervention and are not produced by the individual receiving the antibodies.[2]]

I have identified the correct type of immunity.[1]

I have justified my response.[2]

13 [Monoclonal antibodies can attach to specific cancer cell antigens and flag them to be detected by immune cells,[1]][thus initiating an immune response.[2]]

I have described a mode of action of monoclonal antibodies.[1]

I have described how they can treat cancer.[2]

14 [Monoclonal antibodies can bind to cytokines to downregulate signalling between immune cells[1]][in an effort to reduce the activation of immune cells involved in the destruction of self cells.[2]]

Other acceptable responses include:

- B and T cell inhibition.

I have described the mode of action of monoclonal antibodies.[1]

I have described how they can treat autoimmune diseases.[2]

15 [Non-maleficence involves the minimisation of preventable harm.[1]] [Approving the use of monoclonal antibodies prior to the completion of clinical trials runs the risk of unknown side effects, and therefore we should not give monoclonal antibodies prior to approval given the unnecessary risk and potential for harm these side effects could cause in patients.[2]]

I have described the bioethical concept of non-maleficence.[1]

I have described the relevance of non-maleficence to the scenario.[2]

Chapter 8 Exam practice

Section A

1 A	**2** D	**3** B	**4** B
5 B	**6** C	**7** B	**8** B
9 A	**10** C	**11** C	**12** A

Section B

13 a [A vaccine is a medical intervention that contains pathogenic antigens, such as an inactivated component or a weakened form of a pathogen,[1]][which triggers the production of antibodies and memory cells, but does not cause disease.[2]]

I have described what vaccines contain.[1]

I have described the purpose of vaccines.[2]

b i [The antibodies would bind to the heavy chain of the tetanospasmin toxin.[1]]

I have correctly identified where antibodies bind to the tetanospasmin toxin.[1]

ii [The antibodies within the sheep blood agglutinate the tetanospasmin toxin, forming large antibody-toxin complexes. This effectively deactivates the toxin, preventing it from causing disease.[1]]

I have explained how antibodies can provide immunity to the tetanospasmin toxin.[1]

c i [The full vaccination schedule requires the individual to receive the tetanus vaccine six times, as there are six injection peaks indicated on the graph.[1]]

I have stated six doses of the vaccine.[1]

ii [Vaccination against the tetanospasmin toxin generates an adaptive immune response that produces specific memory cells targeting the toxin.[1]][This enables a faster and larger immune response in subsequent encounters with tetanospasmin.[2]] [Repeated immunisations, as opposed to a single vaccination, increase the total amount of these memory cells and increase the effectiveness of future immune responses, promoting longer-lasting immunity.[3]]

I have described how the vaccine works.[1]

I have explained how the presence of memory cells affect future responses to the toxin.[2]

I have explained why repeated immunisations are necessary.[3]

14 a [Booster vaccines are required when existing memory cell levels become depleted.[1]][They stimulate existing memory cells to produce more memory cells, thereby providing the individual with immunity to whooping cough for longer. In doing so, the adults can help prevent the spread of *B. pertussis* to children.[2]]

I have identified the necessity of booster vaccines.[1]

I have explained the function of booster vaccines.[2]

I have used key biological terminology such as: booster vaccines, memory cells.

b [Vaccines stimulate the production of long-lasting memory cells specific to a pathogen.[1]][Therefore, on subsequent exposure to the pathogen, the memory cells can facilitate a greater and more rapid immune response, eliminating the pathogen quickly so that the person does not develop symptoms of the disease.[2]]

I have described how vaccines work.[1]

I have explained how vaccines can reduce the likelihood of developing disease.[2]

c i [Antibodies against *B. pertussis* produced within the mother can be passed to the foetus via the placenta, which is connected to the foetus through the umbilical cord, providing the foetus with some immune protection against the bacterium.[1]]

✓ ✗ I have explained how a pregnant mother could provide their foetus with protection.[1]

ii [This is a form of natural passive immunity,[1]][as the antibodies are produced without medical intervention, and are produced outside of the foetus.[2]]

✓ ✗ I have identified the correct type of immunity.[1]

✓ ✗ I have justified my response.[2]

iii [High vaccination rates can protect unvaccinated infants due to the creation of herd immunity,[1]][which reduces the number of susceptible hosts in a given community, thereby preventing whooping cough from being naturally spread in the population.[2]]

✓ ✗ I have identified that protective herd immunity is formed.[1]

✓ ✗ I have described the purpose of herd immunity.[2]

d [Transmission of whooping cough could be reduced through proper hand hygiene, which involves washing hands with soap,[1]] [and wearing personal protective equipment such as masks.[2]]

Other acceptable responses include:

- quarantine of whooping cough cases, or suspected cases.
- generating herd immunity through vaccination.

✓ ✗ I have identified one method of reducing transmission.[1]

✓ ✗ I have identified a second method of reducing transmission.[2]

15 a [Afghanistan.[1]]

✓ ✗ I have identified the correct country.[1]

b i [Decreased vaccination rates against the polio virus[1]][and the evolution of the polio virus.[2]]

Other acceptable responses include:

- globalisation and travel.

✓ ✗ I have suggested one reason for the re-emergence of polio.[1]

✓ ✗ I have suggested a second reason for the re-emergence of polio.[2]

ii [No, because there isn't a sudden or unusual increase in the occurrence of polio in this time period in the given regions shown in the graph.[1]]

✓ ✗ I have described why the graph does not support the occurrence of a pandemic.[1]

c [Given that the Indigenous Australians had not previously encountered the introduced diseases, they had a decreased immunity compared to the Europeans. This meant that they experienced more significant disease when compared to the Europeans, many of whom had already been infected and had developed immunity to the diseases that were introduced.[1]]

✓ ✗ I have explained why the diseases had such a significant effect on the Indigenous Australians when compared to the Europeans.[1]

16 a [Omalizumab will have two antigen-binding sites with a complementary shape to the IgE antibody. This will allow it to prevent IgE from binding with its complementary allergen and reduce degranulation of mast cells and histamine release, alleviating the symptoms of persistent allergic asthma.[1]]

✓ ✗ I have described how omalizumab could be used to treat persistent allergic asthma.[1]

b [Production would commence with the stimulation of a mouse's immune system against the molecule of interest (IL-5) and the extraction of plasma B cells from the mice.[1]][Then, the plasma B cells are fused with cancerous cells to create a hybridoma, which produces large amounts of monoclonal antibodies against IL-5.[2]] [Monoclonal antibodies can then neutralise IL-5, preventing the recruitment of eosinophils.[3]]

✓ ✗ I have described the extraction of plasma B cells from mice.[1]

✓ ✗ I have described the fusion of plasma B cells with cancerous cells.[2]

✓ ✗ I have described the neutralisation of IL-5 and the prevention of eosinophil recruitment.[3]

9A The gene pool

Theory review questions

1 gene pools, allele frequencies, same species, same location

2 C

3 C

4 I-missense mutation; II-silent mutation; III-nonsense mutation; IV-frameshift mutation; V-block mutation

SAC skills questions

5 A **6** B **7** C **8** C

9 B

Exam-style questions

Within lesson

10 a i [A nonsense mutation has occurred, which involves a single nucleotide substitution resulting in the production of a stop codon that prematurely terminates the translation of the mRNA transcript.[1]]

✓ ✗ I have described the mutation which has occurred.[1]

ii [ACC to ACT.[1]]

Other acceptable responses include:

- ACC to ATC.

✓ ✗ I have identified a possible change in the DNA template strand.[1]

iii [Due to the production of a stop codon, the polypeptide chain will be prematurely terminated during translation,[1]] [resulting in a polypeptide for the fibrillin-1 protein which is shorter than normal.[2]]

✓ ✗ I have identified that the polypeptide chain will be prematurely terminated.[1]

✓ ✗ I have described the effect of premature termination on fibrillin-1.[2]

b i [A missense mutation has occurred, which involves a single nucleotide substitution resulting in the production of a codon that codes for a different amino acid.[1]]

✓ ✗ I have described the mutation which has occurred.[1]

ii [ACA to CCA.[1]]

Other acceptable responses include:

- ACG to CCG.

✓ ✗ I have identified a possible change in the DNA template strand.[1]

iii [Due to the substitution of glycine for cysteine, this will change the primary structure of fibrillin-1, potentially leading to abnormal bonding between amino acids, resulting in a different tertiary structure.[1]] [Due to the change in fibrillin-1's 3D structure, fibrillin-1 may no longer be functional.[2]]

✓ ✗ I have described the effect of the mutation on the polypeptide chain.[1]

✓ ✗ I have related the effect of the mutation to fibrillin-1.[2]

c [Due to the redundancy of the genetic code, where several different codons are able to code for the same amino acid,[1]] [some mutations may still result in the production of the same amino acid, leaving the resultant protein (e.g. fibrillin-1) unchanged.[2]]

✓ ✗ I have identified that the genetic code is redundant.[1]

✓ ✗ I have described how redundancy allows for mutated codons to code for the same amino acid.[2]

Multiple lessons

11 A

12 a [AGA.[1]]

✓ ✗ I have stated the DNA triplet for this anticodon.[1]

b [This mutation is a missense mutation as it has changed the anticodon to code for a different amino acid.[1]] [This would have occurred in germline cells as it has been passed down to future offspring to produce a population of insecticide-resistant fruit flies.[2]]

✓ ✗ I have described a missense mutation.[1]

✓ ✗ I have explained where this mutation occurred.[2]

c [The creation and introduction of new alleles into a population increases genetic diversity by creating increased allele variation between individuals.[1]]

✓ ✗ I have described the effect of new alleles on genetic diversity.[1]

13 a [5′ AUG CAU GGC UUU AUG CAA GAA CUG AUA UAG 3′.[1]]

✓ ✗ I have correctly written the pre-mRNA sequence from 5′ to 3′.[1]

b [Six amino acids would be present in the polypeptide chain expressed.[1]] [This is because while there are seven exons, the stop codon does not code for an amino acid, leaving six amino acids.[2]]

✓ ✗ I have identified the correct number of amino acids.[1]

✓ ✗ I have justified my response.[2]

c i [To observe this change, the GTT codon must mutate into ATT through a nucleotide substitution, creating a nonsense mutation.[1]] [This is because ATT is a STOP triplet, signalling for the ribosome to cease translation. Therefore, fewer exons are translated and the mutated polypeptide is shorter than the non-mutated polypeptide, only having four amino acids.[2]]

✓ ✗ I have identified the mutation occurring.[1]

✓ ✗ I have explained that this will create a stop codon.[2]

ii [During post-transcriptional modifications, alternative splicing may occur, where exons are spliced together in varying patterns, resulting in a shorter strand of mRNA.[1]]

✓ ✗ I have suggested another possible reason for producing a shortened polypeptide.[1]

Key science skills and ethical understanding

14 a [The independent variable is the strength of UV radiation[1]] [and the dependent variable is the number of mutations in the tumour suppressor gene of each mouse's skin cells.[2]]

✓ ✗ I have identified the independent variable.[1]

✓ ✗ I have identified the dependent variable.[2]

b [The number of mutations in the tumour suppressor gene will increase as the strength of UV radiation is increased, because UV radiation is a mutagen.[1]]

✓ ✗ I have stated that there should be an increase in mutations if UV strength is increased.[1]

c [Mutagen.[1]]

✓ ✗ I have identified what agent UV light is.[1]

d [Random mutations can uncontrollably occur.[1]] [This would affect the reliability of the experiment as the scientists would not be able to distinguish between mutations caused by UV radiation and random mutations, possibly leading to an over-estimation of the mutation frequency.[2]]

Other acceptable responses include:

- Skin cell samples should be taken from the same part of the mouse. Some parts of the skin might be more exposed to UV radiation than others which would influence mutation rate in the tumour suppressor gene of those skin cells.
- Radiation time must be the same between each treatment. If this is not kept the same between treatments then the comparisons between strengths cannot be made.
- Genetic variation at the tumour suppressor gene locus may exist within mice cultures. This would lead to measurements of mutations being skewed.

✓ ✗ I have identified an uncontrolled variable.[1]

✓ ✗ I have explained its effect on the results of the experiment.[2]

e [Non-maleficence involves the minimisation of harm.[1]] [In this experiment, scientists should ensure that the mice are exposed to the least amount of pain as possible, perhaps euthanising them if the tumours induced are causing significant amounts of suffering.[2]]

✓ ✗ I have described the bioethical concept of non-maleficence.[1]

✓ ✗ I have described the relevance of non-maleficence to the scenario.[2]

9B Environmental selection pressures

Theory review questions

1 B

2 bird; green mussels; grey shell colour

3 I-heritability; II-selection pressure; III-variation; IV-selective advantage

4 C

5 C

SAC skills questions

6 A **7** A **8** B **9** C

10 A

Exam-style questions

Within lesson

11 C **12** B **13** A

14 a [Variation in shell thickness existed in the population of blue mussels prior to the introduction of shore crabs.[1]] [When the shore crab was introduced in the south, mussels with thin shells were preyed upon more,[2]] [while thick-shelled mussels were conferred a selective advantage.[3]] [Over time, the thick-shelled mussels became more common as they passed on their advantageous trait to subsequent generations.[4]]

✓ ✗ I have stated that variation existed in the population.[1]

✓ ✗ I have identified that the selection pressure negatively impacted thin-shelled mussels.[2]

✓ ✗ I have identified that thick-shelled mussels were conferred a selective advantage.[3]

✓ ✗ I have explained the consequences of this on the phenotype of the southern population of mussels.[4]

b [The thickness of shells in the northern mussel population would also increase.[1]] [This is because they would be exposed to the same selection pressure as mussels in the south, and assuming that the same gene for thick shells exists in the northern population, natural selection favouring thick shells would occur there as well.[2]]

✓ ✗ I have stated that the shell thickness of the northern mussels would increase.[1]

✓ ✗ I have explained why this would occur.[2]

Multiple lessons

15 a [Allele frequency is the proportion of certain alleles in a gene pool.[1]]

✓ ✗ I have defined allele frequencies.[1]

b i [The Illinois birds have lower genetic variation than the birds from other states.[1]] [This means that Illinois birds are at greater risk of extinction because they are less likely to have advantageous phenotypes that may help them survive new selection pressures.[2]]

Other acceptable responses include:

- Populations with lower genetic variation are at risk of inbreeding, which can lead to disadvantageous phenotypes persisting in the population. States with prairie chicken populations that are large and genetically diverse do not face these problems.

✓ ✗ I have stated that Illinois birds have lower genetic variation than birds from other states.[1]

✓ ✗ I have explained why low genetic variation increases the risk of extinction.[2]

ii [Due to the presence of different selection pressures, different alleles are selected for in the Illinois and Minnesotan populations, thereby affecting allele frequencies.[1]]

✓ ✗ I have suggested a reason why the average number of alleles per gene locus is different.[1]

c [Fit individuals from the Minnesotan, Kansas, or Nebraskan populations could be introduced into the Illinois population for interbreeding.[1]]

✓ ✗ I have identified a measure to increase the amount of genetic diversity in the Illinois bird population.[1]

d [In the pre-human settlement population of prairie chickens, variation in traits existed.[1]][Upon human settlement, some individuals had traits that were advantageous for survival under the new environmental conditions and conferred a selective advantage: for example, better vision or the ability to find and digest different food.[2]][Individuals with these advantageous phenotypes survived and had more offspring who inherited these traits than individuals without these phenotypes.[3]][Over generations, the surviving population of prairie chickens had more of these advantageous alleles than historic populations.[4]]

✓ ✗ I have stated that variation existed in the prairie chicken population.[1]

✓ ✗ I have identified possible selection pressures.[2]

✓ ✗ I have described the selective advantage conferred to individuals with advantageous alleles/phenotypes.[3]

✓ ✗ I have explained the consequences of this on the presence of the advantageous allele within the population.[4]

Key science skills and ethical understanding

16 a [Predatory fish.[1]]

✓ ✗ I have named a selection pressure.[1]

b [They are possibly more likely to attract females and so more likely to reproduce and contribute to the next generation.[1]]

Other acceptable responses include:

- No advantage in being camouflaged since there are no predators.

✓ ✗ I have suggested a possible reason for having brightly coloured spots.[1]

c i [Variation in the original laboratory population of guppies with respect to the colour of spots existed.[1]][Predatory fish would have served as a selection pressure against the guppies,[2]] [thereby conferring a selective advantage to guppies with dull coloured spots compared to brightly coloured.[3]][Guppies with dull coloured spots would have a higher rate of survival and reproduction. Over time, the dull coloured guppies would have become more common as they passed on the advantageous allele to their offspring.[4]]

✓ ✗ I have stated that variation existed in the guppy population.[1]

✓ ✗ I have identified the selection pressure.[2]

✓ ✗ I have described the selective advantage conferred to individuals with dull coloured spots.[3]

✓ ✗ I have explained the consequences of this on the presence of the advantageous phenotype within the population.[4]

ii [The independent variable involves the presence or absence of predatory fish.[1]][The dependent variable is the percentage of guppies with brightly coloured spots or dull coloured spots after 10 generations.[2]]

✓ ✗ I have identified the independent variable.[1]

✓ ✗ I have identified the dependent variable.[2]

iii [Temperature of the water[1]][and the food source.[2]]

Other acceptable responses include:

- the colour and makeup of the stream bed
- velocity of the water
- water salinity
- water pH
- sunlight

✓ ✗ I have named one controlled variable.[1]

✓ ✗ I have named a second controlled variable.[2]

d i [To confirm that the change in frequency of phenotypes is not due to something specific to the laboratory.[1]]

✓ ✗ I have identified why the scientists carried out the experiment in the wild.[1]

ii [Beneficence involves maximising benefits while minimising harms.[1]][By moving guppies from dangerous streams to safe streams, scientists can justify the movement of guppies as they are providing the guppies with a safer environment to live in.[2]]

✓ ✗ I have described the bioethical concept of beneficence.[1]

✓ ✗ I have described the relevance of beneficence to the scenario.[2]

9C Genetic drift and gene flow

Theory review questions

1 D

2 Increases: III; IV
Decreases: I; II; V; VI

3 I-bottleneck effect; II-founder effect; III-gene flow

4 low; inbreeding; low adaptive potential; extinction; smaller; larger

SAC skills questions

5 A **6** A **7** B **8** C

9 A

Exam-style questions

Within lesson

10 A **11** D

12 a [Gene flow can increase the genetic diversity of a population through immigration introducing new alleles into a population.[1]] [However, through emigration, genetic diversity can decrease as alleles are leaving a population.[2]]

✓ ✗ I have explained how gene flow can increase genetic diversity.[1]

✓ ✗ I have explained how gene flow can decrease genetic diversity.[2]

b [Due to the shortened distance between populations B and C, there is a higher chance of migration between these populations when compared to populations A and B, which are relatively far away from each other.[1]]

✓ ✗ I have suggested a possible reason for the difference in gene flow.[1]

Multiple lessons

13 a [The Dutch settlers were the founding population[1]] [which meant their high prevalence of Huntington's disease was passed onto future Afrikaner generations due to the founder effect.[2]]

✓ ✗ I have identified that the founder effect is responsible for the high frequency of the allele.[1]

✓ ✗ I have explained the effect of the founder effect on the frequency of the allele.[2]

b [New alleles could be introduced through either immigration[1]] [or mutations[2]].

✓ ✗ I have identified one possible source of new alleles.[1]

✓ ✗ I have identified a second possible source of new alleles.[2]

c [Because Huntington's disease develops during adulthood, individuals are still capable of surviving and reproducing for a limited time, allowing them to pass on the allele for Huntington's disease.[1]]

✓ ✗ I have identified that individuals are still capable of surviving and reproducing.[1]

14 a [When the population size of blue whales decreases, the genetic diversity of the blue whales is also likely to decrease.[1]] [Therefore, they may lose advantageous alleles which confer a selective advantage against particular environmental selection pressures, which lowers their adaptive potential.[2]]

✓ ✗ I have identified that a decrease in the population can lead to a decrease in genetic diversity.[1]

✓ ✗ I have explained how decreased genetic diversity affects the ability to adapt to new environmental selection pressures, adaptive potential.[2]

b [If boat traffic continues to grow, increased collisions between blue whales and boats could lead to increased deaths and thus extinction.[1]] [Another possible outcome includes the emigration of blue whales out of Chilean Patagonia into waters with decreased boat traffic.[2]]

✓ ✗ I have identified one possible future outcome.[1]

✓ ✗ I have identified a second possible future outcome.[2]

c [The sample used in the breeding program (four blue whales) is small and would cause the founder effect.[1]] [The genetic diversity of the resulting offspring from this breeding program would be as low as the limited diversity in the initial four blue whales.[2]] [Low genetic diversity is dangerous for a population since there will be a lower chance of advantageous alleles existing in the gene pool, so the whales will not be safeguarded against future environmental selection pressures that may threaten the species' survival.[3]]

Other acceptable responses include:

- Inbreeding between closely related founder blue whales would increase detrimental alleles in the population. Detrimental alleles reduce the fitness of individuals and cause further reductions in the population size until the species becomes extinct.

✓ ✗ I have identified that the founder effect would occur in the breeding population.[1]

✓ ✗ I have explained how the resulting population will have low genetic diversity.[2]

✓ ✗ I have outlined how future fitness may be affected by low genetic diversity.[3]

Key science skills and ethical understanding

15 a [The independent variable is the distance between rock-wallaby colonies[1]] [and the dependent variable is the amount of gene flow between the populations .[2]]

✓ ✗ I have stated the independent variable.[1]

✓ ✗ I have stated the dependent variable.[2]

b [Gene flow involves the introduction of new alleles into a population through new individuals immigrating into and breeding with a population.[1]] [Therefore, genetic diversity can increase through an increase in immigration.[2]]

✓ ✗ I have explained what gene flow is.[1]

✓ ✗ I have explained how this increases genetic diversity.[2]

c [Genetic diversity is critical for the survival of the brush-tailed rock-wallaby, as variation increases the chance of having an advantageous allele which confers a selective advantage in the face of new environmental selection pressures.[1]][Populations with low genetic diversity may not have this crucial allele, so are at greater risk of extinction.[2]]

Other acceptable responses include:

- Populations with low genetic diversity often have high rates of inbreeding, which allow for the persistence of deleterious alleles.

I have outlined the importance of variation.[1]

I have explained the risk of low genetic diversity.[2]

d [Rock-wallabies were not exposed to the founder effect as the map shows the current population distribution being within the historical population distribution.[1]]

I have explained that the current population is within the historical population.[1]

9D Speciation

Theory review questions

1 fertile/viable; fertile/viable; selection pressures; genetic differences

2 B

3 III; IV; I; II

4 Allopatric speciation: V; VI
Sympatric speciation: II
Both: I; III; IV

SAC skills questions

5 A **6** B **7** B **8** A

9 D

Exam-style questions

Within lesson

10 a i [Initially, populations of Galápagos finches became isolated by a geographical barrier (e.g. the ocean).[1]][Over time, the isolated populations were exposed to different selection pressures [2]][and once they accumulated sufficient differences from one another and could no longer interbreed to form viable and fertile offspring, a new species was formed.[3]]

I have stated that the populations became geographically isolated.[1]

I have identified the presence of different selection pressures acting on the populations.[2]

I have explained how a new species is formed.[3]

ii [Due to the presence of varying habitats on each island providing different selection pressures, certain populations of Galápagos finches within an island may have been driven towards specific phenotypes,[1]][thereby allowing differences to accumulate despite the absence of a geographical barrier preventing gene flow.[2]][Once sufficient differences accumulated and the populations could no longer interbreed to form viable and fertile offspring, a new species was formed.[3]]

I have identified the presence of different selection pressures acting on the populations.[1]

I have identified the key characteristic of sympatric speciation.[2]

I have described when speciation occurs.[3]

b [The scientists could breed individual populations with one another.[1]][If they cannot produce offspring or the offspring they produce are infertile, then they are considered separate species.[2]]

Other acceptable responses include:

- comparison of DNA sequences
- comparison of structural features
- comparison of amino acid sequences

I have designed a method to determine whether they are the same species.[1]

I have stated the results that would support the conclusion.[2]

c [Finches may have migrated from their original island to another island by flying.[1]]

I have suggested a possible explanation for how one species may be found on a number of different islands.[1]

Multiple lessons

11 C **12** A

13 a [After Pedra Branca Rock became separated from Tasmania, lizard populations on the two islands would have become geographically separated from each other by the ocean.[1]][Therefore, over time, due to the presence of different selection pressures, differences would begin to accumulate.[2]][Once the two populations could no longer interbreed to produce viable and fertile offspring, a new species formed, giving rise to the Pedra Branca Skink.[3]]

I have stated that the populations became geographically isolated.[1]

I have identified the presence of different selection pressures acting on the populations.[2]

I have explained how a new species is formed.[3]

b [Due to the presence of a geographical barrier (i.e. the ocean), the Pedra Branca Skink cannot migrate from Pedra Branca Rock to Tasmania or mainland Australia.[1]]

I have suggested a possible reason for the absence of the Pedra Branca Skink on Tasmania and mainland Australia.[1]

c i [As a result of the ice age, the bottleneck effect may have occurred, dramatically decreasing both population size and genetic diversity of the lizards.[1]]

✓ ✗ I have identified the decrease in population size and genetic diversity.[1]

ii [Small populations are exceptionally vulnerable to new selection pressures due to their low genetic diversity,[1]][decreasing the chances of an advantageous phenotype being present in the population to be selected for, leaving them vulnerable to extinction.[2]]

Other acceptable responses include:

- Increased prevalence of inbreeding, which can allow for deleterious alleles to persist within the population, leaving a population vulnerable to extinction.

✓ ✗ I have identified that small populations have a low genetic diversity.[1]

✓ ✗ I have described the effect of a low genetic diversity on survival.[2]

iii [Mutations, which involve alterations to DNA, can introduce new alleles.[1]]

✓ ✗ I have described one mechanism that introduces new alleles into a population.[1]

iv [To reduce the chances of extinction, breeding or conservation programs can be established in an effort to increase population numbers.[1]]

✓ ✗ I have suggested one possible method to reduce the chances of extinction.[1]

14 a [Sympatric speciation. This is because the populations were not geographically isolated.[1]]

✓ ✗ I have identified sympatric speciation and justified my answer by referring to the lack of geographical isolation.[1]

b [Both allopatric and sympatric speciation require the presence of selection pressures which help cause the accumulation of differences between populations.[1]][However, while allopatric speciation relies on the presence of a geographical barrier preventing gene flow, sympatric speciation does not require a geographical barrier.[2]]

✓ ✗ I have identified one point of comparison between sympatric and allopatric speciation.[1]

✓ ✗ I have identified one point of difference between sympatric and allopatric speciation.[2]

Key science skills and ethical understanding

15 a [They are not the same species as they could not produce a viable and fertile offspring with each other.[1]]

✓ ✗ I have justified why they are not the same species.[1]

b [Initially, the Australian population would have become isolated by a geographical barrier from the Mozambican population.[1]][Over time the isolated Australian population was exposed to different selection pressures[2]][and accumulated sufficient differences to the original Mozambican population to be considered a new species.[3]]

✓ ✗ I have stated the populations are geographically isolated.[1]

✓ ✗ I have identified the presence of different selection pressures on the population.[2]

✓ ✗ I have explained how the new species is formed.[3]

c [The two populations were not exposed to different selection pressures despite their distance.[1]]

Other acceptable responses include:

- Isolation of these two populations may have occurred more recently than with the Australian population meaning that not enough time has passed for them to diverge into separate species.
- The cattle tick may have been introduced from one of these countries to the other via migration or artificial means.

✓ ✗ I have suggested a reason as to why the populations are not different species.[1]

d [The introduced Argentinian population may outcompete the Australian population, thereby leading to a decline in the Australian population numbers.[1]]

Other acceptable responses include:

- The introduced Argentinian population may not be able to adapt to the environmental selection pressures in Australia, leading to their death.
- The introduced Argentinian population may become a pest species, where they have no predators and are allowed to reproduce uncontrolled.

✓ ✗ I have suggested one biological implication.[1]

9E Selective breeding

Theory review questions

1 B

2 Natural selection: V
Selective breeding: I; II; III; IV

3 C

4 C

5 B

SAC skills questions

6 A **7** A **8** B **9** A

10 A

Exam-style questions

Within lesson

11 B **12** C

Multiple lessons

13 B **14** A **15** D

16 a [Humans have selected for dogs that express desired traits based on the functions they perform (e.g. slender bodies for running, sensitive noses for tracking) and bred them together.[1]][Over generations of selective breeding, humans continue to select for greater expressed forms of these traits, eventually resulting in the array of modern dog breeds observed today.[2]]

- I have outlined the selection stage of selective breeding.[1]
- I have outlined the continued breeding of dogs with desirable traits.[2]
- I have referred to the scenario in my response.

b [In a population of randomly mating wolves, the selection pressure is determined by the natural environment whereas in forming a domesticated population of dogs, humans select for a desired trait, so the selection pressure is deliberately human-imposed.[1]]

- I have described the difference between selective breeding and natural selection.[1]
- I have used comparative language such as: whereas.

c i [One unintended consequence includes inbreeding, which can increase the expression of deleterious recessive alleles within a population.[1]][Another unintended consequence includes a reduced adaptive potential, leaving populations susceptible to new selection pressures that may arise.[2]]

- I have described one potential consequence of selective breeding.[1]
- I have described a second potential consequence of selective breeding.[2]

ii [Selective breeding decreases genetic diversity as only individuals which are phenotypically similar are allowed to breed.[1]]

- I have explained how selective breeding decreases genetic diversity.[1]

17 a i [Variation can arise through the production of new alleles via mutations[1]][or through sexual reproduction.[2]]

- I have identified one way natural variation can exist in a population.[1]
- I have identified another way natural variation can exist in a population.[2]

ii [Post-transcriptional modifications to the transcribed pre-mRNA through alternative splicing can produce many different strands of mature mRNA, leading to the production of different proteins which can influence milk production.[1]]

Other acceptable responses include:

- Post-translational changes to the protein such as alternative folding may produce a different functional 3D protein.
- Cows' different diets can lead to different milk production.

- I have identified that post-transcriptional modifications can lead to the production of different strands of mature mRNA.[1]
- I have used key biological terminology such as: post-transcriptional modifications, pre-mRNA, alternative splicing.

b [To be considered the same species, two individuals from a population must be able to interbreed to produce viable and fertile offspring.[1]][Therefore, farmers could determine whether a new species has been formed by breeding two individuals together and seeing if they are able to produce viable and fertile offspring.[2]]

Other acceptable responses include:

- Genetic testing, where a comparison of the individuals' genetic material is carried out.

- I have defined the term 'species'.[1]
- I have described how farmers could determine whether a new species has been formed.[2]

c [Selective breeding reduces genetic diversity within a population as it drives the genotypes of a population towards a specific allele.[1]][Therefore, the population of farm animals introduced onto an isolated island will have a lower adaptive potential and would struggle to adapt to new environmental selection pressures imposed by their new environment, decreasing their survivability.[2]]

Other acceptable responses include:

- Inbreeding can increase the presence of deleterious alleles, decreasing the survivability of the farm animals.

- I have identified that selective breeding decreases genetic diversity.[1]
- I have explained how decreased genetic diversity can impact survivability.[2]

Key science skills and ethical understanding

18 a [Weismann could use selective breeding to form a population of long-tailed mice. By establishing a population of mice with long tails and breeding them together,[1]][the frequency of the allele coding for increased tail length will increase over time, leading to a population of long-tailed mice.[2]]

- I have identified that long-tailed mice would need to be bred together.[1]
- I have explained how breeding long-tailed mice increases tail length over time.[2]

b [Selection for the desired trait over generations will only occur if that trait is heritable.[1]][As cutting off tails with a knife does not affect the gametes of mice, cutting off a tail is not heritable.[2]]

✓ ✗ I have stated that artificially selected traits must be heritable.[1]

✓ ✗ I have linked my response to Weismann's experiment.[2]

c i [That repeated matings between individuals with stunted or malformed tails will result in a population of mice with stunted or missing tails because these traits are heritable.[1]]

✓ ✗ I have stated a hypothesis based on the principles of selective breeding.[1]

ii [Mice in a control group would not have undergone selective breeding.[1]]

✓ ✗ I have stated what would have been the experimental conditions for the control group of this experiment.[1]

d [Non-maleficence involves the minimisation of preventable harm.[1]] [Therefore, in order to prevent harm when conducting his experiment, Weismann could have used a local anaesthetic to prevent suffering in the mice which had their tails removed.[2]]

✓ ✗ I have described the bioethical concept of non-maleficence.[1]

✓ ✗ I have described the relevance of non-maleficence to the scenario.[2]

9F Evolving Pathogens

Theory review questions

1 B

2 III; I; II; IV

3 A

4 antigenic drift; small; antigenic shift; sudden; antigenic shift; recombination

SAC skills questions

5 A **6** A **7** A **8** C

9 B

Exam-style questions

Within lesson

10 B **11** A

12 a i [Due to the premature discontinuation of the course of antibiotics, remaining colonies of *Mycobacterium tuberculosis* (especially antibiotic-resistant ones) may have survived, allowing them to continue replicating. Therefore, due to the persistence of *M. tuberculosis* within the patient's body, the symptoms of *M. tuberculosis* infection returned.[1]]

✓ ✗ I have explained that *M. tuberculosis* bacteria may have persisted due to discontinuation of the course of antibiotics.[1]

ii [The discontinued use of antibiotics prior to completing the entire course may contribute to the development of antibiotic-resistant bacteria by giving the bacteria an increased amount of time to accumulate mutations, allowing for genes conferring antibiotic resistance to potentially arise.[1]]

✓ ✗ I have explained how the patient's actions may have contributed to the development of antibiotic-resistant bacteria.[1]

b [Susceptible and antibiotic-resistant *M. tuberculosis* bacteria would have existed in the population prior to the use of antibiotics.[1]] [When the bacteria were exposed to the antibiotic, which acted as a selection pressure, bacteria susceptible to the antibiotic were destroyed.[2]] [The antibiotic-resistant bacteria were conferred a selective advantage[3]] [and were able to continue replicating, increasing the allele frequency of antibiotic resistance and rendering the course of antibiotics unsuccessful.[4]]

✓ ✗ I have identified that variation would have existed in the original population.[1]

✓ ✗ I have identified the selection pressure as the exposure to the antibiotic.[2]

✓ ✗ I have identified that antibiotic-resistant bacteria are conferred a selective advantage.[3]

✓ ✗ I have explained that the continued replication of bacteria would have rendered the course of antibiotics ineffective.[4]

Multiple lessons

13 a i [Mutations, which are permanent changes to DNA sequences, may have caused the formation of the gene for antibiotic resistance.[1]]

✓ ✗ I have described mutations.[1]

ii [Susceptible and resistant *E. coli* bacteria would have existed in the population prior to the use of antibiotics.[1]] [When they were exposed to the antibiotic, which acted as a selection pressure, bacteria susceptible to the antibiotic were destroyed.[2]] [The bacteria with antibiotic resistance were conferred a selective advantage,[3]] [and were able to continue replicating, increasing the allele frequency of antibiotic resistance and the prevalence of antibiotic-resistant *E. coli*.[4]]

✓ ✗ I have identified that variation would have existed in the original population.[1]

✓ ✗ I have identified the selection pressure as the exposure to the antibiotic.[2]

✓ ✗ I have identified that antibiotic-resistant bacteria are conferred a selective advantage.[3]

✓ ✗ I have explained that the continued replication of bacteria would have increased the prevalence of antibiotic resistance.[4]

iii [Hand hygiene.[1]]

Other acceptable responses include:

- Avoiding inappropriate use of antibiotics.
- Completing entire courses of antibiotics.
- Appropriate sanitation.

✓ ✗ I have suggested a possible method to prevent the formation of antibiotic-resistant *E. coli*.[1]

b [After phagocytosis of *E. coli* by antigen-presenting cells, they would present the pathogenic antigens of *E. coli* to helper T cells.[1]] [These helper T cells would subsequently secrete cytokines that activate similarly selected B cells, leading to the processes of clonal expansion and differentiation, producing large quantities of memory B cells and antibody producing plasma cells.[2]] [These plasma cells would then secrete large quantities of antibodies capable of neutralising the *E. coli* bacteria.[3]]

✓ ✗ I have described antigen-presentation.[1]

✓ ✗ I have described the processes of clonal expansion and differentiation.[2]

✓ ✗ I have described the production of antibodies against *E. coli*.[3]

14 a [By using attenuated pathogens or deactivated pathogenic antigens, vaccinations are capable of stimulating the adaptive immune response and the production of memory cells without causing disease.[1]] [Therefore, upon re-exposure to the pathogen that was vaccinated against, the memory cells are capable of generating a rapid immune response, preventing the formation of disease.[2]]

✓ ✗ I have described that vaccinations stimulate the production of memory cells.[1]

✓ ✗ I have explained the significance of memory cells in preventing the formation of disease.[2]

b [The purpose of the campaign to vaccinate individuals against the influenza virus includes the establishment of herd immunity.[1]] [Herd immunity serves to protect vulnerable individuals in the community (e.g. those with allergies to the vaccine or the immunocompromised) by reducing the availability of susceptible hosts that would otherwise spread the influenza virus.[2]]

✓ ✗ I have identified herd immunity.[1]

✓ ✗ I have described the purpose of herd immunity.[2]

c [Antigenic drift involves slow and gradual changes to the surface antigens of the influenza virus.[1]] [Due to these changes, previously generated memory cells may no longer be as effective at recognising the influenza virus, decreasing immunity, leading to the requirement of yearly influenza vaccinations.[2]]

✓ ✗ I have described the process of antigenic drift.[1]

✓ ✗ I have described the consequence of antigenic drift on immunological memory and vaccination requirements.[2]

d [A pandemic caused by the influenza virus may occur due to antigenic shift, which involves sudden and significant changes to the surface antigens of the influenza virus, typically resulting in the formation of a completely new virus subtype.[1]] [Therefore, it is unlikely for immunity to exist within the community, allowing the influenza virus to infect a significant number of individuals and spread across the world, resulting in a pandemic.[2]]

✓ ✗ I have described the process of antigenic shift.[1]

✓ ✗ I have described the consequence of antigenic shift.[2]

Key science skills and ethical understanding

15 a [The dependent variable is the level of growth of each bacterial colony.[1]] [The independent variable is the type of bacteria grown on the Petri dish.[2]]

✓ ✗ I have identified the dependent variable.[1]

✓ ✗ I have identified the independent variable.[2]

b [The concentration of vancomycin applied[1]] [and the sizes of the initial bacterial colonies.[2]]

Other acceptable responses include:

- Environmental conditions (e.g. temperature).

✓ ✗ I have identified one controlled variable.[1]

✓ ✗ I have identified a second controlled variable.[2]

c [Researchers could use appropriate personal protective equipment such as gloves, safety glasses, and lab coats,[1]] [and they could apply appropriate hand hygiene, to destroy any potential pathogens that may have colonised their hands.[2]]

Other acceptable responses include:

- Conducting the experiment within a closed environment to prevent the escape of the pathogen.

✓ ✗ I have described one method of preventing self-infection.[1]

✓ ✗ I have described a second method of preventing self-infection.[2]

d [Due to the high use of antibiotics and high prevalence of patients with bacterial infections in hospitals, they can often be the source of antibiotic-resistant bacteria.[1]]

✓ ✗ I have suggested a possible reason.[1]

e [Non-maleficence involves the minimisation of preventable harm where possible.[1]] [One potential bioethical issue involves the failure of the researchers to undertake appropriate precautions when culturing the potentially antibiotic-resistant bacteria, which could lead to the uncontrolled spread of the bacteria within the community.[2]]

✓ ✗ I have described the bioethical concept of non-maleficence.[1]

✓ ✗ I have described a potential bioethical issue with respect to non-maleficence.[2]

Chapter 9 SAC practice

1 [The production of large quantities of fish faeces[1]][and leftover fish food.[2]]

Other acceptable responses include:

- Proximity to Maria Island National Park.
- Mass death events of salmon.

✓ ✗ I have identified one reason for the opposition of additional ocean cages.[1]

✓ ✗ I have identified a second reason for the opposition of additional ocean cages.[2]

2 [The low oxygen levels were caused by the huge quantity of salmon which require oxygen to respire within Macquarie Harbour.[1]]

Other acceptable responses include:

- Lower layers of the ocean naturally have less oxygen content, as organic matter sinks and decomposes on the seafloor.
- Decomposition of waste products (e.g. fish faeces, dead fish) contributed to the depletion of oxygen, leading to low oxygen levels.

✓ ✗ I have explained the cause of low oxygen levels within Macquarie Harbour.[1]

3 [Variation in the optimal temperature of water exists in the population of salmon.[1]][Therefore, as salmon are subjected to warmer temperatures, which serve as a selection pressure,[2]][those that have genetic traits that make them more suited towards warmer waters are conferred a selective advantage.[3]][Salmon suited towards warmer waters would have a higher rate of survival and reproduction, allowing them to pass on the advantageous allele for warm water survival to their offspring, increasing the frequency of the allele over time.[4]]

✓ ✗ I have identified that variation exists in the original population.[1]

✓ ✗ I have identified the selection pressure.[2]

✓ ✗ I have identified the group of salmon with the selective advantage.[3]

✓ ✗ I have identified that the frequency of the advantageous allele will increase over time.[4]

4 [Mass death events of salmon can lead to a bottleneck effect, which decreases genetic diversity due to the drastic reduction in population size.[1]]

✓ ✗ I have described the effect of mass death events on the genetic diversity of the salmon.[1]

5 [The survivability of the salmon population is decreased due to the reduction in genetic diversity caused by the mass death events. This results from a lowered adaptive potential,[1]][which decreases their ability to adapt to new environmental selection pressures as it is unlikely for advantageous alleles that help them survive in the new conditions to exist.[2]]

Other acceptable responses include:

- A reduction in population size caused by the mass death events can lead to an increased prevalence of inbreeding, which allows for deleterious alleles to persist within the population, also decreasing survivability.

✓ ✗ I have identified that a reduction in genetic diversity will lead to a lowered adaptive potential.[1]

✓ ✗ I have explained the impact of a lowered adaptive potential on the survivability of the salmon.[2]

6 [Selective breeding to increase the size of salmon would involve farmers choosing to mate especially large salmon.[1]][Therefore, over many generations, the frequency of the allele coding for increased salmon size will increase, thus increasing the average salmon size.[2]]

✓ ✗ I have described that selective breeding involves selecting for a desirable trait.[1]

✓ ✗ I have described the effect of selective breeding on allele frequency.[2]

7 [Selective breeding would decrease the genetic diversity of the salmon as only individuals which are phenotypically similar are allowed to breed.[1]]

✓ ✗ I have explained how selective breeding decreases genetic diversity.[1]

8 [The allele for resistance against amoebic gill disease may have first arisen through a mutation, which involves changes in the DNA sequence.[1]]

✓ ✗ I have described mutations.[1]

9 [Yes, due to the presence of a geographical barrier separating salmon between cages, different species may arise through the process of allopatric speciation.[1]][If different environmental selection pressures are present, differences will accumulate over time, and once the two populations are no longer able to interbreed to form viable and fertile offspring, a new species is formed.[2]]

✓ ✗ I have identified that allopatric speciation may occur.[1]

✓ ✗ I have described the process in forming a new species via allopatric speciation.[2]

10 [Vaccines may be extremely difficult to develop due to the presence of antigenic shift,[1]][which involves small and gradual changes in the surface antigens of the virus.[2]]

✓ ✗ I have identified the process of antigenic shift.[1]

✓ ✗ I have described the process of antigenic shift.[2]

11 [Based on the graph, infections from the infectious salmon anaemia virus increased significantly during Jul 2007 to Dec 2008 from only three infections to over 28 respectively.[1]][Shortly after, however, the number of infections significantly dropped, with zero infections recorded in Apr 2010,[2]][before slowly increasing once again, reaching over ten in Jul 2011. The number of infections subsequently decreased again, with one infection recorded in Jan 2013.[3]]

✓ ✗ I have described the maximum point on the graph.[1]

✓ ✗ I have described the minimum point on the graph.[2]

✓ ✗ I have described the remainder of the graph.[3]

✓ ✗ I have used data in my response.

12 [Non-maleficence involves the minimisation of harms where possible.[1]] [Therefore, in order to limit harm to other salmon farms, the eradication strategy employed to control the spread of the infectious salmon anaemia virus is appropriate as it minimises the risk of infection spreading to neighbouring farms.[2]]

Other acceptable responses include:

- As the eradication of all salmon leads to the death of both healthy and infected salmon, a more targeted approach should be employed to minimise harm to the healthy individuals.

✓ ✗ I have described the bioethical concept of non-maleficence.[1]

✓ ✗ I have described the relevance of non-maleficence to the scenario.[2]

Chapter 9 Exam practice

Section A

1 D	**2** B	**3** C	**4** C
5 C	**6** A	**7** A	**8** A
9 C	**10** B		

Section B

11 a [Without predation, a significant environmental selection pressure for the kākāpō is removed. This reduces the struggle of the population to survive.[1]]

✓ ✗ I have identified a benefit of the absence of predation on the population.[1]

✓ ✗ I have referred to selection pressures.

b [The introduction of new environmental selection pressures may lead to extinction of the kākāpō.[1]] [This is due to the small population size of the kākāpō, which decreases its genetic diversity, and makes it unlikely for individuals to possess advantageous alleles that will help it survive under new conditions.[2]]

✓ ✗ I have suggested that the kākāpō may be threatened with extinction.[1]

✓ ✗ I have explained why the kākāpō would be threatened with extinction.[2]

12 a [The separation of rainforests leads to the generation of geographical barriers between populations, preventing gene flow and facilitating the process of allopatric speciation.[1]] [Therefore, due to the presence of different selection pressures, differences between the populations can accumulate, allowing for the formation of new species when they can no longer interbreed to form viable and fertile offspring.[2]]

✓ ✗ I have identified the process of allopatric speciation and the requirement for a geographical barrier.[1]

✓ ✗ I have described the process of allopatric speciation.[2]

b i [Bottleneck effect.[1]]

✓ ✗ I have identified the correct process.[1]

ii [Severe reductions in population size can drastically decrease genetic diversity, decreasing the survivability of a population.[1]] [This is caused by a lowered adaptive potential, which involves the decreased ability for a population to adapt to new environmental selection pressures due to the absence of advantageous alleles which help individuals survive in the new conditions.[2]]

Other acceptable responses include:

- Reduction to population sizes can increase the prevalence of inbreeding, which allows for deleterious alleles to persist in a population.

✓ ✗ I have identified that reductions in population size cause decreases in genetic diversity.[1]

✓ ✗ I have explained the impact of decreased genetic diversity on survivability.[2]

c i [Due to the founder effect, a small unrepresentative sample of lizards from the lizard population of rainforest A may have broken off and inhabited rainforest B.[1]] [As unrepresentative samples are not reflective of the gene pool of the original population, it would be expected that the genetic diversity of the population inhabiting rainforest B was significantly lower than that of the population inhabiting rainforest A.[2]]

✓ ✗ I have described the process of the founder effect.[1]

✓ ✗ I have explained how the founder effect may have led to the difference in genetic diversity.[2]

ii [Sympatric speciation involves the formation of species without the need for a geographical barrier preventing gene flow.[1]] [Therefore, due to the presence of different selection pressures in the separated forests, different species may still be able to form in the presence of gene flow.[2]]

✓ ✗ I have identified that sympatric speciation may occur.[1]

✓ ✗ I have described the process of sympatric speciation.[2]

13 a [Small populations are likely to have small gene pools, so individuals may inadvertently breed with close relatives.[1]] [Low heterozygosity is a strong indicator of low genetic diversity.[2]] [By analysing population size and heterozygosity, scientists can estimate the population's adaptive potential and risk of extinction, as populations with a higher genetic diversity are more likely to survive in adverse conditions.[3]]

✓ ✗ have outlined the purpose of testing the population size.[1]

✓ ✗ I have outlined the purpose of testing the amount of heterozygous individuals in the population.[2]

✓ ✗ I have explained the purpose of combining the data of both these tests and outlined the benefits of high genetic diversity.[3]

b [Scientists could identify whether gene flow occurred by comparing the types of alleles present in each population.[1]] [If there is a high similarity then it is likely that gene flow has occurred.[2]]

✓ ✗ I have explained the significance of comparing allele frequencies between populations.[1]

✓ ✗ I have identified what a high similarity in allele frequencies represents.[2]

c i [The trend for both male and female pygmy-possums appears to be roughly U-shaped.[1]][The female population started with 80 known living possums in 1996 and dropped down to 0 in 2001, but increased back up to 60 in 2013. For the male population, it began at 7 known possums that decreased to 0 in 2001 and then increased back up to 36 in 2013.[2]][The overall trend shows that the female population has always been significantly greater than the male population.[3]]

✓ ✗ I have stated that the trend is parabolic/U-shaped.[1]

✓ ✗ I have described the trends in the female and male pygmy-possum populations.[2]

✓ ✗ I have explained the overall relationship between the male and female populations.[3]

✓ ✗ I have referred to specific population numbers in my response.

ii [2010 and 2011.[1]]

✓ ✗ I have identified two consecutive years where the translocation likely occurred.[1]

iii [Gene flow.[1]]

✓ ✗ I have identified the correct evolutionary process.[1]

iv [The translocated individuals will introduce new alleles into the population to increase genetic diversity and population size.[1]]

✓ ✗ I have suggested a benefit of the translocation.[1]

14 a [Both susceptible and cephalexin-resistant bacteria would have existed prior to the application of cephalexin.[1]][When exposed to cephalexin, which acts as the selection pressure, all susceptible bacteria are destroyed while the bacteria with cephalexin resistance are conferred a selective advantage.[2]][The cephalexin-resistant bacteria are able to continue replicating, passing on the advantageous allele onto other bacteria and increasing the prevalence of cephalexin-resistant bacteria.[3]]

✓ ✗ I have identified that variation would have existed in the original population.[1]

✓ ✗ I have identified the selection pressure as the exposure to cephalexin and that cephalexin-resistant bacteria are conferred a selective advantage.[2]

✓ ✗ I have explained that the continued replication of bacteria would have increased the prevalence of cephalexin resistance.[3]

b i [No, cephalexin cannot be used to combat viral infections as it is an antibiotic, which is only capable of targeting bacteria.[1]]

✓ ✗ I have explained that cephalexin cannot be used to combat viral infections.[1]

ii [New subtypes of viruses can be formed through the process of antigenic shift,[1]][where significant and sudden changes occur in the surface antigens of viruses, often through a combination of various different existing strains.[2]]

✓ ✗ I have identified the process of antigenic shift.[1]

✓ ✗ I have described the process of antigenic shift.[2]

iii [Due to the presence of antigenic drift, which involves small and gradual changes to the surface antigens of viruses, vaccinations against viruses are extremely difficult to develop, as newly developed vaccines may be immediately rendered obsolete due to changes in the surface antigens.[1]]

✓ ✗ I have suggested a possible reason for why vaccinations against viruses are incredibly difficult to develop.[1]

10A The fossil record

Theory review questions

1 I-half-life; II-radioisotope; III-sedimentary rock; IV-transitional fossil; V-radiocarbon dating; VI-geological timescales; VII-index fossil; VIII-law of fossil succession; IX-Cambrian explosion; X-permineralisation

2 II; I; IV; III; V

3 D

4 C

5 D

6 A

SAC skills questions

7 A **8** A **9** A **10** C

11 B

Exam-style questions

Within lesson

12 D **13** C **14** C **15** C

16 D **17** C **18** B

19 a [Radiocarbon dating techniques, where the ratio of carbon-14 to carbon-12 atoms is measured and compared to current atmospheric carbon-14 levels to calculate the age of the fossil.[1]]

✓ ✗ I have described the radiocarbon decay technique.[1]

b [This researcher is incorrect. Mould fossils are fossils that form when a living thing decomposes underneath sediment, creating a cavity in the shape of the dead organism.[1]][In this case, the fossilised carcass showed little decomposition and appeared to be in a similar condition to when the organism had died.[2]]

✓ ✗ I have explained why it is not a mould fossil.[1]

✓ ✗ I have linked my answer to the information provided by showing that this fossil had little decomposition.[2]

Multiple lessons

20 a [The scientists would have used an absolute dating method such as radiometric dating to calculate the absolute age of the mollusc fossil.[1]][Using radiometric dating, the scientists would have measured the amount of a suitable radioisotope relative to its breakdown product and then calculated the age of the molluscs using the radioisotope's half-life.[2]]

✓ ✗ I have identified a technique which could be used to calculate the absolute age of the molluscs.[1]

✓ ✗ I have identified what the scientists would have measured if they used radiometric dating.[2]

b [Using the law of fossil succession the relative age of the Osteichthyan fossil could be estimated from the absolute age of the molluscs.[1]][Fossils found in the same layer as the molluscs are approximately the same age, whereas fossils in deeper layers are older, and higher layers are younger.[2]]

✓ ✗ I have stated that the law of fossil succession could be used.[1]

✓ ✗ I have explained how the mollusc's absolute age could be used to determine the relative age of the Osteichthyan fossil.[2]

c [The relative age of the Osteichthyan fossil is greater than 400 million years old.[1]]

✓ ✗ I have stated the relative age of the Osteichthyan fossil.[1]

d [No, as the molluscs may not meet the requirements of an index fossil.[1]]

✓ ✗ I have stated if these molluscs could be used as an index fossil.[1]

e [The Cambrian explosion (~535 mya), which describes a period of rapid diversification of multicellular life, characterised by the evolution of hardened body parts such as shells or bones.[1]]

Other acceptable responses include:

- The evolution of multicellularity (~900 mya) and subsequent specialisation of tissues and organs.

✓ ✗ I have identified an important step in evolution from ancient unicellular eukaryotes to Osteichthyans.[1]

f [One possible selection pressure that could have made it advantageous for Osteichthyans to develop a hard, bony skeleton could have been the presence of a new predator, especially one that possessed a jaw and teeth for biting its prey.[1]]

Other acceptable responses include:

- Changing environmental conditions, such as rougher waters, that require stronger body structures for survival.

✓ ✗ I have suggested a possible selection pressure.[1]

Key science skills and ethical understanding

21 a [The absolute age will accurately describe the fossil's age in years, whereas the relative age only indicates the order of formation for each fossil.[1]][Therefore, to accurately describe stickleback evolution, the absolute age should be calculated.[2]]

✓ ✗ I have explained what the absolute and relative age describes.[1]

✓ ✗ I have concluded whether the scientists should calculate the relative or absolute age.[2]

b [The absolute age of the top layer fossils: 5 730 years old.[1]]
[The absolute age of the middle layer fossils: 11 460 years old.[2]]
[The absolute age of the lower layer fossils: 22 920 years old.[3]]

✓ ✗ I have calculated the age of the top layer fossils.[1]

✓ ✗ I have calculated the age of the middle layer fossils.[2]

✓ ✗ I have calculated the age of the lower layer fossils.[3]

c [The question states that sticklebacks with large dorsal spines and pelvic bones are common in the ocean, while sticklebacks with small spines and pelvises are common in freshwater.[1]][Given that the saltwater variety were common ~11 460 years ago, it is likely that the lake had high levels of saltwater, or was inundated by the ocean, at this time.[2]][However, the variety with small dorsal spines and pelvic bones were common ~22 920 and 5 730 years ago, suggesting the lake was once again freshwater at this time.[3]]

✓ ✗ I have identified the habitats of each stickleback phenotype.[1]

✓ ✗ I have used evidence to describe the lake environment ~12 000 years ago.[2]

✓ ✗ I have used evidence to describe the lake environment ~5 730 and ~22 920 years ago.[3]

10B Evidence of relatedness

Theory review questions

1 differently; common ancestor; vestigial structures

2 A

3 D

4 True: II; IV
False: I; III

SAC skills questions

5 B **6** B **7** B **8** A

Exam-style questions

Within lesson

9 C

10 a [Homologous structures.[1]]

✓ ✗ I have identified that the animals' legs are homologous structures.[1]

b [Short legs evolved first in the order Monotremata[1]][because both short-beaked echidnas and platypuses have short legs, while only short-beaked echidnas have spines.[2]]

✓ ✗ I have identified that short legs evolved first in the order Monotremata.[1]

✓ ✗ I have justified my answer.[2]

c [Scientists could compare the fossil's amino acid sequence with the amino acid sequences of short-beaked echidnas and platypuses.[1]][The animal with more similarity in amino acid sequence with the fossil is more closely related to the fossil.[2]]

Other acceptable responses include:

- Scientists could compare the fossil's DNA sequence with the DNA sequences of short-beaked echidnas and platypuses. The animal with more similarity in DNA sequence with the fossil is more closely related to the fossil.

✓ ✗ I have determined that scientists could use amino acid sequences.[1]

✓ ✗ I have explained how the relatedness between the fossil and the animals could be determined using amino acid sequence analysis.[2]

Multiple lessons

11 B **12** B **13** A

14 a [Vestigial limbs once served a purpose in an ancestral species, however in modern species serve no function.[1]]

✓ ✗ I have explained the term vestigial limbs.[1]

b [Snakes evolved from legged lizards, however fully formed legs were disadvantageous for serpentine movement.[1]][As such, smaller legged individuals were favoured and natural selection resulted in the almost complete removal of limbs in modern snakes.[2]][As these vestigial limbs provide no selective advantage or disadvantage, they remain present throughout subsequent generations.[3]]

✓ ✗ I have discussed selection pressures surrounding legs in snake evolution.[1]

✓ ✗ I have discussed changes in leg size during snake evolution.[2]

✓ ✗ I have discussed the selection pressures on vestigial limbs in snakes.[3]

c i [As the sedimentary layer is of igneous origin, the scientists could calculate the age via radiometric dating.[1]][By measuring the relative amount of radioisotope and stable products, the absolute age of the rock can be calculated.[2]]

✓ ✗ I have stated a suitable form of dating.[1]

✓ ✗ I have explained the radiometric dating process.[2]

ii [Carbon-14's half-life is too short to date a fossil that is 100 million years old.[1]]

✓ ✗ I have attributed this to the short half-life of carbon-14.[1]

iii [The scientists would have calculated the absolute age of sedimentary igneous rock in which *Tetrapodophis amplectus* was found.[1]][The scientists could then conclude that because the fossil is found in the same layer as the rock, it has the same relative age as the rock.[2]]

✓ ✗ I have stated that radiometric dating was used to date the sedimentary layer.[1]

✓ ✗ I have stated that the age of the fossil is approximately the age of the sedimentary layer.[2]

iv [As *Tetrapodophis amplectus* is a transitional fossil, the most recent common ancestor of lizards and snakes occurred more than 100 mya.[1]]

✓ ✗ I have stated how this information could be used to determine the age of a common ancestor.[1]

15 a [Individuals 1 and 2.[1]][There is only one nucleotide site that differs between them, which is at site F. This is the fewest number of sequence differences between any of the samples, indicating that they are the most closely related.[2]]

- I have stated the correct two individuals.[1]
- I have justified my response by referring to the data.[2]

b [This is a nonsense mutation.[1]][A nonsense mutation in a protein-coding gene would cause translation to stop prematurely and create a short polypeptide.[2]][This polypeptide would likely not function properly.[3]]

- I have stated the correct type of mutation.[1]
- I have explained the effect of this mutation on translation.[2]
- I have explained the effect of this mutation on protein structure.[3]

Key science skills and ethical understanding

16 a [Trial 2. This is because among the three animals, kangaroos are the least related to humans.[1]][The less relatedness present between humans and the animal, the more differences will be present in the amino acid sequences between humans and the animal.[2]]

- I have explained the trial that will have the least amount of precipitate formed.[1]
- I have further explained my answer with reference to the difference in amino acid sequences.[2]

b [Human albumin mixed with the antibodies.[1]]

- I have identified the control.[1]

c [The students applied the concept of non-maleficence.[1]][This is because the students used the needles to minimise the harm done to the rabbits.[2]]

- I have identified the bioethical concept applied by the students.[1]
- I have explained my response.[2]

10C Phylogenetic trees

Theory review questions

1 lineage; taxon

2 W-root; X-node; Y-branch; Z-leaf

3 B

4 D

5 A

SAC skills questions

6 B **7** C **8** D **9** D

Exam-style questions

Within lesson

10 C **11** B **12** C **13** B

14 a [In both diagrams, the closest relatives of *Ceratophyllum* are the eudicots.[1]][In both diagrams, all of the plants share a common ancestor.[2]]

Other acceptable responses include:

- Eudicots are all classed as Mesangiospermae.
- Mesangiospermae is the closest relative of Austrobaileyales.

- I have identified one similarity between the two phylogenetic trees.[1]
- I have identified another similarity between the two phylogenetic trees.[2]

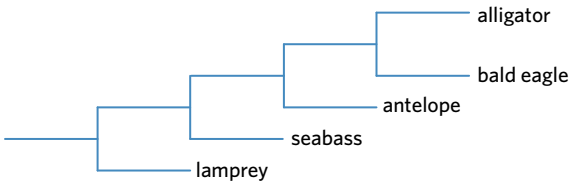
b [The Nymphaeales are more closely related to *Amborella* in the first tree, whereas the Nymphaeales are more closely related to all of the other groups in the second tree.[1]][Chloranthales is more closely related to magnoliids in the second tree, whereas Chloranthales is more closely related to monocots, *Ceratophyllum*, and eudicots in the first tree.[2]]

Other acceptable responses include:

- In the first tree, the monocots diverged early from the other Mesangiospermae, whereas in the second tree, the magnoliids and Chloranthales diverged before the monocots.

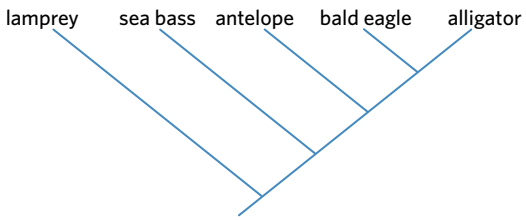
- I have identified one difference between the two phylogenetic trees.[1]
- I have identified another difference between the two phylogenetic trees.[2]

15 a



Other acceptable responses include:

- Students may represent their phylogenetic tree in a range of formats, including the following:



- I have correctly drawn a phylogenetic tree to show the relatedness between the animals.

b [Bald eagles are the most closely related to alligators.[1]]

- I have identified the animal that is the most closely related to alligators.[1]

Multiple lessons

16 A

17 **a** [By comparing the similarities and differences between homologous structures, scientists can deduce relationships between organisms and construct a phylogenetic tree.[1]]

✓ ✗ I have stated that homologous structures (not analogous) must be compared.[1]

b **i** [Species 3 since it shares a common ancestor with species 11 more recently than species 12.[1]]

✓ ✗ I have explained why species 3 is more closely related to species 11.[1]

ii [One species in each of the four *Anolis* groups of the phylogenetic tree demonstrate a trunk-ground lifestyle.[1]][It is likely that the ancestor was not a trunk-ground lizard, and this lifestyle evolved independently in the four trunk-ground species.[2]]

✓ ✗ I have referred to the relevant part/s of the phylogenetic tree in my answer.[1]

✓ ✗ I have explained that the independent evolution of trunk-ground lifestyles in the four species is most likely.[2]

18 **a** [The amino acid sequences of their protein coats can use used to determine the relatedness between two virus strains.[1]][The more similarity presence, the more related they are.[2]]

✓ ✗ I have identified the molecular evidence that can be used.[1]

✓ ✗ I have explained how the identified molecular evidence is used to determine the relatedness.[2]

b **i** [Strains 7 and 8[1]][diverged most recently and so would have had the least amount of time to accumulate molecular changes.[2]]

✓ ✗ I have identified the correct strains.[1]

✓ ✗ I have justified my response by referring to the accumulation of differences.[2]

ii [There are two divergences that occurred close together in time.[1]][Due to insufficient data, it may be difficult to determine which divergence occurred first, and this is indicated using three branches.[2]]

✓ ✗ I have explained that two divergences occured close together in time.[1]

✓ ✗ I have explained how uncertainties in data may cause two divergence to occur close together in time.[2]

c **i** [Since strains 3 and 4 recently diverged, the transmission to palm civet hosts likely occurred in their recent ancestor.[1]]

✓ ✗ I have identified that a single transmission event to palm civets occurred.[1]

ii [Since strains 5 and 6 are not closely related, the transmission to human hosts must have occurred twice independently.[1]][Since all other virus strains are found in horseshoe bats, it is likely that the ancestral coronavirus used horseshoe bats as a host.[2]]

✓ ✗ I have stated that the two strains in humans likely evolved independently.[1]

✓ ✗ I have identified the host of the ancestral virus.[2]

Key science skills and ethical understanding

19 **a** [The most recent divergence occurred between mammals and reptiles.[1]]

✓ ✗ I have identified the correct divergence event.[1]

b **i** [*Tiktaalik* would have developed lungs and sturdy bones in its limbs.[1]][Its lungs would allow it to take in gulps of air for oxygen while its sturdy limbs would allow *Tiktaalik* to prop itself up above shallow waters to take in gulps of air.[2]]

Other acceptable responses include:

- *Tiktaalik* may have had eyes on top of its head, allowing it to see above the water easily.

✓ ✗ I have given two examples of structural features that support the transitional fossil hypothesis.[1]

✓ ✗ I have explained the survival advantage on land of each feature.[2]

ii [If *Tiktaalik* were dated to be older than ancestral fish then this would suggest that *Tiktaalik* did not evolve from fish as the hypothesis suggests.[1]]

Other acceptable responses include:

- It has features that are not found in either ancestral fish or tetrapods.

✓ ✗ I have given a reasonable example that refutes the hypothesis.[1]

iii [Justice,[1]][which involves the consideration of different people's opinions and positions. This is because both groups of scientists need to put different opinions into consideration.[2]]

Other acceptable responses include:

- Integrity, which involves the accurate representation of the facts, whether favourable or unfavourable. This is because both groups should publish their results even if they are contradictory.

✓ ✗ I have identified the bioethical concept that should be followed.[1]

✓ ✗ I have explained my answer with relevance to the bioethical concept.[2]

Chapter 10 SAC practice

1 [Physical protection from scavengers and decomposers (e.g. fungi, bacteria),[1]][areas of rapid sediment accumulation,[2]] [and constant cool temperatures.[3]]

Other acceptable responses include:

- low oxygen availability
- low light exposure

✓ ✗ I have described one condition that increases the likelihood of fossilisation.[1]

✓ ✗ I have have described a second condition.[2]

✓ ✗ I have have described a third condition.[3]

2 [Absolute dating involves measuring the relative amounts of radioisotopes compared to the products they degrade into. This reveals the absolute age (in years) of a fossil.[1]]

✓ ✗ I have briefly described absolute dating.[1]

3 [The huge size of Australia[1]][and the small number of research groups looking for the fossils.[2]]

✓ ✗ I have identified one challenging factor.[1]

✓ ✗ I have identified another challenging factor.[2]

4 [^{14}C radioactive isotopes should not be used to estimate the absolute age of the oldest known eucalypt fossils in South America.[1]][This is because it is widely accepted that the oldest eucalypt fossils have been dated to millions of years ago, while the dating period of ^{14}C radioactive isotopes is only 1 000–50 000 years, which means that the isotopes have broken down completely in the fossils.[2]]

✓ ✗ I have determined whether ^{14}C radioactive isotopes should be used.[1]

✓ ✗ I have explained my answer with reference to the time when the fossils are commonly dated to.[2]

5 [Justice,[1]][which involves the consideration of different people's opinions and positions. This is because each group of scientists needs to consider the different opinions.[2]]

Other acceptable responses include:

- Integrity, which involves the accurate representation of the facts, whether favourable or unfavourable. This is because both groups should publish their results even if they are contradictory or unfavourable.

✓ ✗ I have identified a bioethical concept that should be followed.[1]

✓ ✗ I have explained my response with relevance to the bioethical concept.[2]

6 [Homologous structures are features present in two or more species that may look and function very differently in each species, but are derived from a common ancestor.[1]][This differs from analogous structures, which are features present in two or more species that fulfil the same function but do not originate from a common ancestor.[2]]

✓ ✗ I have explained homologous structures.[1]

✓ ✗ I have compared this to analogous structures.[2]

✓ ✗ I have used comparative language such as: this differs from.

7 [Homologous structures.[1]][Even though epicormic buds help species in the *Eucalyptus* genus survive bushfires whilst helping species in the X genus fight fungal infections (different functions),[2]][the two genera have a common ancestor.[3]]

✓ ✗ I have stated whether epicormic buds are homologous or analogous structures.[1]

✓ ✗ I have described the two different functions of epicormic buds in the two genera.[2]

✓ ✗ I have stated that the two genera have a common ancestor.[3]

8 [Bark thickness.[1]]

✓ ✗ I have determined a potential structure that can be used to look for species closely related to the genus Eucalyptus.[1]

9 [Scientists analysed DNA sequences instead of amino acid sequences because the genera are closely related, meaning that they are likely to share very similar sequences for certain proteins.[1]][Comparing nucleotide sequences allows scientists to look for silent mutations that may have accumulated without altering the amino acid sequence which is more useful for comparing close relatives.[2]]

✓ ✗ I have explained that the genera are closely related.[1]

✓ ✗ I have explained the benefit of analysing DNA sequences.[2]

10 [*Angophora hispida*.[1]]

✓ ✗ I have identified the most closely related species to *Angophora floribunda*.[1]

11 [*Angophora*.[1]]

✓ ✗ I have identified the most closely related genus to *Corymbia*.[1]

Chapter 10 Exam practice

Section A

1	A	**2**	C	**3**	C	**4**	B
5	A	**6**	D	**7**	B	**8**	B
9	A	**10**	D	**11**	B	**12**	D

Section B

13 a i [The foot structures are homologous.[1]]

I have correctly identified the relationship between the foot structures.[1]

ii [As the leg structures are homologous, this is an example of divergent evolution.[1]]

I have correctly identified the pattern of evolution.[1]

b [You would not find any *Equus* fossils older than three million years old in South America.[1]] [Because the *Equus* genus originated in North America, they could only migrate to South America over land, and North and South America joined together only three million years ago.[2]]

I have stated the maximum age of *Equus* fossils in South America.[1]

I have explained why you would not see *Equus* fossils before this time.[2]

c [In absolute dating the age is calculated very accurately, usually by measuring relative amounts of radioisotopes within the fossils.[1]] [In comparison, relative dating approximates a fossil's age by associating it with another specimen within the same or another sedimentary layer.[2]]

I have explained absolute dating techniques.[1]

I have explained relative dating techniques.[2]

I have used comparative language such as: in comparison.

14 a i [Mexican long-tongued bat.[1]]

I have identified the correct species.[1]

ii [This bat feeds in narrow spaces with small gaps,so a shorter echolocation signal would allow the bat to detect nearby objects.[2]]

Other acceptable responses include:

- Since these bats don't eat insects, they don't need a longer echolocation signal to detect prey that is far away.
- A shorter signal is less likely to be heard by predators.

I have given a reasonable explanation for the shorter echolocation signal being advantageous.[1]

b [Velvety free-tailed bat.[1]]

I have identified the correct species.[1]

15 a

Species	Environment	Condition
D. opatum	Near the coast of south-eastern Australia	[Submerged in highly mineralised groundwater[1]]
P. cinereus	Cave in western Queensland	[Lack of light, wind, and water[2]]
A. cignorum	Near the coast of south-eastern Australia	[Rapid burial in sediment[3]]

Other acceptable responses include:

Species	Environment	Condition
D. opatum	Near the coast of south-eastern Australia	• Rapid burial in sediment • Lack of oxygen • Lack of decomposers and scavengers
P. cinereus	Cave in western Queensland	• Constant cool temperature • Constant humidity • Lack of decomposers or scavengers
A. cignorum	Near the coast of south-eastern Australia	• Lack of decomposers or scavengers • Lack of oxygen • Submerged in highly mineralised groundwater

I have identified one environmental condition for *D. opatum*.[1]

I have identified one environmental condition for *P. cinereus*.[2]

I have identified one environmental condition for *A. cignorum*.[3]

b [Structural morphology.[1]]

Other acceptable responses include:

- comparative anatomy

I have correctly named the study in question.[1]

c [Finding homologous structures between the skulls would indicate the two species are related.[1]] [The lower jaw structure appears to be homologous between the two species as it is a similar shape.[2]]

Other acceptable responses include:

- the teeth of the lower jaw
- the teeth of the upper jaw
- the molars of the lower and upper jaw

I have identified that the presence of homologous structures would indicate the two species are related.[1]

I have stated a piece of evidence the scientists could have used.[2]

d [The absolute age of the skull is approximately 2 865 years old.[1]]

I have correctly calculated the absolute age of the skull.[1]

e [Personal error.[1]]

✓ ✗ I have correctly identified the type of error.[1]

---

16 a [Analogous structures.[1]]

✓ ✗ I have stated what type of structures the skin extensions are.[1]

---

b [Convergent evolution.[1]]

✓ ✗ I have determined the process of evolution that the two species have gone through.[1]

---

c [These two species do not share a recent common ancestor. However, they have been exposed to similar selection pressures due to living in similar environments.[1]][Gliding is an efficient way of moving between trees, and removes the threat of ground-dwelling predators.[2]][As such, individuals with greater gliding ability are able to reproduce more and pass their gliding genes onto offspring,[3]][and repeated selection over generations has facilitated the independent evolution of the analogous structure of skin extensions.[4]]

✓ ✗ I have explained that the two species have been exposed to similar environments and selection pressures.[1]

✓ ✗ I have explained the selective advantage of gliding.[2]

✓ ✗ I have explained the reproductive advantage of gliding.[3]

✓ ✗ I have discussed effects on subsequent generations of each species over time.[4]

✓ ✗ I have used key biological terminology such as: common ancestor, selection pressures, reproduce, selection, evolution, analogous structure.

---

17 a i [Crocodiles.[1]]

✓ ✗ I have determined the most closely related species to turtles.[1]

---

ii [Approximately 250 million years ago.[1]]

✓ ✗ I have identified when zebrafish and pufferfish diverge from their most recent common ancestor.[1]

---

iii [Coelacanth.[1]]

✓ ✗ I have identified the species that is more closely related to lungfish.[1]

---

b [Scientists should compare the DNA sequences.[1]][This is because snakes and lizards share a very recent common ancestor, meaning that they are likely to share very similar sequences for certain proteins.[2]][Comparing nucleotide sequences, looking for silent mutations that may have accumulated without altering the amino acid sequence will help assess the relatedness between the two species.[3]]

✓ ✗ I have determined the technique that scientists should use.[1]

✓ ✗ I have explained how close relatedness can affect the amino acid sequencing.[2]

✓ ✗ I have explained the benefit of using DNA sequencing.[3]

11A Defining Human

Theory review questions

1 B

2 I-bipedal; II-mammals; III-primates; IV-prehensile; V-hominins

3 B

4 D

5 D

SAC skills questions

6 A **7** A **8** A **9** C

10 A

Exam-style questions

Within lesson

11 B **12** B **13** A **14** B

15 a [Two features that identify this skull as hominoid are its cranium size, which is larger than other primates, and its Y5 molar teeth, which are unique to hominoids.[1]]

I have stated two features that are unique to the hominoid superfamily.[1]

I have used comparative language such as: larger.

b [Hominoids have a broader rib cage and do not have a tail.[1]] [These features indicate they are more closely related to *Homo sapiens* than other primate species.[2]]

Other acceptable responses include:

- Hominoids have a shorter spine between the rib cage and pelvis.
- Shoulder blades that sit further back.
- Fully rotating shoulder joints.

I have stated two structural features that are present in hominoids.[1]

I have stated that these features indicate they are more closely related to *Homo sapiens*.[2]

16 a [Skeleton 1 is the hominin skeleton.[1]] [We can infer this due to the posture of the skeleton, including a centralised foramen magnum.[2]]

Other acceptable responses include:

- Flatter face.
- Larger brain case.
- Smaller arm to leg ratio.
- Presence of an S-shaped spine.

I have correctly stated which skeleton is the hominin skeleton.[1]

I have justified this by referencing a hominin feature present in the image.[2]

b

Skeletal structure	Differences	Significance
1. Pelvis	Hominins have more shallow, bowl-shaped pelvises compared to hominoids.	This provides support for the upper body of hominins while walking upright.
2. Spine	Hominins have an S-shaped whereas hominoids have a C-shaped spine.	This allows hominins to stay upright for extended periods of time.

Other acceptable responses include:

Foot	Hominin feet have two arches and a larger heel compared to hominoids.	This makes upright locomotion more energy-efficient for hominins.
Rib cage	Hominins have a more barrel-shaped rib cage compared to hominoids.	This allows hominins to remain upright for longer periods of time.
Angle of femur	Hominins have a greater angle of femur compared to hominoids.	This increases stability when walking and standing upright for hominins.

I have completed the table appropriately.

I have used comparative terms such as: compared, whereas.

I have used key biological terminology such as: bowl-shaped pelvis, S-/C-shaped spine, barrel-shaped rib cage, angle of femur.

Multiple lessons

17 A

18 a [W.[1]]

I have correctly identified W as the common ancestor.[1]

b [The presence of an opposable thumb and toe on the hands and feet, allowing *Ardipethecus* to grab and swing from branches.[1]]

I have identified one possible morphological feature.[1]

Key science skills and ethical understanding

19 a [Chimpanzee. This is because the chimpanzee has less nucleotide differences to the human at this particular DNA region.[1]]

I have explained which species is most closely related to humans.[1]

b [DNA sequences from the same gene can be compared between two species.[1]][Changes in the sequence from one nucleotide to another accumulate over time via mutations and more nucleotide changes indicate a more distant relatedness.[2]]

- I have stated that the same gene from each species are compared.[1]
- I have identified that more nucleotide changes indicate more distant relatedness.[2]

c [Skeletal structure/morphology.[1]]

- I have identified another bit of structural information that can be obtained from the skeletons.[1]

d [This is an example of a systematic error,[1]][given that the error relates to uncalibrated or faulty equipment providing inaccurate data. Systemic errors influence a measurements accuracy, and tend to be consistent or repeatable until the faulty equipment is fixed.[2]]

- I have correctly identified the type of error that has taken place.[1]
- I have explained why this is a systematic error, with reference to the measurements accuracy and/or the error's repeatability.[2]

11B Hominin evolution

Theory review questions

1 A

2 True: II, IV, V
False: I, III

3 A

4 I-change in pelvis shape; II-shorter arms; III-longer legs

SAC skills questions

5 B **6** B **7** A **8** A

9 A

Exam-style questions

Within lesson

10 A **11** B **12** D **13** A

14 C **15** C

16 **a** [Yes, it would be bipedal because it was only from 2 million years ago and bipedalism evolved in hominins around 4 million years ago.[1]]

- I have explained why it would be bipedal with reference to the timeframe of hominin evolution.[1]
- I have referred to the scenario in my response.

b [A shorter arm to leg ratio supports a bipedal rather than arboreal lifestyle as it demonstrates the reduced need for available contact points on the forelimbs traditionally used for grasping and moving through trees.[1]][Longer arms were more actively selected for when hominins were arboreal, mostly as an adaptation for vertical climbing or hanging, while shorter arms improve energy efficiency when walking upright.[2]]

- I have identified that a shorter arm to leg ratio removes available contact points on the forelimbs.[1]
- I have explained my response in further detail.[2]

Multiple lessons

17 D

18 **a** [Among the ancestors of *Homo floresiensis*, variation in height would have existed.[1]][On the Indonesian island where they lived, there may have been a selection pressure (e.g. scarcity of food), which conferred a selective advantage to individuals that were smaller and shorter due to their ability to conserve energy.[2]][Over time, shorter *H. floresiensis* would have become more common as they passed on the advantageous allele to their offspring.[3]][In comparison, *H. sapiens* and *H. neanderthalensis* would have been exposed to different selection pressures that made it advantageous to be taller.[4]]

- I have stated that variation existed in the original population.[1]
- I have identified a possible selection pressure and advantageous phenotypes.[2]
- I have explained the consequences of this over time.[3]
- I have compared this to *H. sapiens* and *H. neanderthalensis*.[4]

b [Permineralisation occurs when different minerals in the groundwater seep into the hard body structures (the bones) of the organism and create a mineral cast of the structures once the organic material has decomposed.[1]][In this case, the adult *H. floresiensis* likely died in the river and was covered by sediment and groundwater. When the soft tissue decomposed, the hard body structures were fossilised by permineralisation.[2]]

- I have briefly explained the process of permineralisation.[1]
- I have referenced my explanation to the formation of this fossil.[2]

Key science skills and ethical understanding

19 **a** [The common ancestor is likely to have belonged to the genus *Australopithecus*.[1]][Based on our understanding of the evolution of hominins, we know that all species within the genus *Homo* evolved from an earlier hominin species known as the australopithecines, who were ancient bipedal ancestors that lived more than 2 million years ago.[2]]

- I have identified the genus *Australopithecus*.[1]
- I have justified my answer with reference to trends in hominin evolution.[2]

b [There would be no *H. neanderthalensis* DNA in modern African *H. sapiens*.[1]][This is because, while they shared a common ancestor, the two species did not interbreed.[2]]

✓ ✗ I have stated that they would not share DNA.[1]

✓ ✗ I have explained why they would not share DNA.[2]

c [One bioethical issue pertains to who has the legal ownership over the fossils,[1]][and whether the information will be made freely available and easily accessible for all people, especially those from the areas in which the fossils were discovered.[2]]

Other acceptable responses include:

- Whether the researchers have the legal right to excavate a foreign site.
- Whether the researchers are fully informed and observant of local customs and traditions, particularly around the cultural significance of a foreign research site.
- Whether the researchers are engaging with and utilising the expertise of local researchers.

✓ ✗ I have identified a relevant bioethical issue.[1]

✓ ✗ I have justified this with reference to a possible ethical justification.[2]

11C The human fossil record

Theory review questions

1 interbreeding or crossbreeding; *Homo neanderthalensis* or the Neanderthals; *Homo denisova* or the Denisovans; transitional fossil

2 D

3 *Australopithecus*: I; III; V
Homo: II; IV

4 III, IV

SAC skills questions

5 A **6** A **7** A **8** A

Exam-style questions

Within lesson

9 C **10** C **11** C **12** D

13 A

14 a [A transitional fossil is a fossil that shows traits common to both its ancestor group and its descendant group.[1]][They are useful in that they are intermediary fossils, capable of demonstrating evolutionary changes between two completely different species that are related on either side of the transitional species.[2]]

✓ ✗ I have defined a transitional fossil.[1]

✓ ✗ I have explained why a transitional fossil is useful.[2]

b [*H. naledi* cannot be a transitional fossil between *Australopithecus* and *Homo* given that the *Homo* genus is presumed to have existed long before the *H. naledi* fossils – early *Homo* species appeared as early as 3 million years ago, while the *H. naledi* fossils are dated to less than 1 million years.[1]]

Other acceptable responses include:

- *Australopithecus* died out long before 1 million years ago.

✓ ✗ I have referenced the diagram to show that *H. naledi* cannot be a link species between these two genera.[1]

Multiple lessons

15 a [A smaller braincase[1]][and a larger arm to leg ratio.[2]]

Other acceptable responses include:

- A wider and less bowl-shaped pelvis.
- Less centralised foramen magnum.
- More pronounced sagittal crest.
- More pronounced brow ridge.
- More sloped face.
- Pronounced chin.
- Larger teeth.
- Shorter legs.

✓ ✗ I have identified one structural feature.[1]

✓ ✗ I have identified a second structural feature.[2]

✓ ✗ I have used comparative language, such as 'smaller' rather than 'small'.

b [This information suggests that the Neanderthals may have interbred with *Homo sapiens* as they made their way out of Africa,[1]][but that they did not interbreed with African humans when sharing that environment.[2]]

✓ ✗ I have identified the interbreeding opportunity between Neanderthals and non-African humans.[1]

✓ ✗ I have explained that interbreeding likely did not occur in Africa.[2]

c [One reason why scientists do not always agree on evolutionary relationships is that the fossil record is incomplete.[1]][This means that as new discoveries of fossils are made, previously held views or understandings of these evolutionary relationships can change or be altered.[2]]

Other acceptable responses include:

- Some fossils are incomplete or only very small fragments are found.

✓ ✗ I have explained that the fossil record has gaps.[1]

✓ ✗ I have outlined how new discoveries can change previously held beliefs.[2]

Key science skills and ethical understanding

16 a [This suggests *Homo sapiens* and Denisovans are not different species since they could interbreed.[1]]

✓ ✗ I have stated the correct implication.[1]

b [Denisovan DNA is not present in African *H. sapiens* because the two populations did not interbreed.[1]][However, Denisovans did interbreed with the ancestors of modern-day Asian and Indigenous Australian *H. sapiens*, which explains the presence of Denisovan DNA in their genomes.[2]]

✓ ✗ I have explained the absence of Denisovan DNA in African *H. sapiens*.[1]

✓ ✗ I have explained the presence of Denisovan DNA in Asian and Indigenous Australian *H. sapiens*.[2]

c [Interbreeding between *H. sapiens* and Denisovans most likely occurred twice independently, once in East Asia and again in Papua New Guinea.[1]][This is evident because, firstly, the different amounts of Denisovan DNA in Papua New Guineans and East Asians may be due to different amounts of DNA being exchanged through two separate interbreeding events.[2]][Secondly, the higher similarity of Siberian Denisovan DNA to the DNA in the East Asian genome compared to the DNA in the Papua New Guinean genome suggests less divergence between the exchanged genetic material.[3]] [This would mean the interbreeding event with East Asians occurred more recently, and therefore separately, to the Papua New Guinean interbreeding event.[4]]

✓ ✗ I have suggested that two separate interbreeding events occurred.[1]

✓ ✗ I have explained how the first finding suggests separate interbreeding events.[2]

✓ ✗ I have explained how the second finding suggests an earlier interbreeding event.[3]

✓ ✗ I have inferred that this more recent interbreeding event would have been separate.[4]

✓ ✗ I have signposted my response by using terms such as: firstly, secondly

✓ ✗ I have used comparative language such as: higher, compared to, fewer

d [This DNA could belong to another unidentified hominin species that humans interbred with in the past.[1]]

Other acceptable responses include:

- These sequences could simply be DNA from Neanderthals or Denisovans that have mutated to become unrecognisable.
- Mutations arose in this group or their ancestors that lead to the presence of the unique sequences.

✓ ✗ I have given a reasonable explanation to account for these findings.[1]

11D Human migration

Theory review questions

1 A

2 U-*Homo erectus*; V-*Homo sapiens*; W-*Homo erectus*; X-*Homo sapiens*; Y-Multiregional hypothesis; Z-Out of Africa hypothesis

3 fossil record; dated; *Homo sapiens*; migratory patterns; DNA evidence; genome-wide analysis; high mutation rate

4 B

SAC skills questions

5 C **6** C **7** B **8** D

Exam-style questions

Within lesson

9 C **10** D

11 a [Early *Homo sapiens* entered a now prehistoric supercontinent called Sahul roughly 50 000–65 000 years ago, which was composed of present-day Australia, Tasmania, and New Guinea.[1]][Sahul would later separate, leaving the populations geographically isolated and making them the longest unbroken example of an indigenous population in the world.[2]]

✓ ✗ I have described Sahul and provided a timeline of between 50 000–65 000 years.[1]

✓ ✗ I have explained that Sahul separated and left the populations geographically isolated.[2]

b [Connection to Country refers to a reciprocal relationship between First Nations people and their ancestral lands and seas: the land provides for the people, while the people manage and sustain the land through their culture, ceremonies, and care.[1]]

✓ ✗ I have described Connection to Country as a reciprocal relationship.[1]

Multiple lessons

12 a [The Out of Africa model. We can tell this as the earliest time period shown is 200 000 years ago when humans are shown in Africa, before spreading outwards at a later date.[1]]

✓ ✗ I have explained that this is the OOA model with reference to the timeline of geographical spread.[1]

b i [*Homo Neanderthalensis* was a hominin, given that it is one of our bipedal relatives from the genus *Homo*.[1]][To be hominin means to be a part of the tribe *Hominini*, which includes us and all of our upright walking ancestors, including those from the genera *Australopithecus* and *Homo*.[2]]

✓ ✗ I have identified the Neanderthals as hominin.[1]

✓ ✗ I have defined what it means to be hominin.[2]

ii [Modern non-African *Homo sapiens* contain a small percentage of Neanderthal DNA because of interbreeding which occurred after *Homo sapiens* left Africa and encountered other hominins.[1]] [This interbreeding occurred once *Homo sapiens* had left Africa, meaning that modern African populations do not show Neanderthal DNA in their genomes.[2]]

✓ ✗ I have identified interbreeding as the means by which Neanderthal DNA entered the genomes.[1]

✓ ✗ I have stated that this interbreeding occurred outside of Africa to explain the absence in modern African populations.[2]

c i [A shorter arm-to-leg ratio.[1]]

Other acceptable responses include:

- A more centralised foramen magnum.
- A less funnel-shaped ribcage.
- A more protruding big toe.
- A shorter, wider pelvis.
- An arched foot.
- A larger heel.

✓ ✗ I have identified a structural difference.[1]

ii [Variation in arm-to-leg ratio existed in the population of *Homo sapiens*.[1]] [When *Homo sapiens* became increasingly bipedal, humans with longer arm-to-leg ratios were less efficient,[2]] [while those with shorter arm-to-leg ratios were conferred a selective advantage.[3]] [Over time, the shorter arm-to-leg ratio became more common as they passed on their advantageous trait to subsequent generations.[4]]

✓ ✗ I have stated that variation existed in the population.[1]

✓ ✗ I have identified that the selection pressure negatively impacted longer arm-to-leg ratios.[2]

✓ ✗ I have identified that short arm-to-leg ratios were conferred a selective advantage.[3]

✓ ✗ I have explained the consequences of this on the phenotype of the population.[4]

Key science skills and ethical understanding

13 a [Aboriginal populations are one of the longest continuous populations on earth, meaning that there has been more time for mutations to accumulate in their mtDNA, resulting in greater genetic variation in their mitochondrial lineages.[1]]

✓ ✗ I have explained that there has been more time for mutations to accumulate in the mtDNA.[1]

b [A model could be a drawing or a 3D structure and is used to approximate an object or event. In this case, it is being used to describe a system of lineage.[1]] [On the other hand, a simulation is the process of using a model to observe and predict what may happen in a real or theoretical system.[2]]

✓ ✗ I have described a model as an approximation of a system or event.[1]

✓ ✗ I have described a simulation as the use of a model to make predictions.[2]

c [Respect encourages the acknowledgment of the intrinsic value of people, including their beliefs, customs, and cultural heritage.[1]] [If the findings were to contradict traditional beliefs, this could represent a challenge to the cultural heritage of Aboriginal communities and could diminish their Connection to Country.[2]]

✓ ✗ I have described respect with reference to beliefs and culture.[1]

✓ ✗ I have explained how contradictory findings could challenge Connection to Country.[2]

✓ ✗ I have used key biological terminology such as: acknowledgement, intrinsic value, contradict.

Chapter 11 SAC practice

1 [The term hominin refers to the subfamily which includes modern humans and their bipedal ancestors.[1]] [Two genera belonging to the hominin subfamily are *Homo* and *Australopithecus*.[2]]

✓ ✗ I have described the hominin subfamily by reference to humans and their bipedal ancestors.[1]

✓ ✗ I have listed two hominin genera.[2]

2 [One structural change was a shortening of the arm-to-leg ratio.[1]] [A second change was the development of a broader and more bowl-shaped pelvis.[2]]

Other acceptable responses include:

- Arched feet.

✓ ✗ I have identified one structural change.[1]

✓ ✗ I have identified a second structural change.[2]

3 [Despite a general trend over time towards a larger brain volume, not every hominin ancestral species had a smaller brain than later species.[1]] [For example, Neanderthals had a larger brain volume than modern humans despite having evolved much earlier.[2]]

✓ ✗ I have explained what is meant by the non-linear trend in brain size evolution.[1]

✓ ✗ I have supported my answer with reference to the Neanderthals' large brain volume.[2]

4 [A correlation study measures the association between two or more variables.[1]] [It differs from a controlled experiment in that the researchers are not trying to identify a causal relationship between variables and typically do not manipulate any of the variables. Rather, the aim is to assess their statistical relationship without any influence from external factors.[2]]

✓ ✗ I have defined a correlation study as measuring the association between variables.[1]

✓ ✗ I have suggested a difference between a correlation study and a controlled experiment.[2]

5 [On average, the skulls from earlier in the Pleistocene will have a smaller brain volume than those from later in the Pleistocene.[1]]

✓ ✗ I have stated a plausible hypothesis.[1]

6 [This specimen would be considered distinct from *H. erectus*[1]][given that, unlike the findings for *H. erectus*, the specimen demonstrates a large frontal region, tall braincase, and flat face – all of which were determined by the results of the study to be typically associated with other MP hominins.[2]]

✓ ✗ I have correctly stated that the specimen would be considered distinct.[1]

✓ ✗ I have justified this response with reference to the information in the study.[2]

7 [One potential source of error would be a random error arising if the researchers were inaccurate with their own measurements, such as estimating and using a tape measure incorrectly.[1]][One way to avoid this would be to refine the measurement process by using highly specialised and calibrated measuring devices.[2]]

Other acceptable responses include:

- Another way to avoid this would be to have each researcher independently measure and compare results to ensure accuracy.

✓ ✗ I have suggested a potential source of error.[1]

✓ ✗ I have suggested refining the measurement process.[2]

8 [Adaptive introgression refers to instances where the incorporation of foreign DNA, typically via interbreeding events, provides some form of fitness advantage for the recipient species and therefore rises to high frequency in the gene pool of that population.[1]][This is especially important as a species migrates into new environments, as it allows that population to adapt to new selection pressures faster.[2]]

✓ ✗ I have correctly described the term adaptive introgression.[1]

✓ ✗ I have explained the importance of adaptive introgression in terms of surviving in new environments.[2]

9 [The Melanesians.[1]]

✓ ✗ I have correctly identified the Melanesians.[1]

10 [One selection pressure could have been exposure to different levels of UV radiation.[1]][As humans migrated out of Africa and further away from the equator, less pigmented, lighter skin was likely selected for as a means of maintaining Vitamin D levels.[2]]

✓ ✗ I have identified a potential selection pressure.[1]

✓ ✗ I have explained this selection pressure with reference to migration into new environments.[2]

11 [One bioethical issue is the use of profits arising from genomic research, and whether that profit is flowing back into indigenous communities equitably.[1]]

Other acceptable responses include:

- The permission to take, use, and profit off indigenous genetic information.
- The outcomes of genomic findings, and whether they adversely impact indigenous land claims.

✓ ✗ I have correctly identified one potential bioethical issue.[1]

12 [Justice prioritises the fair and equitable consideration of different parties involved in a decision or course of action.[1]][In this case, future researchers might consider ways in which the profits of their research can flow back into the indigenous communities they surveyed, and look for ways to better financially compensate participants.[2]]

✓ ✗ I have described the bioethical concept of justice.[1]

✓ ✗ I have explained how future researchers might uphold justice for future indigenous participants.[2]

Chapter 11 Exam practice

Section A

1 D	**2** C	**3** B	**4** C
5 B	**6** C	**7** D	**8** D
9 C	**10** B	**11** C	**12** D
13 D	**14** D	**15** B	

Section B

16 a [Most primates possess prehensile hands and feet, typically consisting of five digits each.[1]]

Other acceptable responses include:

- A large cranium relative to body weight.
- Large numbers of sensitive touch receptors in their fingertips.
- Flexible spines and a large degree of rotation around the hips and shoulders.

✓ ✗ I have identified a feature shared by primates.[1]

b [Arboreal locomotion refers to movement in or amongst trees.[1]][*A. sediba*'s relatively long arms compared to its body would have helped it swing and move through the trees effectively due to a high number of contact points and a heavy reliance on its arms for strength and movement.[2]]

✓ ✗ I have explained what is meant by arboreal locomotion.[1]

✓ ✗ I have identified a structural feature of *A. sediba* that would have facilitated this movement.[2]

c [Forward facing eyes capable of 3D colour-vision allow primates to better locate objects within their field of vision, such as predators and prey.[1]]

✓ ✗ I have identified an evolutionary advantage of this adaptation.[1]

d [Bipedalism.[1]]

✓ ✗ I have correctly identified bipedalism.[1]

e [One structural difference is that *H. sapiens* developed shorter arms than *A. sediba*.[1]][This change is significant because, unlike *A. sediba*, *H. sapiens* did not use arboreal habitats and therefore needed fewer available contact points on the forelimbs, 'freeing' the arms for other tasks, such as carrying children, preparing food, and building tools.[2]]

Other acceptable responses include:

- Longer legs – energy efficient bipedalism.
- Shorter, more bowl-shaped pelvis – supporting the upper body while walking.
- Larger skull/braincase – housing an increasingly large and complex brain.
- Increase in the arch of the foot – improved bipedal force and leverage.

✓ ✗ I have identified one structural difference between *H. sapiens* and *A. sediba*.[1]

✓ ✗ I have explained the significance of this change.[2]

17 a [The average brain volume of *Homo sapiens* is approximately 1350 cc.[1]]

✓ ✗ I have correctly identified that the average brain volume of *Homo sapiens* is between 1300–1400 cc.[1]

b [Human beings are mammalian primates belonging to the genus *Homo*.[1]]

✓ ✗ I have defined humans as belonging to class Mammalia, order Primates, and genus *Homo*.[1]

c [The two outliers in this graph are *Homo floresiensis* and *Homo neanderthalensis*.[1]][These outliers exist because the evolution of brain volume in hominins has not been perfectly linear. While brain volume has generally tended to increase with time, some hominins have had smaller (*H. floresiensis*) or larger (*H. neanderthalensis*) average brain volumes in relation to those hominin species that lived in similar time periods.[2]]

✓ ✗ I have identified the two outliers.[1]

✓ ✗ I have explained that brain volume evolution in hominins has not been exactly linear.[2]

18 a [Skull B.[1]]

✓ ✗ I have correctly identified the gorilla skull.[1]

b [The presence of a sagittal crest, relatively smaller cranial capacity, and larger canine teeth.[1]]

Other acceptable responses include:

- A larger brow ridge.
- A less central foramen magnum.
- A U-shaped/rectangular dental arch.

✓ ✗ I have identified three features of the gorilla skull that differentiate it from the *Homo sapiens* skull.[1]

✓ ✗ I have used comparative language in my response such as: smaller, larger

19 a [The Out of Africa hypothesis.[1]][Given that there is no Neanderthal DNA in populations found in Africa, it can be assumed that *Homo sapiens* first evolved in Africa where there were no Neanderthal populations.[2]][Populations of *H. sapiens* then moved out of Africa and encountered other *Homo* species. This is supported by the presence of *Homo neanderthalensis* DNA in all modern humans except African populations.[3]]

✓ ✗ I have correctly identified the Out of Africa hypothesis.[1]

✓ ✗ I have explained that humans first evolved in Africa by referencing the lack of Neanderthal DNA in African populations.[2]

✓ ✗ I have explained human migration out of Africa by reference to the presence of Neanderthal DNA in non-African populations.[3]

b i [A more prominent brow ridge[1]][and a relatively smaller brain case.[2]]

Other acceptable responses include:

- Larger canine and molar teeth.
- A less central foramen magnum.

✓ ✗ I have identified a first difference.[1]

✓ ✗ I have identified a second difference.[2]

✓ ✗ I have used comparative language in my response such as: more, relatively.

✓ ✗ I have not included features common to all primates in my response.

ii [A more C-shaped spine[1]][and a larger arm-to-leg ratio.[2]]

Other acceptable responses include:

- A more funnel-shaped ribcage.
- A longer, narrower pelvis.
- A less protruding big toe.
- A smaller heel.
- A flatter foot.

✓ ✗ I have identified a first structual feature.[1]

✓ ✗ I have identified a second structual feature.[2]

✓ ✗ I have used comparative language in my response such as: more, larger.

✓ ✗ I have not included features common to all primates in my response.

c i [Migration occurred at some point less than 80 000 years ago.[1]]

✓ ✗ I have correctly stated that migration occurred less than 80 000 years ago.[1]

ii [These findings might diminish the traditional understanding of the Aboriginal Dreaming, which believes that the Aboriginal people have been in Australia since the time of creation, not just 80 000 years ago.[1]]

✓ ✗ I have identified the challenge to the traditional Aboriginal belief of creation.[1]

GLOSSARY

#

3′ poly-A tail a chain of adenine nucleotides added to the 3’ end of pre-mRNA during RNA processing p. 90

5′ methyl-G cap a molecule added to the 5’ end of pre-mRNA during RNA processing p. 90

A

absolute age an estimate of the age (in years) of a fossil or rock p. 546

absolute dating a dating technique used to determine the absolute age of a fossil by measuring the relative amounts of radioisotopes to their products. Also known as radiometric dating p. 544

accurate how close a measurement is to the true value p. 11

acetyl-CoA the product of the link reaction where pyruvate is conjugated to coenzyme A, creating the primary input into the Krebs cycle p. 302

activation energy the energy required to initiate a reaction p. 130

activator protein a protein coded for by a regulatory gene that increases gene expression p. 102

active immunity protection against a disease created by antibodies and memory cells formed by a person’s own adaptive immune system p. 421

active site the part of an enzyme where the substrate binds p. 129

active transport the movement of molecules across a semipermeable membrane requiring an energy input p. 110

adaptive potential the ability for a population to adjust to new environmental selection pressures p. 497, 517

adaptive radiation the rapid divergent evolution of a species, thereby producing a wide array of species/forms p. 571

ADP adenosine diphosphate, the unloaded form of ATP p. 143

advantageous phenotype a biochemical, physical, or behavioural trait that increases an organism’s fitness in its local environment p. 484

aerobic cellular respiration cellular respiration that occurs in the presence of oxygen. Involves three stages, during which glucose and O2 are converted into ATP, CO2, and water p. 299

afferent lymphatic vessel thin-walled structures that collect lymph from the tissues of the body and deliver it to lymph nodes p. 406

affinity the tendency of a molecule/atom to bind or react with another molecule/atom p. 256, 271

agarose gel a sponge-like gel used in gel electrophoresis that contains pores for DNA fragments to move through p. 189

agglutination the clumping of particles together. In the immune system, antibodies can help clump pathogens together p. 393

aim the objective of an investigation or experiment p. 5

airborne transmission the spread of pathogens through air via small particles (traditionally <5 μm) p. 445

allele an alternate form of a gene p. 474

allele frequency the proportion of certain alleles in a gene pool p. 474, 484, 496

allergen a non-pathogenic antigen that triggers an allergic reaction p. 356

allergic reaction an overreaction of the immune system to a non-pathogenic antigen p. 356

allopatric speciation the geographic separation of a population from a parent population resulting in the formation of a new species p. 505

allosteric site a region on an enzyme that is not the active site p. 142, 272, 324

alpha helix an organised coiled secondary structure of proteins p. 59

alternative splicing the process where different exons may be spliced, resulting in a single gene producing multiple different mRNA strands p. 91

amino group the functional group on amino acid molecules that is made up of one nitrogen and two hydrogens (NH_2) p. 58

amplify to increase the quantity of a molecule by making many copies p. 182

anaerobic fermentation a metabolic pathway that occurs in the absence of oxygen. Involves glycolysis, followed by further reactions that convert pyruvate into lactic acid in animals, or ethanol and CO_2 in yeasts p. 299, 314

analogous structures features present in two or more species that fulfil the same function but do not originate from a common ancestor p. 558

anecdote evidence involving a personal account or report of a previous experience that may provide a certain level of support for a position p. 23

anneal the joining of two molecules, for example two complementary DNA strands during the cooling phase of PCR p. 183

antibiotic medications used to kill bacteria or slow their growth p. 446

antibiotic resistance gene gene which confers antibiotic resistance p. 205

antibody a protein produced by plasma cells during the adaptive immune response that is specific to an antigen and combats pathogens in a variety of ways. Also known as immunoglobulin p. 57, 390

anticodon the sequence of three nucleotides on a tRNA molecule that recognises a specific sequence of three nucleotides (codon) on an mRNA strand p. 92

antigen any molecule that may trigger an immune response p. 354

antigen-antibody complex a structure formed by the complementary binding between antigen and antibody molecules p. 393

antigen-presenting cell a subgroup of phagocytes that display the antigens from consumed pathogens on their surface and interact with the adaptive immune system p. 377, 390,404

antigenic drift small and gradual mutations in the genes encoding for viral surface antigens p. 525

antigenic shift sudden and significant mutations in the genes encoding for viral surface antigens p. 525

antimicrobial agent an agent that kills or slows the growth of microorganisms. Examples include antiseptics, disinfectants, antifungals, antivirals, and antibacterial agents p. 522

antimicrobial resistance the ability of a microorganism to survive exposure to an antimicrobial agent p. 446, 522

antiparallel a characteristic of DNA strands describing how each strand runs in an opposite direction to the other. One strand runs in a 3' → 5' direction and the other runs in a 5' → 3' direction p. 69

antiseptic a substance that is applied to living tissue to kill or slow the growth of microorganisms p. 445

antivenom a medical treatment containing antibodies specific to the toxins present in venomous bites or stings p. 424

antiviral medications used to treat viral infections p. 446

apoptosis the controlled death of cells in the body. Also known as programmed cell death p. 395, 457

applied ethics the application of ethical theories to real–life moral problems and contexts p. 34

arable land land that is suitable for growing crops p. 281

arboreal living in or amongst trees p. 605

arm to leg ratio the ratio of arm length to leg length. Tree-dwelling hominids have longer arms and shorter legs, or a larger arm to leg ratio p. 608

artificial active immunity protection against a disease created by antibodies and memory cells produced by an individual's own immune system after medical intervention. Also known as artificially acquired active immunity p. 422

artificial immunity protection against a disease formed as a result of medical intervention. Also known as induced immunity p. 420

artificial passive immunity protection against a disease created by antibodies from an external medical source. Also known as artificially acquired passive immunity p. 424

ATP adenosine triphosphate, a high energy molecule that, when broken down, provides energy for cellular processes p. 143, 242, 299

ATP synthase an enzyme in the inner mitochondrial membrane that uses the concentration gradient of H^+ to synthesise ATP from ADP and P_i p. 303

autoantibodies antibodies directed against an organism's own tissues p. 458

autoimmune disease a disease in which an individual's immune system initiates an immune response against their own cells p. 356, 454

autoreactive a cell that recognises a self-tissue or self-antigen as non-self p. 458

B

B lymphocyte a type of lymphocyte that plays an important role in humoral immunity and differentiates into plasma cells and B memory cells p. 390, 455

B memory cell a differentiated B lymphocyte that is responsible for providing long-lasting immunological memory of an antigen p. 391

bacterial conjugation the process in which bacteria exchange genetic material via direct cell-cell contact p. 523

bacterial transformation the process by which bacteria take up foreign DNA from their environment. Scientists use this process to introduce recombinant plasmids into bacteria p. 204

bacteriophage a virus that infects prokaryotic organisms p. 171

bacterium (pl. bacteria) a single-celled prokaryotic, microscopic organism that frequently grows in clusters. It can live symbiotically with other organisms and/or act as pathogens p. 357

band a line seen in the gel after running gel electrophoresis that corresponds to a collection of DNA fragments of a specific size p. 190

base pair (bp) a unit of measurement that corresponds to one nucleotide p. 191

beneficence an ethical concept that seeks to maximise benefits when taking a particular position or course of action p. 38

beta-pleated sheet an organised folded secondary structure of proteins p. 59

bias an inclination to favour a particular position or outcome p. 3

biochemical pathway a series of enzyme-catalysed biochemical reactions in which the product of one reaction becomes the substrate of the next reaction. Also known as a metabolic pathway p. 131, 142

bioethanol a type of biofuel that is produced via the anaerobic fermentation of plants such as sugarcane or corn p. 333

bioethical approach a decision–making framework that helps guide ethical behaviour p. 34

bioethical issue an ethical dilemma pertaining to biology that typically involves a decision–making process between two or more choices or options for an action p. 34

bioethics the study of ethical issues pertaining to biology and medicine p. 34

biofuel fuel created from organic material known as biomass p. 332

biomass organic material, including plants, animal by-products, and biological waste material. Biomass can be sourced from many industries, including farming, forestry, and food manufacturing p. 332

biped an individual that moves on two legs (upright-walking) p. 605

bipedalism using two legs for walking upright p. 596

block mutation a mutation that affects a large chunk of DNA, or an entire gene p. 476

blunt end the result of a straight cut across the double-stranded DNA by an endonuclease resulting in no overhanging nucleotides p. 163, 174

bone marrow semi-solid tissue found within bones. Serves as the primary site of the creation of red blood cells and leukocytes p. 404

booster vaccine a vaccination given to a person later in time after they have completed their initial vaccination program to enhance their existing immunity against a disease. Also known as a booster shot p. 423

bottleneck effect the reduction in genetic diversity that occurs when a large proportion of a population is removed due to a chance event p. 495

branch a line on a phylogenetic tree that represents an evolutionary path p. 569

brow ridge a bony ridge above the eye sockets. It is found in all primates, but is greatly reduced in *Homo sapiens* p. 597

buffer an ion-rich solution that carries electrical current through the agarose gel p. 189

bulk transport a type of active transport that uses vesicles to move large molecules or groups of molecules into or out of the cell p. 110

bundle-sheath cell a plant cell type that is the site of most of the Calvin cycle in C4 plants p. 257

C

C3 plants plants with no evolved adaptation to minimise photorespiration p. 257, 267, 281

C4 plants plants that minimise photorespiration by separating initial carbon fixation and the remainder of the Calvin cycle over space p. 257, 267, 281

CAM plants plants that minimise photorespiration by separating initial carbon fixation and the remainder of the Calvin cycle over time p. 259, 267, 281

Cambrian explosion a period (~ 535 mya) of rapid diversification of multicellular life, characterised by the evolution of hardened body parts such as shells or bones p. 541

cancer a disease caused by the uncontrolled replication of cells with the ability to migrate to other parts of the body p. 454

canine teeth a type of tooth in mammals that is relatively long and pointed p. 597

carbon fixation the process in living organisms where inorganic carbon, typically within carbon dioxide, is converted into organic compounds such as glucose. Carbon fixation is a central part of the light-independent stage of photosynthesis p. 255

carbon neutral a state in which there is no net release of carbon dioxide into the atmosphere, meaning that there is a balance between the amount of CO_2 that is emitted during combustion of a fuel and how much was originally absorbed during the formation process of that fuel p. 332

carboxyl group the functional group on amino acid molecules that contains a hydroxyl group (OH) and an oxygen double-bonded to a carbon atom p. 58

cast fossil fossil formed when a mould fossil is filled with sediment p. 542

catalyse to increase the rate of a reaction p. 129, 304

catalyst a substance capable of increasing the rate of a reaction without being used up p. 129

categorical variable a factor that is qualitative, typically describing a characteristic such as gender, birth order (1st, 2nd, 3rd), or nationality p. 18

causation when change in one variable leads to reliable change in another p. 23

cell-mediated immunity an adaptive immune response in which infected or abnormal cells are destroyed by cytotoxic T cells. Also known as T cell immunity p. 390

cellular pathogen a pathogen that has a cellular structure and exhibits the processes of a living organism. Examples include bacteria, fungi, protozoa, and parasites p. 356

cellular respiration the process by which cells create usable energy in the form of ATP from a series of biochemical reactions, involving the breakdown of glucose p. 299

cerebrum the largest part of the brain, which comprises two-thirds of the brain's entire weight and is responsible for a large range of vital functions including sensory processing, motor control, and visual and spatial learning p. 607

chemical barrier a component of the first line of defence that features the use of enzymes, toxins, and acids to protect against pathogen invasion p. 367

chemotaxis the attraction of phagocytes towards a pathogen p. 379

chimeric an organism or cell containing genetic material from another organism or cell p. 453

chitinases enzymes that occur in a number of different plants and have antifungal properties p. 368

chlorophyll a chemical found in the thylakoids of chloroplasts. It is responsible for absorbing light energy in photosynthesis p. 241

chloroplast a membrane-bound organelle only found in plant and photoautotroph cells that is the site of photosynthesis p. 241

chromosome a structure made of protein and nucleic acids that carries genetic information p. 69

cilium (pl. cilia) thin, hair-like projection that protrudes from eukaryotic cells p. 369

circulatory system a collection of tissues and organs involved in the transportation of substances around the body. Composed of the lymphatic and cardiovascular systems p. 403

cisgenic organisms a genetically modified organism that contains foreign genetic material from a sexually compatible donor organism, typically from the same species p. 217

clonal expansion the process in which many copies of a lymphocyte are generated p. 390

clonal selection the process in which B and T cells encounter an antigen that matches their antigen-binding site, and then generate many copies of themselves p. 390, 404

coding strand the strand of DNA not transcribed by RNA polymerase, contains an identical sequence to the mRNA strand produced (except thymine is replaced with uracil in mRNA) p. 89

codon the sequence of three nucleotides in mRNA coding for one amino acid p. 80

codon the sequence of three nucleotides in mRNA coding for one amino acid p. 92

coenzyme a non-protein organic cofactor that assists enzyme function. They release energy and are recycled during a reaction p. 143

coenzyme A a large organic non-protein molecule that plays a key role in the modification of pyruvate to allow it to enter the Krebs cycle. Also known as CoA p. 302

cofactor any organic or inorganic molecule, such as a coenzyme or metal ion, that assists enzyme function p. 143

combustion a chemical reaction between a fuel and an oxidant (typically oxygen) that produces heat and gas. Also known as burning p. 332

competition interactions between organisms in which both are negatively impacted when vying for the same limited resource. Can exist within or between species p. 484

competitive inhibition the hindrance of an enzyme by blocking the active site and preventing the substrate from binding p. 141

competitive inhibitor a molecule that hinders an enzyme by blocking the active site and preventing the substrate from binding p. 272, 324

complement cascade a complex sequence of events which occurs after the activation of complement proteins p. 379

complement proteins a number of different types of proteins found in the blood that opsonise, cause lysis, and attract phagocytes to invading pathogens p. 378, 456

complementary base pairing describes which nucleotides can form hydrogen bonds with each other. C pairs with G, A pairs with T (or U in RNA) p. 69

condensation reaction a reaction where two monomers join to form a larger molecule, producing water as a by-product p. 58, 68, 92

conformational change a change in the three-dimensional shape of macromolecules such as proteins p. 103, 130, 137

conjugated monoclonal antibodies monoclonal antibodies with other molecules (e.g. chemotherapy drugs or radioisotopes) attached to them p. 456

Connection to Country a reciprocal relationship between First Nations people and their ancestral lands and seas p. 631

consequences-based approach an approach to bioethics that aims to maximise positive outcomes while minimising negative outcomes p. 36

conserved genes genes that have remained largely unchanged throughout evolution, and are found across the genome's of many different species p. 560

contagious a property of a pathogen or disease meaning that it can be transmitted from one organism to another p. 433, 442

control group a group of individuals/samples that are not exposed to the independent variable. Also known as an experimental control, control treatment, or the control p. 8

controlled experiment an investigation into the effect of an independent variable on a dependent variable, while keeping all other factors constant p. 3

controlled variable a factor that is kept constant throughout the experiment. Also known as a constant variable p. 5

convergent evolution the process in which distantly related species evolve similar traits over time due to the action of similar selection pressures p. 558

correlation when there is a relationship between two variables p. 23

Country an area that is traditionally owned and looked after by an Aboriginal language group or community, or by certain people within that group. The term may indicate more than simply a geographical area – it is also a concept that can encompass the spiritual meaning and feelings of deep connection and attachment associated with that area p. 4, 632

cranium the part of the skull that covers the brain p. 595

CRISPR short, clustered repeats of DNA found in prokaryotes which protects them against viral invasion p. 172, 280

CRISPR-associated protein 9 (Cas9) an endonuclease that creates a blunt end cut at a site specified by guide RNA (gRNA) p. 172, 280

CRISPR-Cas9 a complex formed between gRNA and Cas9 which can cut a target sequence of DNA. Bacteria use this complex for protection from viruses and scientists have modified it to edit genomes p. 171

crista (pl. cristae) the folds of the inner membrane of a mitochondrion. The site of the electron transport chain p. 300

cuticle a waxy protective film covering the surface of a plant leaf p. 367

cytochrome c an enzyme found in mitochondria that carries electrons in aerobic and anaerobic respiration reactions p. 560

cytokine a signalling molecule released by cells (typically in the immune system) which aids in communication between immune cells and helps protect against pathogens p. 377, 390

cytosol the aqueous fluid that surrounds a cell's organelles inside the plasma membrane p. 300

cytotoxic T cell (Tc) a differentiated T lymphocyte that is responsible for the destruction of infected or abnormal cells p. 394

D

dating period the range of time since fossilisation in which a particular radioisotope series can be used. Beyond this period, most of the radioisotope will have broken down into its products, meaning that it is too difficult to estimate the fossil's age p. 547

defensins small peptides that are toxic to microbes and fungi p. 368

degenerate a property of the genetic code which means that a single amino acid can be coded for by more than one codon p. 476

degranulation the release of granule contents from a cell p. 378

deleterious used to describe alleles that have an overall negative effect on individual fitness when expressed p. 475

deleterious allele an allele that has an overall negative effect on individual fitness when expressed p. 517

deleterious mutation a change in DNA that negatively affects an individual p. 175

denature the disruption of a molecule's structure by an external factor such as heat p. 137, 183, 269, 323

dendritic cell a type of leukocyte that engages in phagocytosis and antigen presentation p. 377

DNA (deoxyribonucleic acid) a double-stranded nucleic acid chain made up of nucleotides. DNA carries the instructions for proteins which are required for cell and organism survival p. 561

dependent variable (DV) the factor/s measured in the experiment that are changed when the IV is manipulated p. 5

desirable trait a heritable phenotype that humans select for during selective breeding p. 516

diabetes a disease where the body cannot properly produce or respond to insulin p. 204

differentiation the process in which cells develop specialised characteristics, typically transforming them from one cell type to another more specialised cell type p. 176, 391

direct physical contact transmission the spread of pathogens through contact between a host and another individual p. 445

disadvantageous allele an allele that encodes for a biochemical, physical, or behavioural trait that lowers an individual's fitness in its local environment p. 484

disinfectant a substance that is applied to non-living materials to kill or slow the growth of microorganisms p. 445

disulphide bond a strong covalent bond occurring between two sulphur atoms p. 59, 391

divergent evolution the process in which a common ancestor evolves into two or more descendant species p. 558

DNA (deoxyribonucleic acid) a double-stranded nucleic acid chain made up of nucleotides. DNA carries the instructions for proteins which are required for cell and organism survival p. 68

DNA profiling the process of identification on the basis of an individual's genetic information p. 192

double helix the structure of double-stranded DNA in the nucleus of eukaryotic cells, where each DNA strand wraps around a central axis p. 70

Dreaming an Aboriginal philosophy that describes the time when Ancestral Spirits (Dreaming Beings) moved over the land and created life and important geographical sites. It explains the origins of the universe, as well as the relationships between humans, animals, and the land on which they live. The Dreaming is passed down through generations and governs familial, relational, communal and spiritual obligations for Aboriginal Australians. It is also known as The Dreamtime p. 632

droplet transmission the spread of pathogens through air and contaminated surfaces via respiratory droplets p. 445

duty- and/or rule-based approach an approach to bioethics that promotes the responsibility of the agent above all else, and places importance on the duty of each individual p. 36

E

ecological niche the specific environmental conditions and resources or selection pressures within a particular environment p. 507

efferent lymphatic vessels thin-walled structures that collect lymph that has drained through lymph nodes, returning it back to circulation p. 406

electrode conductors of electricity that are attached to both ends of a gel allowing an electrical current to pass through it p. 190

electron transport chain the third stage of aerobic cellular respiration, in which a series of protein complexes embedded in the inner membrane of a mitochondrion harness the stored energy in NADH and $FADH_2$ to generate large amounts of ATP p. 300

electroporation a method that involves delivering an electric shock to bacterial membranes to increase their membrane permeability and increase the likelihood of bacterial transformation p. 206

elongate to synthesise a longer polynucleotide p. 183

embryo an early stage of development in an organism. In humans, used to refer to the organism during the first eight weeks of development p. 176

emerging disease an infectious disease that is new to appear in the human population, or that is rapidly increasing in incidence p. 433

emigration the movement out of a population p. 498, 630

end-product inhibition a form of inhibition where the final product in a series of reactions inhibits an enzyme in an earlier reaction in the sequence p. 324

endonuclease an enzyme that breaks the phosphodiester bond between two nucleotides in a polynucleotide chain p. 163, 172

environmental selection pressure a factor in the environment (e.g. limited resources, deforestation, changing temperature, predation) that impacts an organism's ability to survive and reproduce p. 484

enzyme an organic molecule, typically a protein, that catalyses (speeds up) specific reactions p. 57, 82, 89, 128, 254, 266

enzyme inhibitor a molecule that binds to and prevents an enzyme from functioning p. 141, 272, 324

enzyme-linked immunosorbent assay (ELISA) an experimental technique used to identify a pathogen by determining the presence of antigens or antibodies in a sample p. 443

enzyme-substrate complex the structure formed when an enzyme and substrate are bound together p. 130

eosinophil a large granular leukocyte responsible for the release of toxic chemical mediators p. 378

epidemic a dramatically increased occurrence of a disease in a particular community at a particular time p. 435, 443, 525

error differences between observed values and the true value p. 3

ethanol a 2-carbon alcohol molecule that is produced along with carbon dioxide during anaerobic fermentation in yeast, bacteria, and plants p. 315

ethanol fermentation the process of anaerobic fermentation in yeasts, where pyruvate produced via glycolysis is converted to ethanol and carbon dioxide. Also known as alcohol fermentation p. 315

ethical concept a specific perspective or lens used to consider multiple angles of an ethical dilemma p. 34

ethics a field of knowledge that helps individuals exercise moral judgment and determine what is right and wrong p. 15, 34

ethidium bromide a fluorescent dye that binds to DNA fragments in a gel and allows them to be easily visualised under ultraviolet light p. 190

evolution the change in the genetic makeup of a population over successive generations p. 486

evolutionary relationship the relatedness of organisms based on shared ancestry p. 568

exocytosis a type of bulk transport that moves large substances out of a cell p. 93, 109

exons regions of DNA that code for proteins and are not spliced out during RNA processing p. 82, 90

experimental group a group of individuals/samples in which the independent variable is manipulated. Also known as the treatment group p. 8

F

faecal-oral transmission the spread of pathogens via oral consumption of contaminated faeces p. 445

femur angle the angle between the top and bottom of the femur when standing. It is greater in hominins when compared to other primates p. 597

fermentation the anaerobic chemical breakdown of high-energy organic molecules, typically via the action of enzymes. For many plants, fermentation involves the conversion of glucose to ethanol and carbon dioxide p. 333

fertile the ability to produce offspring p. 506

first-generation biofuels biofuels produced from edible food crops such as corn or sugarcane. These compete directly with agricultural land p. 335

first line of defence a component of the innate immune system characterised by the presence of physical, chemical, and microbiological barriers to keep pathogens out of the host organism p. 366

fitness a measure of how well an organism survives and reproduces in its environment p. 484

flavin adenine dinucleotide (FAD) a coenzyme that acts as a proton (H^+) and electron carrier in cellular respiration. FAD can cycle between its FAD and $FADH_2$ forms, depending on the reaction it takes part in p. 302

flora naturally occurring, non-pathogenic bacteria present in an organism p. 369

fomites an inanimate object that, when contaminated with a pathogen, can transmit that pathogen to a new host p. 445

food vs fuel debate a central concern of large-scale biofuel manufacturing that questions the validity of using arable farmland to produce fuel, rather than food p. 335

foramen magnum the hole in the base of the skull through which the spinal cord passes. A more centralised foramen magnum indicates bipedal locomotion p. 597, 605

forward primer a DNA primer that binds to the 3' end of the template strand and reads the DNA in the same direction as RNA polymerase p. 184

fossil the preserved body, impressions, or traces of a dead organism p. 542

fossil fuel fuel that formed over tens of millions of years from the remains of dead organic material. Fossil fuels are considered non-renewable p. 331

fossil record the information derived from fossils. The fossil record is arranged in chronological order and helps us map the history of life on Earth, placing species in the appropriate geologic time frame p. 540

fossil succession the principle that fossils of the same age will be in the same layer of sedimentary rock, and fossils found in a higher or lower sedimentary layer will be younger or older, respectively. Also known as faunal succession p. 544

fossilisation the process by which an organism becomes a fossil p. 542

founder effect the reduction in genetic diversity that occurs when a population is derived from a small unrepresentative sample of the original population p. 496

frameshift mutation a mutation that involves the insertion or deletion of one or two nucleotides, altering every codon from that point forward p. 476

fungus (pl. fungi) a eukaryotic organism characterised by spore production and a chitinous cell wall. It can act as a pathogen and cause a number of different diseases in humans p. 357

G

gall an abnormal outgrowth of tissue in plants designed to limit the spread of an invading pathogen p. 367

gel electrophoresis a technique that separates DNA fragments based on their molecular size p. 189

gene a section of DNA that carries the code to make a protein p. 69, 79, 476

gene expression the process of reading the information stored within a gene to create a functional product, typically a protein p. 82, 88, 102

gene flow the flow of alleles in and out of a population due to the migration or interbreeding of individuals between two populations p. 498

gene knock-in a technique in gene editing where scientists substitute or add nucleotides in a gene p. 176

gene knockout a technique in gene editing where scientists prevent the expression of a target gene to understand its function in an organism p. 175

gene of interest a gene scientists want to be expressed in recombinant bacteria. This gene often encodes a protein we wish to produce in commercial quantities. Also known as the desired gene p. 204

gene pool the complete set of alleles present within a particular population p. 474, 496

gene regulation the control of gene expression, typically achieved by switching transcription on or off p. 101

gene therapy repairing genetic mutations by replacing a defective gene with a healthy one p. 175

genetic code the set of rules by which information is encoded in genetic material p. 80

genetic diversity the variation in genetic makeup or alleles within a population p. 475, 486, 495

genetic drift a random event that dramatically alters a population's gene pool p. 495

genetic engineering the process of using biotechnology to alter the genome of an organism, typically with the goal of conferring some desirable trait p. 216

genetic engineering technologies refers to the artificial alteration of an organism's genome via the exchange of foreign genetic material, typically from another organism. This is often done external to the organism via the use of a transfer vector such as a plasmid. Also known as genetic recombination technologies p. 217

genetic modification the manipulation of an organism's genetic material using biotechnology p. 175, 205, 281

genetic testing screening an individual's DNA for anomalies that may make them susceptible to a particular disease or disorder p. 192

genetically modified organism (GMO) an organism with genetic material that has been altered using genetic engineering technology p. 217

genome the complete set of DNA housed within an organism p. 69, 561

genotype the genetic composition of an organism at a particular gene locus p. 475

genus (pl. genera) a taxonomic rank above species and below family. Modern humans belong to the genus *Homo* p. 605

geographic barrier a physical factor that prevents gene flow, and thereby stops two populations from breeding together p. 507

germline cell a cell involved in the generation of gametes in eukaryotes p. 476

glucanase an enzyme plants use to defend against fungi p. 368

glucose a simple 6-carbon sugar molecule with the formula $C_6H_{12}O_6$ p. 299

glycolysis the first stage of aerobic cellular respiration in which glucose is converted to two pyruvate molecules p. 299

Golgi apparatus an organelle made of flattened sacs of membrane involved in modifying, sorting, and packaging proteins. Also known as the Golgi body or Golgi complex p. 110

granum (pl. grana) a stack of thylakoids p. 242

guide RNA (gRNA) RNA which has a specific sequence determined by CRISPR to guide Cas9 to a specific site p. 173

H

haemoglobin (Hb) a protein found in red blood cells that is responsible for the transport of oxygen in the body p. 560

half-life the time taken for half the mass of a radioisotope sample to break down into its products p. 547

heat shock a method that involves rapidly increasing and decreasing the temperature to increase membrane permeability in order to enhance the likelihood of bacterial transformation p. 206

herd immunity protection against a disease conferred to non-immune individuals when a high percentage of a population is immune to the same disease. Herd immunity is often achieved through high rates of vaccination p. 425

heritability the transmission from parent to offspring (i.e. encoded in genes) p. 476, 484

heterozygous having different alleles for the same gene on homologous chromosomes p. 193

histamine a molecule released by mast cells that plays a key role in inflammation p. 378

hominins members of the taxonomic tribe Hominini that includes modern humans and our upright-walking ancestors p. 594

hominoids members of the superfamily Hominoidea that includes apes and humans p. 594

Homo sapiens the species name for modern humans p. 592

homologous structures features present in two or more species that may look and function very differently in each species, but are derived from a common ancestor p. 558

homozygous having identical alleles for the same gene on homologous chromosomes p. 193, 517

host an organism that harbours a pathogen p. 444

host organism the organism which researchers wish to genetically modify p. 217

humoral immunity an adaptive immune response in which extracellular pathogens are targeted by specific antibodies produced by plasma cells. Also known as B cell immunity p. 390

hybridoma the product of the fusion between a mouse's extracted plasma cell and a myeloma cell p. 455

hydrolysis a chemical reaction in which water is used to break down the chemical bonds of a substance p. 333

hydrophilic having a tendency to be attracted to and dissolve in water p. 58

hydrophobic having a tendency to repel and be insoluble in water p. 58

hyphae branching filaments of a fungus which help absorb nutrients from the environment p. 357

hypothesis a testable statement that describes how experimenters expect the dependent variable to change as the independent variable changes p. 4

I

iatrogenic describes a disease caused by medical intervention p. 445

immigration the movement into a population p. 498

immune deficiency a state in which the immune system is no longer able to protect the body against infection or disease. Also known as immunodeficiency p. 458

immunological memory the ability of the immune system to quickly and aggressively combat a previously encountered pathogen due to the presence of T and B memory cells p. 389

immunosuppression a reduction in the ability of the immune system to generate an immune response p. 458

immunotherapy medical interventions that treat disease by modulating the immune system, typically by either amplifying or reducing an immune response p. 453

inbreeding sexual reproduction between two related individuals p. 497

incidence the frequency of a disease in a population p. 433

independent variable (IV) the factor/s that is/are manipulated in an experiment p. 5

index fossil a group of widespread fossils which existed for a short period and have a known age. Can be used as a reference to easily determine the age of unknown fossils p. 545

indirect physical contact transmission the spread of pathogens via contaminated objects or vectors p. 445

infectious disease an illness caused by a pathogen that can be transmitted between individuals p. 432

inference conclusions or assumptions reached by analysing and extrapolating from evidence p. 616

inflammatory response a series of biochemical events that occur in the body as a result of infection and/or trauma. Characterised by swelling, redness, pain, and heat in the affected tissue p. 378

innate immune system a component of the immune system that is composed of generalised and non-specific defences and/or responses to pathogens. Also known as the non-specific immune system p. 366

inorganic a compound that does not contain a carbon-hydrogen bond, e.g. carbon dioxide p. 255

insulin a hormone secreted by the pancreas to control blood glucose levels p. 204

integrity an ethical concept that encourages a full commitment to knowledge and understanding as well as the honest reporting of all sources of information and results p. 38

interbreeding when two individuals living in different populations mate and have offspring p. 498, 618

interferon a cytokine released by virally infected cells that increases the viral resistance of neighbouring uninfected cells p. 378

introns non-coding regions of DNA that do not code for proteins. They are spliced out during RNA processing p. 82, 90

J

justice an ethical concept that encourages fair consideration of competing claims, and ensures that there is no unfair burden on a particular group from an action p. 38

K

key science skills (KSSs) the set of capabilities that VCE Biology students must learn to design, conduct, analyse, and report valid experiments p. 3

kilobase (kb) a unit of measurement that corresponds to one thousand nucleotides. May also be written as kbp p. 190

Krebs cycle the second stage of aerobic cellular respiration, where multiple reactions occur to create ATP, NADH, $FADH_2$, and the waste product CO_2. Also known as the citric acid cycle or TCA cycle p. 299

L

lactic acid a 3-carbon molecule that is the product of anaerobic fermentation in animals. Also known as lactate p. 315

lactic acid fermentation the process of anaerobic fermentation in animals, where pyruvate produced via glycolysis is converted to lactic acid p. 315

lane the column of the gel corresponding to each sample of DNA p. 192

leaf the end of a branch that shows the current (or final) form of a species p. 569

leukocytes a group of blood cells responsible for protecting the body against pathogens and foreign material. Also known as white blood cells p. 376

ligase an enzyme that joins molecules, including DNA or RNA, together by catalysing the formation of phosphodiester bonds p. 164, 204

light-dependent stage the first stage of photosynthesis, where light energy splits water molecules into oxygen and hydrogen inside the thylakoid membranes. Also known as the light-dependent reactions p. 242, 254

light-independent stage the second stage of photosynthesis where carbon dioxide is used to form glucose in the stroma of a chloroplast. Also known as the Calvin cycle, the dark stage, or the light-independent reactions p. 244, 254

limiting factor a factor that prevents the rate of reaction from increasing p. 140, 266

lineage a direct sequence of species that evolved from a common ancestor p. 568

lymph a pale fluid that flows through the lymphatic system and has a high concentration of leukocytes p. 404

lymph node a small secondary lymphoid tissue found throughout the body where antigen-presenting cells activate the adaptive immune system p. 390, 404

lymphatic capillaries the smallest form of lymphatic vessel. Located in the spaces between cells p. 406

lymphatic system a large network of vessels and tissues throughout the body that form an important component of both the circulatory and immune systems p. 390, 403

lysis the disintegration or rupturing of a cell p. 357, 379

M

macrophage a type of leukocyte found throughout the body that engages in phagocytosis and antigen presentation p. 377

major histocompatibility complex (MHC) proteins a group of proteins present on the surface of all self-cells that enables the immune system to distinguish it from non-self material. Also known as self-antigens p. 355

major histocompatibility complex I (MHC I) proteins expressed on all nucleated cells in the body. These mark cells as 'self' so that the immune system doesn't attack them p. 355

major histocompatibility complex II (MHC II) proteins expressed on antigen-presenting cells, which interact with T helper cells in the process of antigen-presentation p. 355

mammals warm-blooded vertebrates belonging to the taxonomic class Mammalia that have mammary glands, hair/fur, three middle ear bones, and one lower jawbone p. 594

mast cell a type of leukocyte responsible for releasing histamine during allergic and inflammatory responses p. 378

membrane attack complex (MAC) a pore formed by complement proteins in the cell membrane of a pathogen, disrupting the membrane and leading to the pathogen's destruction p. 379, 393, 456

mesophyll cell a plant cell type found in leaves that contain large amounts of chloroplasts p. 241, 257

messenger RNA (mRNA) RNA molecules that are produced during transcription and carry genetic information from the nucleus to the ribosomes p. 70, 80, 89

metathinking the practice of reflecting upon and evaluating the way we think, including the different strategies and tools for problem-solving and learning p. 34

method the steps followed in a scientific investigation p. 7

methodology the strategy or overarching framework followed in a scientific investigation p. 7

microbiological barrier a component of the first line of defence in which the presence of normal flora limits the growth of pathogenic bacteria p. 368

missense mutation a mutation in which a nucleotide is substituted for another, changing the codon and coding for a different amino acid. Therefore, there can potentially be an effect on protein structure p. 476

mitochondrial DNA (mtDNA) circular DNA found in mitochondria p. 560

mitochondrial matrix the space inside the inner membrane of a mitochondrion. The site of the Krebs cycle p. 300

mitochondrion (pl. mitochondria) a double-membrane-bound organelle that is the site of the second and third stages of aerobic cellular respiration p. 111, 300

moieties a two way division of society into maternal and paternal groups p. 632

molecular homology the study of the similarities in the nucleotide sequences of DNA or amino acid sequences in proteins between organisms to establish relatedness p. 560

monoclonal antibodies (mAbs) identical laboratory-made antibodies produced by plasma cell clones p. 453

monomer a molecule that is the smallest building block of a polymer p. 58, 68

morphological clades combinations of various physical characteristics that are unique to particular geographical regions across a wide timespan p. 631

motor protein a protein that converts chemical energy into mechanical work p. 57

mould fossil fossil formed when a living thing decomposes underneath sediment, creating a cavity in the shape of the dead organism p. 542

multiregional hypothesis a model for the geographical spread of *Homo sapiens* which suggests that separate human populations evolved independently from earlier hominins that had spread throughout Eurasia and experienced gene flow. Also known as the regional continuity model p. 629

mutagen an agent that can cause mutations in DNA p. 475

mutation a permanent change to a DNA sequence p. 475, 523

myeloma cells rapidly-dividing cancerous plasma cells which are fused with extracted B cells from mice to produce hybridomas p. 455

N

NADPH a coenzyme that is a proton (H^+) and electron carrier in photosynthesis p. 242

naked monoclonal antibodies monoclonal antibodies that do not have any other molecules attached to them p. 456

natural active immunity protection against a disease created by antibodies and memory cells produced by an individual's own immune system without medical intervention. Also known as naturally acquired active immunity p. 421, 436

natural immunity protection against a disease formed without medical intervention p. 420

natural killer (NK) cell a type of leukocyte responsible for the recognition and destruction of damaged and/or infected host cells p. 377

natural passive immunity protection against a disease created by antibodies from an external non-medical source. Also known as naturally acquired passive immunity p. 421

natural selection a mechanism through which organisms that are better adapted to their environment have an increased chance of surviving and passing on their alleles p. 484, 515

neutrophil the most common type of leukocyte in the body. Engages in phagocytosis of pathogens and foreign material, as well as the release of cytokines p. 376

nicotinamide adenine dinucleotide (NAD) a coenzyme that acts as a proton (H^+) and electron carrier in cellular respiration. NAD can cycle between its NAD^+ and NADH forms, depending on the reaction it takes part in p. 301

node the splitting point between two branches on a phylogenetic tree, representing a speciation event p. 569

non-cellular pathogen a pathogen that neither has a cellular structure nor exhibits the processes of a living organism. Examples include viruses and prions p. 356

non-competitive inhibition the hindrance of an enzyme by binding to an allosteric site and changing the shape of the active site to prevent the substrate from binding p. 142

non-competitive inhibitor a molecule that hinders an enzyme by binding to an allosteric site and changing the shape of the active site to prevent the substrate from binding p. 272, 324

non-maleficence an ethical concept that discourages causing harm – or when harm is unavoidable, ensuring that the harm is not disproportionate to the benefits from any position or course of action p. 38

non-renewable refers to a resource that is replenished at a slower rate than it is being used, meaning that it will eventually run out p. 331

non-self antigen a molecule from outside the body that is recognised by the immune system and initiates an immune response. Also known as a foreign antigen p. 355

non-specific describes a component of the immune system that responds the same way to all pathogens p. 367

nonsense mutation a mutation in which a nucleotide is substituted for another, changing the codon to a stop codon, prematurely ceasing translation of the gene's mRNA. Therefore, there is an effect on protein structure p. 476

normal flora naturally occurring, non-pathogenic microbes present in an organism p. 524

nuclear DNA DNA that is located in the nucleus of a cell p. 70, 561

nucleic acid the class of macromolecule that includes DNA and RNA. All nucleic acids are polymers made out of nucleotide monomers p. 67

nucleotide the monomer subunit of nucleic acids. Made up of a nitrogen-containing base, a five-carbon sugar molecule (ribose in RNA and deoxyribose in DNA), and a phosphate group p. 68, 561

numerical variable a factor that is measured as a number such as height, count of population, and age p. 18

O

operator a short region of DNA that interacts with repressor proteins to alter the transcription of an operon p. 82, 102

operon a cluster of linked genes that all share a common promoter and operator and are transcribed at the same time p. 102

opinion the personal belief or viewpoint of an individual which typically has not been verified as fact p. 3

opposable digit a digit (either the thumb, big toe, or both) that is able to touch all the other digits on the same appendage p. 595

opsonisation the mechanism by which complement proteins attach to the surface of pathogens, making them easier to phagocytose p. 379

optimal the point at which for a given condition (e.g. temperature), the maximum function of an enzyme occurs. Also known as optimum p. 137, 268, 322

organic a compound containing a carbon-hydrogen bond, e.g. glucose p. 255

origin of replication (ORI) a sequence found in prokaryotes that signals the start site of DNA replication p. 205

Out of Africa hypothesis a model for the geographical spread of *Homo sapiens* which suggests that humans first developed and evolved in Africa before migrating outwards and expanding their colonies, replacing the earlier hominins that had spread prior. Also known as the African replacement model p. 629

outbreak a sudden and unexpected increase in the occurrence of a disease p. 435, 442

outlier a reading that varies drastically from other results p. 11

overhanging nucleotides unbonded nucleotides on the ends of the DNA strand resulting from a staggered cut p. 163

oxalic acid a substance produced by some plants that can be toxic if ingested p. 368

P

pandemic an epidemic that has spread across multiple countries and/or continents p. 435, 443, 525

parasite an organism that lives in or on another organism, usually deriving nutrition from the host organism p. 357

passive immunity protection against a disease created by antibodies from an external source p. 421

pathogen an agent that causes disease p. 432, 442, 354

peptide bond the chemical bond linking two amino acids p. 58, 92

peptide hormone a protein signalling molecule that regulates physiology or behaviour p. 57

permineralised fossil fossil formed when mineral-rich groundwater deposits minerals like silica and calcite into organic material, creating a mineral relic p. 542

personal error mistakes or miscalculations due to human fault. Can be eliminated by performing the experiment again correctly p. 15

pH a scale used to measure the acidity or basicity of an aqueous solution p. 323

phagocyte a group of leukocytes responsible for the endocytosis and destruction of pathogens, foreign material, and cell debris p. 376

phenols secreted by wounded plants to repel or kill invading microorganisms p. 368

phenotype the physical or biochemical characteristics of an organism that are the result of gene expression and the environment p. 475

phosphodiester bond a strong covalent bond linking a five-carbon sugar to a phosphate group p. 68

photoautotroph an organism capable of undertaking photosynthesis p. 241

photolysis the process in which molecules are broken down by the action of light p. 242

photorespiration a wasteful process in plants initiated by Rubisco that limits photosynthesis p. 255, 271, 281

photosynthesis the process of capturing light energy to power the production of glucose and oxygen from carbon dioxide and water p. 241

phylogenetic tree a diagram used to show the relatedness between organisms p. 568

phylogenetics the study of the relatedness between organisms p. 568

physical barrier a component of the first line of defence that features solid or fluid obstacles that block pathogen entry such as skin or mucus p. 367

placebo a substance that has no active ingredients or side effects p. 9

plant tissue culture a range of techniques used to grow plant cells, tissues, or organs under sterile conditions using a nutrient culture medium, such as an agar plate or nutrient broth of known composition. It is widely used to produce clones of a plant p. 219

plasma cell a differentiated B lymphocyte that is responsible for the generation and secretion of antibodies during the humoral response p. 391

plasma membrane the phospholipid bilayer with embedded proteins which separates the intracellular environment from the extracellular environment p. 110

plasmid a small, circular loop of DNA separate from the chromosome, typically found in bacteria p. 203

plasmid vector a piece of circular DNA that is modified to be an ideal vector for bacterial transformation experiments p. 204

plateau to reach a state where no further change occurs p. 266

point mutation a mutation that alters a single nucleotide in a DNA sequence p. 476

polymer a large molecule that is made up of small, repeated monomer subunits p. 58, 67

polymerase an enzyme that synthesises a polymer from monomers, such as forming a DNA strand from nucleic acids p. 165

polymerase chain reaction (PCR) a laboratory technique used to produce many identical copies of DNA from a small initial sample p. 182

polypeptide a long chain of amino acids. Proteins can be made of one or many polypeptides p. 56

polyploidy when an organism contains additional sets of chromosomes in its genome p. 508

population a group of individuals of the same species living in the same location p. 12, 474, 484, 496

power grip a type of grip involving the palm and the fingers, used by primates (to varying extents) for moving and manipulating objects. The power grip generates more force due to the significant use of the palm p. 595

precise two or more measurements that closely align with each other p. 11

precision grip a type of grip involving the tips of the thumb and finger, used by primates (to varying extents) for precise manipulation of objects of various sizes p. 595

precursor messenger RNA (pre-mRNA) the immediate product of transcription of a DNA sequence. Requires modifications before it can undergo translation p. 89

prehensile the ability to grasp objects p. 595

primary data results collected from experiments, interviews, or surveys undertaken by the researcher p. 17

primary immune response the reaction of the adaptive immune system to an antigen it has not previously been exposed to p. 422

primary lymphoid tissue components of the lymphatic system that are responsible for the production and maturation of lymphocytes. Includes bone marrow and the thymus p. 404

primary structure the first level of protein structure, which refers to the sequence of amino acids in a polypeptide chain p. 59

primates the highest order of mammals, comprised of about 400 different living species who share a number of features including opposable digits and binocular vision p. 594

primer a short, single strand of nucleic acids that acts as a starting point for polymerase enzymes to attach p. 165, 183

prion an abnormally folded protein in the nervous system of a mammal that induces other proteins to misfold. Causes a number of neurodegenerative diseases p. 357

product the transformed molecule created in a reaction p. 129

promoter the sequence of DNA to which RNA polymerase binds p. 82, 89, 102

prosthetic group a non-protein group bound to a protein. For example, a vitamin or ion p. 60

protein a biomacromolecule made of amino acid chains folded into a 3D shape p. 56

proteome all the proteins that are expressed by a cell or organism at a given time p. 57

protospacer a short sequence of DNA extracted from a bacteriophage by Cas1 and Cas2, which has yet to be incorporated into the CRISPR gene p. 172

protospacer adjacent motif (PAM) a sequence of two-six nucleotides that is found immediately next to the DNA targeted by Cas9 p. 173

protozoa a phylum of single-celled eukaryotes that can cause disease p. 357

pyruvate a three-carbon molecule that can be formed from the breakdown of glucose via glycolysis p. 301

Q

quaternary structure the level of protein structure where multiple polypeptide chains bond together, or other non-protein groups are added to form a fully functional protein p. 59

R

R-group the variable portion of an amino acid molecule. It can be one of twenty variations and determines the identity of the amino acid p. 58

radioactive isotope a radioactive atom of a specific element. This atom breaks down into a predictable and stable product. Also known as a radioisotope p. 546

radiocarbon dating a form of absolute dating used to determine the age of a fossil by measuring the properties of radiocarbon, a radioactive isotope of carbon. Also known as carbon dating and radioactive carbon dating p. 547

random coil an irregular secondary structure of proteins that is neither an alpha helix nor a beta-pleated sheet p. 59

random error variation in results caused by uncontrollable conditions between replicates, resulting in a less precise spread of readings. Can be reduced using more replicates or refining the measurement process p. 11

rate the speed at which a chemical reaction proceeds p. 266

raw data results that have not been processed, manipulated, or formatted for use p. 17

re-emerging disease an infectious disease that was previously under control but that is now increasing in incidence p. 433

reactant a molecule that undergoes a transformation into a product. When enzymes are involved, the reactant is called a substrate p. 129

reading frame the order in which nucleotide triplets or codons are divided into a consecutive, non-overlapping sequence p. 476

receptor protein a protein within or on the surface of a cell that binds with signalling molecules, leading to a change in cellular activity p. 57

recessive allele a trait that can be masked by a dominant allele on a homologous chromosome p. 517

recognition site a specific target sequence of DNA upon which restriction endonucleases act p. 163

recombinant plasmid a circular DNA vector that is ligated to incorporate a gene of interest p. 204

regulatory gene a segment of DNA responsible for producing proteins that control the expression of other genes p. 102

relative age the age of a fossil as determined by relative dating techniques. Describes the age of a fossil compared to other fossils, instead of a fossil's exact age in years p. 544

relative dating a dating technique used to determine the relative age of a fossil by comparing its position to other fossils or rock in surrounding rock strata (layers) p. 544

reliable describes an experiment, tool, or measurement that produces similar results when repeated and reproduced p. 3

renewable refers to a resource that can typically be replenished at the same (or faster) rate than it is being used, meaning it is unlikely to run out p. 332

repeatable an experiment/measurement in which scientists, using the methods they designed, can obtain the same result multiple times p. 8

replicates multiple measurements that are exposed to the same level of the IV, are very close in value, and are close to the 'true' value of the quantity being measured p. 11

replication the process of running your test/experiment multiple times p. 10

reporter gene gene with an easily identifiable phenotype that can be used to identify whether a plasmid has taken up the gene of interest p. 205

representitive a sample that accurately reflects the characteristics of the larger population p. 12

repressor protein a protein coded for by a regulatory gene that prevents gene expression by binding to its operator p. 82, 102

reproducible an experiment/measurement in which a group of scientists, using methods designed by others, can obtain the same results as another group's experiment p. 8

research question a testable, achievable, and specific question that an investigation sets out to answer p. 4

reservoir a population of animals or environment in which a pathogen normally lives p. 433

respect an ethical concept that encourages the acknowledgment of the intrinsic value of living things, and considers the welfare, beliefs, customs, and cultural heritage of both the individual and the collective p. 38

respiratory droplets droplets (traditionally >5 ×m) produced by breathing, talking, vomiting, and coughing. They may contain saliva, mucus, and other substances from the respiratory tract, including cells/particles of pathogens p. 445

restriction endonuclease any enzyme that acts like molecular scissors to cut nucleic acid strands at specific recognition sites. Also known as a restriction enzyme p. 163, 183, 204

reverse primer a DNA primer that binds to the 3' end of the coding strand and reads the DNA in the reverse direction to RNA polymerase p. 184

Rhesus antigen an antigen on the surface of red blood cells that can cause an immune response if not matched correctly between donor and receiver p. 393

ribosomal RNA (rRNA) RNA that is a key structural component of ribosomes, which assemble proteins p. 70, 89

ribosome an organelle made of rRNA and protein that is the site of protein synthesis. Can be free in the cytosol or attached to the rough endoplasmic reticulum p. 89, 109

RNA (ribonucleic acid) a single-stranded nucleic acid chain made up of nucleotides. Includes mRNA, rRNA, and tRNA p. 68

RNA polymerase the enzyme responsible for constructing a pre-mRNA sequence from a DNA sequence during transcription p. 82, 89

root represents the most recent common ancestor for all members of a phylogenetic tree p. 569

rough endoplasmic reticulum (RER) a membranous organelle shaped like a series of connected, flattened cylinders that folds and transports proteins via its attached ribosomes p. 110

Rubisco a pivotal enzyme involved in initial carbon fixation during the light-independent stage of photosynthesis p. 254, 271, 281

S

Sagittal crest a ridge of hard bone running lengthwise (front to back) along the top of the skull. A pronounced sagittal crest indicates strong jaw muscles p. 597, 607

sample a subset of the larger population being studied p. 12

saturation point the point at which a substance (e.g. an enzyme) cannot receive more of another substance (e.g. a substrate) p. 139, 266, 323

second line of defence a component of the innate immune system characterised by the non-specific and immediate response to injury and pathogens by a variety of cells and molecules p. 367, 375

second-generation biofuels biofuels produced from non-edible crops such as agricultural and forestry residues and municipal waste. These typically compete less with agricultural land p. 335

secondary data results from sources other than the researcher's own investigations p. 17

secondary immune response the heightened reaction of the adaptive immune system to an antigen it has previously been exposed to p. 422

secondary lymphoid tissue components of the lymphatic system that are responsible for the maintenance of mature lymphocytes and the activation of the adaptive immune response. Includes lymph nodes and the spleen p. 404

secondary structure the level of protein structure where the amino acid chain forms either alpha-helices, beta-pleated sheets, or random coils p. 59

secretory products the substances inside a vesicle that are being transported out of the cell p. 110

sediment naturally occurring solid material, such as earth and rock, that is broken down into very fine pieces and typically settles at the bottom of liquid p. 542

sedimentary rock rock that has formed through the accumulation of sediment that hardens under pressure p. 542

selective advantage an organism conferred a beneficial allele, which increases its chances of survival against a specific environmental selection pressure p. 484

selective breeding the changing of a population's gene pool due to humans altering the breeding behaviour of animals and plants to develop a selected trait. Also known as artificial selection p. 515

serology the study of blood serum, typically to determine the presence of antibodies and/or antigens p. 443

serum the fluid and solute component of blood that excludes blood cells, platelets, and clotting factors p. 443

short tandem repeats (STRs) short, repeated sequences of nucleotides found in the non-coding regions of nuclear DNA p. 193

silenced describes a gene that is prevented from being expressed p. 217

silent mutation a mutation in which a nucleotide is substituted for another, changing the codon, but still coding for the same aminoacid. Therefore, there is no effect on protein structure p. 476

somatic cell any cell in an organism that is not a germline cell p. 476

spacer short sequences of DNA obtained from invading bacteriophages that are added into the CRISPR sequence p. 172

species a group of individuals who are able to breed with each other and produce viable and fertile offspring p. 505

spleen an organ located in the upper abdomen that serves a variety of functions in the immune system and the regulation of red blood cells p. 404

spliceosome the enzyme that removes introns from the pre-mRNA molecule and joins exons together during RNA processing p. 91

splicing process where introns are cut out of a pre-mRNA molecule, and exons are joined together p. 90

standard ladder a mixture of DNA fragments of known length that are used to infer the size of fragments in a sample p. 189

start codon the sequence of three nucleotides in mRNA that signals the start of translation p. 80, 92

sterile surgically clean and free from contamination by microorganisms. Also known as aseptic p. 16

sticky end the result of a staggered cut through double-stranded DNA by an endonuclease resulting in overhanging nucleotides p. 163

stoma (pl. stomata) a small pore on the leaf's surface that opens and closes to regulate gas exchange p. 270, 241, 256, 367

stop codon the sequence of three nucleotides in mRNA that signals the end of translation p. 80, 92

storage protein a protein that is a reserve of amino acids and metal ions p. 57

stratum (pl. strata) a layer of sedimentary rock p. 544

stroma the fluid substance that makes up the interior of chloroplasts. It is the site of the light-independent stage of photosynthesis p. 244

structural gene a segment of DNA that doesn't code for regulatory proteins, but instead codes for proteins that play a role in the structure or function of a cell or organism p. 102

structural morphology the study of physical structures to establish relatedness p. 557

structural protein a type of protein that confers strength and shape to cells p. 57

substrate the reactant of a reaction catalysed by an enzyme p. 129, 255

sugar-phosphate backbone a strong covalently linked chain of five-carbon sugar molecules and phosphate groups in a nucleic acid chain p. 68

sympatric speciation the divergence of a species from an original species without the presence of a geographical barrier p. 505

systematic error errors which cause results to differ by a consistent amount each time, typically due to faulty equipment or calibration, resulting in a less accurate result. Can be reduced by calibrating and maintaining instruments p. 15

T

T helper cell (Th) a type of differentiated T lymphocyte that supports the functioning of a number of different immune cells, including the cloning and differentiation of selected T and B cells p. 390

T lymphocyte a type of lymphocyte that plays an important role in cell-mediated immunity. It differentiates into cytotoxic T cells, T memory cells, and T helper cells p. 389

T memory cell a differentiated T lymphocyte that is responsible for providing long-lasting immunological memory p. 395

***Taq* polymerase** a heat-resistant DNA polymerase enzyme isolated from the bacteria Thermus aquaticus, which amplifies a single-stranded DNA molecule by attaching complementary nucleotides p. 183

TATA box a type of promoter region p. 82

taxon (pl. taxa) a unit of biological classification into which related organisms are classified. Taxa are arranged in a hierarchical rank from kingdom down to species, where members of a specific taxon typically share certain morphological characteristics p. 568, 592

template strand the strand of DNA transcribed by RNA polymerase to produce a complementary pre-mRNA strand p. 89

termination sequence a sequence of DNA that signals the end of transcription p. 82, 89

tertiary structure the functional 3D shape of a polypeptide chain p. 59

thermal cycler a laboratory apparatus which alters the temperature in pre-programmed steps for temperature-sensitive reactions like PCR p. 183

third line of defence a subset of the immune system within vertebrates that is composed of the humoral and cell-mediated responses which create a specific immune response and form immunological memory. Also known as the adaptive immune system or specific immune response p. 389

thylakoid a flattened sac-like structure housed inside the chloroplast. Each thylakoid is made up of a chlorophyll-containing membrane enclosing a lumen. Thylakoids are the location of the light-dependent stage of photosynthesis p. 242

thymus a primary lymphoid organ located in the chest. Serves as the site of T cell maturation p. 404

tonsils the name given to the two lymph nodes that reside at the back of the throat p. 404

totem emblems or symbols that represent the spiritual connection (Dreaming) between Aboriginal people and Country. Totems can take a range of different forms, such as animals, plants, and landscapes p. 632

totemic relationships shared kinship between specific totems and the family, clan, individual and/or language group p. 632

trace fossil fossil or structure indicating the presence of organisms, rather than the organisms themselves (e.g. nests, footprints, and burrows) p. 543

transcription the process whereby a sequence of DNA is used as a template to produce a complementary sequence of mRNA p. 79, 89

transcription factor proteins that bind to the promoter region and control the functioning of RNA polymerase p. 89

transfer RNA (tRNA) RNA that recognises specific codons on the mRNA strand and adds the corresponding amino acid to the polypeptide chain during protein synthesis p. 70, 89

transformed data results that have been converted from their raw format into a more visually comprehensible format that is easier to analyse p. 17

transgene a gene that has been artificially introduced into the genome of a separate organism (usually of another species) p. 219

transgenic organism a genetically modified organism that contains foreign genetic material from a separate species (or recombinant DNA from the same species that has been manipulated before introduction) p. 217

transitional fossil a fossil that shows traits that are common to both its ancestral group and its descendant group. They are particularly important when the descendant species is physically very distinct from the ancestral species, such that the transitional fossil can help demonstrate evolutionary changes between the two p. 546, 616

translation the process where an mRNA sequence is read to produce a corresponding amino acid sequence to build a polypeptide p. 79, 89

transmission the passing of a pathogen from an infected host to another individual or group p. 444

transport protein a protein that moves substances across membranes or within organisms p. 57

trendline a line that shows the main pattern followed by a set of points on a graph. Also known as a line of best fit p. 20

trichomes small hairs on the surface of plants used to deter pathogens and/or insects p. 367

triplet the sequence of three nucleotides in DNA coding for one amino acid p. 80

true value the value that would be obtained by a perfect measurement without the influence of errors p. 11

U

unbiased a sample or measurement that is unaffected by a scientist's expectations p. 12

uncertainty a quantification of the error associated with a measurement, often represented by the symbol '±' after a reading p. 15

uncontrolled variable a factor that is not kept constant or accounted for throughout the experiment. Also known as an extraneous variable p. 5

unrepresentative sample a small selection of individuals from a larger group that does not reflect the characteristics of the larger group p. 496

V

vaccination program a series of vaccinations designed to create long-term immunity to a disease. Also known as a vaccination schedule p. 422

vaccine a medical treatment typically containing antigens designed to stimulate a person's adaptive immune system to create immunity to a pathogen without actually causing disease p. 422

valid a measurement or experiment that actually tests what it claims to be testing p. 8

vasodilation the widening of blood vessels p. 380

vector an organism that is not affected by a disease but spreads it between hosts p. 204, 445

vertical transmission spread of pathogens from mother-to-child during gestation, during childbirth, or post-birth due to close physical contact and breastfeeding of a newborn p. 445

vesicle a small fluid-filled organelle enclosed in a phospholipid membrane that transports substances around the cell p. 110

vestigial structures features that have lost all or most of their usefulness as a result of evolution by natural selection p. 559

viable able to survive p. 506

viral recombination the combination of surface antigens from two or more different strains of a virus to form a completely new virus subtype p. 525

virtues-based approach an approach to bioethics that emphasises the individual goodness of the agent, and promotes acting in accordance with the values of a 'moral' person, such as honesty and compassion p. 36

virulence the potential of a pathogen or disease to cause serious illness or harm p. 433, 442, 524

virus a non-cellular, infectious agent composed of genetic material enclosed in a protein coat that requires a host cell to multiply p. 171, 357

W

well an indent in the gel into which a DNA sample is loaded p. 189

worm an invertebrate belonging to phyla including Nematoda and Platyhelminthes that can cause disease in its host by acting as a parasite p. 357

X

xylem vascular tissue in plants responsible for transporting water and minerals from the roots to the leaves p. 241

Y

yeast unicellular eukaryotic organisms from the kingdom Fungi p. 314

yield the amount of agricultural product harvested per area of land p. 281

Z

zoonosis an infectious disease that is caused by a pathogen that has transferred from an animal to a human p. 433

zygote the diploid cell formed by the combination of two haploid gamete cells p. 175

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