Solutions Book 2026

HKDSE Biology: Electives

Paper 2 · 2012–2025 · Sample and Practice Papers

Understand the ideas through examples, then apply them to complete examination questions.

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Chapter 1

From understanding to independent answers

Guide Using the student and solutions books

This book introduces concepts through concrete examples and diagrams, then develops recognisable question types through complete worked examples and focused practice. Read the relevant concept pages first, follow the starting point, reasoning and answer of each example, then attempt the exercises independently. The Student Book contains all concept explanations and complete worked examples. The Solutions Book adds answers and explanations for every exercise in exactly the same order.

The four elective areas are Human Physiology: Regulation and Control, Applied Ecology, Microorganisms and Humans, and Biotechnology. All Paper 2 questions are included. A whole Paper 2 question often spans several concepts. Each area begins with complete examples and continues with exercises of related types; use the areas that you study. The sources are the 2012–2025 papers, Sample Paper and Practice Paper: 64 whole questions. Different languages, scans and subparts of the same question are not counted as additional questions. The compulsory and elective books contain 817 whole questions in total. Stars describe the combination of knowledge and reasoning required by a whole question; they are not official HKEAA difficulty or frequency ratings.

Questions, explanations, captions and labels in this edition are presented in English.

'Describe' asks you to state observed changes; 'explain' requires the mechanism; 'according to the information' limits your conclusion to what the supplied evidence supports. Each question lists its year, paper and original number. Solutions also identify the physical source-page numbers of the marking references used.

Source	Whole questions
2012	4
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This English edition uses the original English question papers for 2012–2024, the Sample Paper and the Practice Paper. The 2025 questions are English translations of the Chinese papers supplied with this collection; their diagrams, numerical data, subparts and mark allocations are retained.

Chapter 2

Graphs, calculations and scientific investigations

Inquiry From observations to evidence-based conclusions

Key ideas

① Bubbles appear when fresh liver is placed in hydrogen peroxide: this is an observation. A glowing splint relights in the gas: this is another observation. Concluding that the gas is oxygen connects the test result with the fact that oxygen supports combustion, so it is an inference.

② Descriptions and explanations answer different questions. A description states what was seen or measured; an explanation states why it happened. "The cell contents have pulled away from the cell wall" describes a visible change. "Water moved out of the cell down a water potential gradient" explains it. When asked to describe, giving only a cause does not answer the question.

③ The independent variable is the condition deliberately changed. The dependent variable is the measured result. Controlled variables are other conditions kept as similar as possible for a fair comparison. When comparing full-fat and semi-skimmed milk, milk type is the independent variable and indicator colour is the observed result. The milk volume, lipase volume and temperature should be the same in both tubes.

④ A control does not always mean "leave out the enzyme". The comparison must isolate the factor being tested. To test whether hydrogen peroxide is the substrate, compare fresh tissue in hydrogen peroxide with the same fresh tissue in water. To test the effect of heating the enzyme, compare fresh and boiled tissue in the same substrate solution.

⑤ Conclusions must stay within the evidence. Testing four tissues cannot establish the enzyme content of all animals and plants. Different final colours do not, on their own, reveal initial reaction rates. When judging statements about a graph, first identify what was actually measured.

A water absorbent above a leaf increases from 2.40 g to 2.49 g; one below increases from 2.43 g to 2.56 g. They absorb 0.09 g and 0.13 g respectively. Subtract each starting value before comparing changes. Subtracting only the final masses gives 0.07 g, which is not the difference in water loss from the two surfaces.

Check your understanding

1. Seeing a glowing splint relight is an _____ identifying the gas as oxygen is an _____.
2. In the comparison of two types of milk, the independent variable is _____, not the final colour.

Check answers:

1. Observation; inference. 2. Type of milk.

How to recognise the problem For "describe", "explain", "according to the graph", "independent variable" or "control", decide whether the answer requires an observation, mechanism, evidence or experimental design.

Key ideas

① Suppose aquatic plants under two colours of light produce oxygen volumes differing by only 0.01 mL, while the measuring instrument has an uncertainty of about 0.03 mL. The difference is smaller than the uncertainty in an individual measurement. Different numerical readings alone do not establish that light colour affected photosynthesis. Repeat the measurements, compare the spread of results and consider measurement precision.

② If another comparison gives a difference of 1 mL, the same 0.03 mL uncertainty is much smaller relative to that difference. This still does not constitute a formal statistical test. Repeat results, variation between samples and experimental design must also be considered.

③ Evaluate an error by asking whether it could affect this conclusion. When investigating water temperature and a fish's breathing frequency, warming may both increase metabolism and reduce dissolved oxygen. Measuring only temperature and breathing frequency leaves dissolved oxygen as an alternative explanation. Measure or control it to separate the two mechanisms.

④ A biotechnology investigation also begins with a question. For parentage testing with DNA fingerprints, establish which bands must have come from the parents, then explain how restriction digestion, electrophoresis and comparison produce interpretable evidence. PCR and electrophoresis are procedures used to obtain or analyse a sample, not themselves the dependent variable.

Check your understanding

1. Two groups differ by 0.01 mL and the instrument uncertainty is about 0.03 mL. Do the unequal numbers alone establish an effect?
2. A fish breathes faster after the water is warmed. Besides increased metabolism, what possible explanation should be checked?

Check answers:

1. No. The difference is smaller than the measurement uncertainty; consider repeats, variation and measurement error. 2. Dissolved oxygen may have decreased, stimulating faster breathing. Measure or control dissolved oxygen.

Chapter 3

Human physiology: regulation and control

Concept V.a Osmoregulation: from blood to urine

Key ideas

① Exercising on a hot afternoon causes water loss in sweat. If that water is not replaced, solutes become relatively more concentrated in the blood and blood water potential falls. The body needs to reduce further water loss; it cannot retrieve sweat that has already left. The kidneys can retain more water and reduce urine volume. Regulating the body's water content to maintain relatively stable conditions around cells is called osmoregulation.

② Water content must be regulated because water can cross cell membranes. If extracellular fluid has excessively high water potential, net water entry can make cells swell. If its water potential is too low, net water loss can make them shrink. Either extreme can impair normal cell activities. Homeostasis maintains a suitable range, not an absolutely identical value every second.

③ Urine forms in the kidneys, passes through the ureters to the urinary bladder for temporary storage, and leaves through the urethra. Blood enters through the renal artery and leaves through the renal vein. Urine and blood follow different routes: urine does not travel to the bladder through the renal vein.

④ The nephron is the basic urine-forming unit. At its beginning, a capillary knot called the glomerulus is enclosed by Bowman's capsule; the renal tubule follows and leads into a collecting duct. Blood pressure in the glomerulus forces some plasma water and small molecules into Bowman's capsule. This is ultrafiltration, and the resulting fluid is glomerular filtrate. Water, urea, glucose and some inorganic ions enter the filtrate, while blood cells and most plasma proteins remain in the blood. Filtration depends on the permeability of the filtration barrier, not on whether a substance is 'useful' or 'waste'. Glucose in normal filtrate is therefore not a kidney error.

⑤ As filtrate passes along the renal tubule, substances still required by the body return from the tubular lumen to the blood. This is reabsorption. Normally, almost all filtered glucose is reabsorbed, together with much of the water and appropriate amounts of inorganic ions. Transport proteins contribute to selective solute reabsorption; some processes require energy from cellular metabolism. Water moves by osmosis down a water-potential gradient. When explaining adaptations of tubular epithelial cells, state both the structural feature and the direction of transport it supports.

⑥ Comparing filtrate with final urine separates the two stages. Ultrafiltration first removes water and many small molecules from the blood; reabsorption retrieves substances that are still needed. Final urine contains water, urea and excess ions. Urea arises from the metabolism of nitrogen-containing substances, and its removal is excretion. Removal of undigested food through the anus is egestion, a different process.

⑦ When water loss during exercise lowers blood water potential, receptors in the hypothalamus initiate regulation. Antidiuretic hormone (ADH) is made in the hypothalamus and released into the blood through the posterior pituitary. More ADH increases water permeability in regions including the collecting ducts. Given the existing water-potential gradient between the tubular fluid and surrounding tissue fluid, more water is reabsorbed into the blood. Urine volume decreases and urine is usually more concentrated; blood water potential gradually rises. As the original deviation is reduced, the stimulus for ADH release weakens. This is negative feedback.

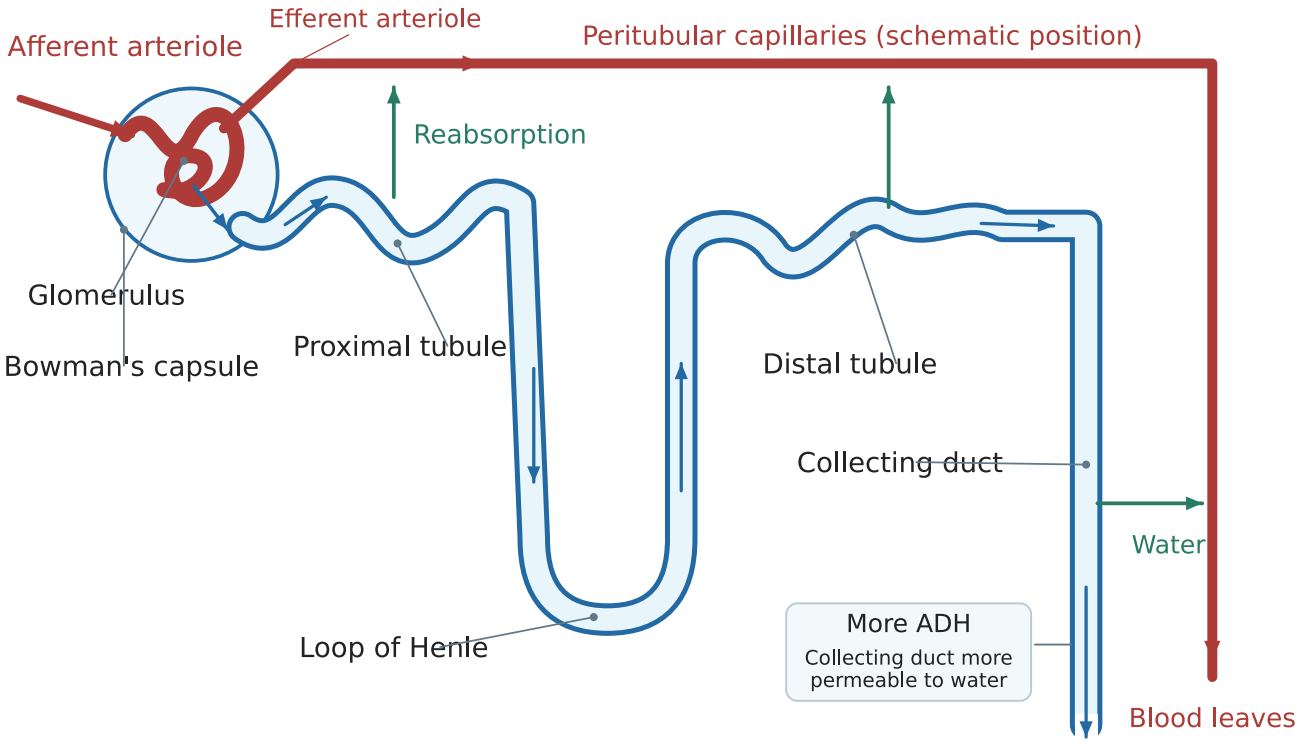
⑧ After drinking more water, other conditions being similar, blood water potential may rise. ADH release falls, collecting ducts become less permeable to water, and less water is reabsorbed. A larger volume of more dilute urine is produced.

This response is opposite to that following water loss. ADH does not make the kidneys manufacture more water, and it must not be confused with glucagon, which raises blood glucose.

⑨ Dialysis uses an artificial membrane for exchange between blood and dialysis fluid. Small molecules such as urea can cross, while blood cells and most plasma proteins are retained. A low urea concentration in dialysis fluid allows net diffusion of urea out of the blood; replacing the fluid maintains the concentration difference. Glucose and relevant ion concentrations are set appropriately to avoid excessive removal of substances normally required. A pressure difference across the membrane can also remove excess water. Dialysis helps maintain the internal environment, but an artificial membrane does not perform every reabsorptive and secretory function of a complete nephron.

Suppose filtrate in a nephron contains 100 relative units of water over a certain period. If 98 units are reabsorbed, 2 remain in urine. If increased ADH raises reabsorption to 99 units, only 1 unit remains. This comparison explains why greater reabsorption reduces urine volume; it does not assume that total glomerular filtration is identical under all conditions.

The nephron: distinguish blood, filtrate and the direction of reabsorption



Process	Direction	Main result
Ultrafiltration	Glomerular blood → Bowman's capsule	Water and many small molecules enter the filtrate
Reabsorption	Tubule / collecting-duct lumen → blood	Useful substances and required water return to the blood

The nephron and capillary positions are simplified. Cells and most plasma proteins normally remain in the blood.

Blue arrows follow filtrate within the tubule; the red pathway represents blood. Ultrafiltration moves substances from blood into Bowman's capsule, while reabsorption returns them from the lumen to blood. Do not reverse these directions.

Check your understanding

1. Movement of water and glucose from the glomerulus into Bowman's capsule is _____ return of glucose from the renal tubule to blood is _____.
2. After water loss, blood water potential usually _____, ADH release _____, and urine volume _____.
3. Urine is stored temporarily in the _____ and leaves the body through the _____.

Check answers:

1. Ultrafiltration; reabsorption. 2. Falls; increases; decreases. 3. Urinary bladder; urethra.

How to recognise the problem Separate ultrafiltration from reabsorption when comparing blood, filtrate and urine. For water loss, drinking or ADH graphs, trace blood water potential, ADH, water reabsorption and urine changes in order.

Concept V.b Regulation of body temperature

Key ideas

① After exercise, you sweat and often feel cooler when air moves over your skin. Much of the cooling comes from evaporation. Sweat droplets remaining on the skin have not necessarily removed the same amount of heat by evaporation. At the same air temperature, humid air can make evaporation more difficult and reduce its cooling effect.

② Body temperature affects enzyme-catalysed reactions and cell activities, so heat production and heat loss must be balanced. Temperature receptors in the skin and within the body supply information to regulatory centres. The hypothalamus helps coordinate changes in blood vessels, sweat glands, muscles and other effectors.

③ When the body is too hot, skin arterioles dilate, allowing more blood through capillaries near the surface and bringing more heat to the surface for loss. Sweat secretion increases, and evaporation removes heat. Do not describe capillaries as actively constricting or dilating in place of the arterioles, which contain smooth muscle. Also distinguish vasodilation from increased heat production.

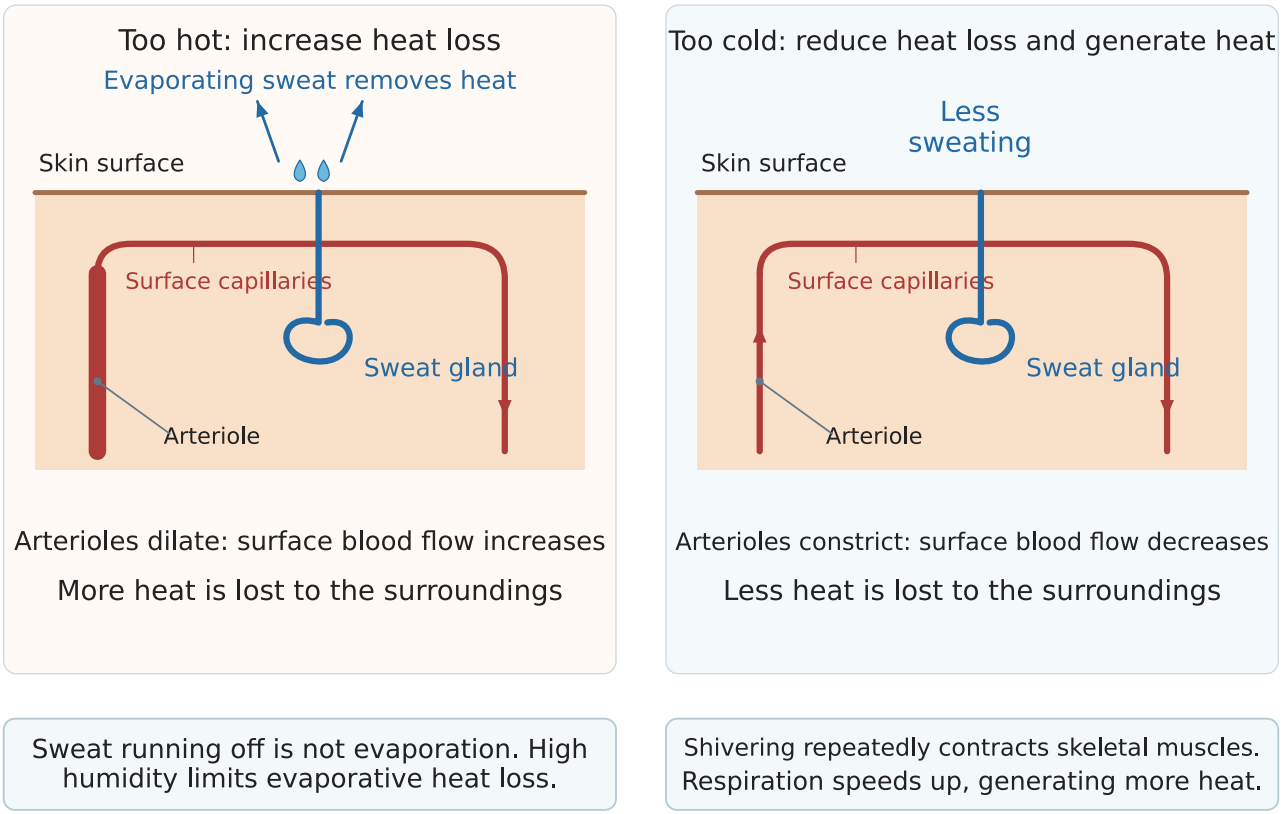
④ In cold conditions, skin arterioles constrict, reducing blood flow near the surface and heat loss. Shivering increases skeletal-muscle metabolism and heat production. Hormones such as thyroxine can increase metabolic rate and contribute to longer-term regulation. Behaviour, including adding clothes, seeking shade or changing posture, also affects heat exchange. Clothing does not actively produce body heat.

⑤ When comparing body-temperature curves, first describe changes over the same interval, then explain them through heat production and loss. Higher body temperature can stimulate greater sweating, while lower air humidity can allow the same amount of sweat to evaporate more readily. Sweat production and evaporation efficiency are distinct variables.

Follow each deviation and response separately

Component	Typical response to overheating	Typical response to cold
Skin arterioles	Dilate; more blood flows near the surface	Constrict; less blood flows near the surface
Sweat glands	Increase secretion; evaporation removes heat	Usually reduce secretion
Shivering	Usually not used to increase heat production	Raises metabolic heat production
Result	Greater heat loss counteracts overheating	Less heat loss and more heat production counteract cooling

Thermoregulation: heat loss and heat production are separate processes



Vessels are simplified. Arterioles change diameter to regulate flow; capillaries do not actively constrict to do this.

Arterioles regulate blood flow through surface capillaries; evaporating sweat removes heat. Vascular changes alter heat loss, whereas shivering increases heat production.

Check your understanding

1. The change of sweat from liquid to vapour, absorbing heat, is _____.
2. In cold conditions, skin arterioles usually _____, reducing heat loss.

Check answers:

1. Evaporation. 2. Constrict.

How to recognise the problem Decide whether body temperature is too high or too low, then follow heat production and heat loss separately. Describe measured changes before inferring regulatory mechanisms.

Concept V.c Regulation of blood gas levels

Key ideas

① After you climb stairs quickly, respiration in leg cells increases, using more oxygen and producing more carbon dioxide. Breathing becomes deeper and faster, while cardiac activity changes to coordinate exchange among lungs, blood and active tissues. Between ‘exercise’ and ‘increased heart rate’ lie metabolic demands and regulatory signals that need to be explained.

② Increasing blood carbon dioxide affects acid–base conditions. Chemoreceptors detect the relevant changes, and the respiratory centre in the medulla changes the signals sent to respiratory muscles. Increased breathing rate and depth deliver more air to the exchange surfaces, supporting carbon dioxide removal and oxygen uptake. As the deviation is reduced, the stimulus weakens. Distinguish the control centre, outgoing nerves and respiratory muscles to explain the completed feedback loop.

③ Stroke volume is the volume pumped out by a ventricle in one contraction. Heart rate is the number of beats per minute. Cardiac output is the volume pumped per minute.

④ cardiac output = stroke volume × heart rate.

⑤ For a stroke volume of 70 cm^3 and a heart rate of 75 beats per minute, 70 cm^3 is pumped each time, 75 times per minute:

⑥ cardiac output = 70 × 75 = 5250 $cm^3\ min^{-1}$.

⑦ If stroke volume later rises to 80 cm^3 while heart rate falls to 60 beats per minute:

⑧ cardiac output = 80 × 60 = 4800 $cm^3\ min^{-1}$.

⑨ Although more blood is pumped per beat, less is pumped per minute. When the two factors change in opposite directions, compare their products rather than only one factor.

⑩ During the cardiac cycle, atria and ventricles contract and relax in sequence. Valves open or close according to pressure differences, maintaining mainly one-way flow. Pacemaker activity in the sinoatrial node initiates the heartbeat: cardiac muscle generates its own rhythm. Nerves and hormones modify the rhythm and contractions. Sympathetic nerves generally increase heart rate and pumping activity, the vagus nerve can lower heart rate, and adrenaline can increase cardiac activity to meet the body's needs.

⑪ During exercise, more blood can be directed to active muscles, supplying oxygen and substrates and removing carbon dioxide. After vigorous exercise, breathing and heart rate may not immediately return to resting values. Recovery still requires oxygen to restore energy and oxygen reserves and process metabolic products. The syllabus term 'oxygen debt' describes this additional recovery requirement; do not attribute all oxygen consumption during recovery to a single reaction.

Check your understanding

1. With a stroke volume of 80 cm^3 and heart rate of 60 beats min^{-1}, cardiac output is _____.
2. Deeper and faster breathing increases carbon dioxide _____ and replenishes oxygen.

Check answers:

1. 4800 $cm^3\ min^{-1}$. 2. Removal.

How to recognise the problem On a ventricular-volume graph, take the difference across one contraction; on a cycle graph, measure a complete cycle. Explain regulation through metabolic demand, detected signals and effectors in that order.

Concept V.d Hormonal control of the reproductive cycle

Key ideas

① Place ovarian changes, uterine-lining thickness and hormone curves on the same menstrual-cycle timeline. Follicle development, ovulation and corpus-luteum formation are connected events. Hormones link these events with preparation of the uterus.

② The anterior pituitary secretes follicle-stimulating hormone (FSH), which promotes follicle development. Developing follicles secrete oestrogen, stimulating thickening of the uterine lining. During much of the cycle, oestrogen inhibits signals including FSH, limiting continued recruitment of more follicles. Before ovulation, however, a sustained high oestrogen level can produce positive feedback and an LH surge, which promotes ovulation. All hormonal effects throughout the cycle cannot be described as the same negative-feedback response.

③ After ovulation, the follicle becomes the corpus luteum, which secretes more progesterone and some oestrogen. These maintain a thick uterine lining suitable for implantation. Without signals sustaining pregnancy, the corpus luteum degenerates, hormone levels fall, and part of the uterine lining is shed as menstruation. Reduced inhibition allows FSH to rise again and initiate another round of follicle development.

④ When interpreting a graph, first locate ovulation and the LH surge, then align the follicular phase, luteal phase and uterine changes. Cycle length varies between individuals; day 14 on a schematic is not a universal ovulation date.

⑤ Hormonal contraception modifies normal regulation with supplied hormones. For example, suppression of FSH and LH changes can inhibit follicle maturation or ovulation. Some effects also involve cervical mucus and the uterine lining.

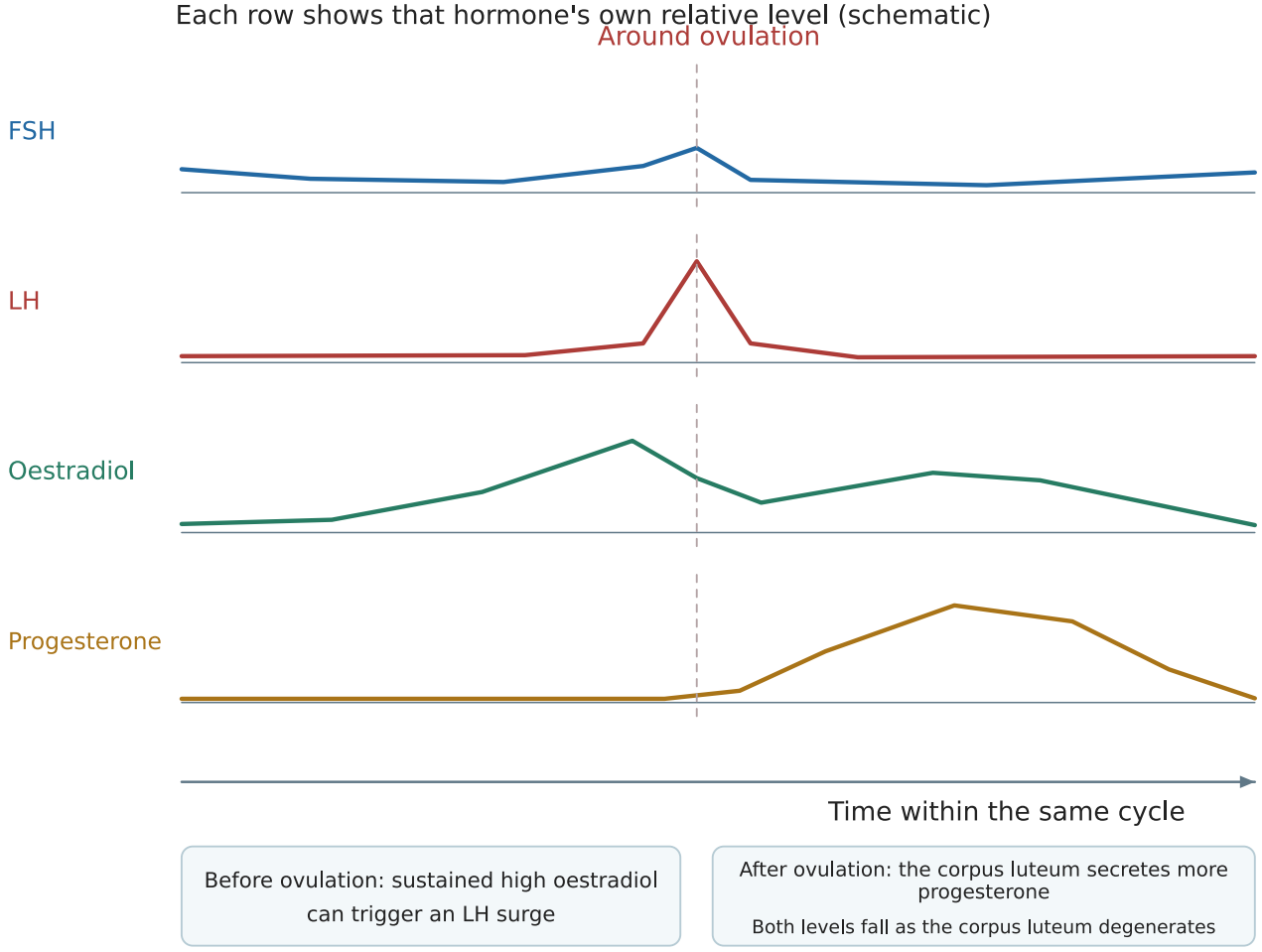
These treatments change the conditions governing reproductive steps; they do not simply kill any gametes already formed.

⑥ Some infertility involves impaired regulation of ovulation, and medical hormone treatment may stimulate follicle development and ovulation. For in vitro fertilisation, distinguish gamete collection, fertilisation outside the body, early embryo culture and embryo transfer. Hormone treatment supports relevant preparatory steps; the same method cannot resolve every cause of infertility.

Align ovarian, hormonal and uterine changes within one cycle

Stage	Ovarian event	Main hormonal relationship	Uterine lining
Menstruation and early follicular phase	A new group of follicles develops	Falling hormones from the previous cycle reduce inhibition of FSH	Part is shed; repair then begins
Before ovulation	The dominant follicle develops	Oestrogen rises; a sustained high level can trigger the LH surge	Thickens
Ovulation	An egg is released from the ovary	The LH surge promotes ovulation	Continues preparation for implantation
Luteal phase	Corpus luteum forms	Progesterone and some oestrogen maintain the lining	Remains thick
Cycle end without pregnancy	Corpus luteum degenerates	Progesterone and oestrogen fall	Hormonal support declines; the next menstruation begins

The menstrual cycle: compare peak timing, not concentrations between hormones



Each hormone curve shows its own relative changes; concentrations cannot be compared numerically between curves. Ovulation is schematic and does not occur on one fixed day for everyone.

Check your understanding

1. The most conspicuous gonadotrophin surge before ovulation is usually _____.
2. The structure that secretes more progesterone after ovulation is the _____.

Check answers:

1. LH / luteinising hormone. 2. Corpus luteum.

How to recognise the problem Put hormones, ovarian structures and the uterine lining on the same timeline. Determine stimulation or inhibition stage by stage; do not force the whole cycle into one feedback pattern.

Applied ecology

Chapter 4

Concept VI.a Human impacts on the environment

Key ideas

① A fish population reproducing every year does not mean that any catch can be sustained indefinitely. If removal repeatedly exceeds replenishment, especially when many immature fish are caught, the population can decline year after year. Fish are renewable resources, but their renewal rate is limited. Coal and oil cannot be replaced rapidly on the timescale of human use and are non-renewable resources.

② Population growth increases demand for food, fresh water, energy and land, and adds pressure to waste disposal. The impact depends on consumption per person, technology and resource distribution as well as population size. Evaluate population policies using environmental, resource and social factors together; one population total does not determine a unique outcome.

③ Fertilisers supply mineral nutrients and can improve crop yield, but excessive application followed by runoff can pollute water. Pesticides reduce pests but may also harm their natural enemies and other non-target organisms. Monoculture, overfishing and unsuitable land use can change species composition and habitats.

④ Urbanisation and industrialisation can reduce available habitats through vegetation clearance, reclamation and fragmentation. Pollutants affect air, water and health through different routes: inhaling air pollutants differs from ingesting pathogens in polluted water.

⑤ Some persistent toxic substances are readily absorbed but poorly eliminated, allowing them to accumulate within an individual. A predator consumes many contaminated prey, so concentrations may increase along a food chain: this is biomagnification. Not every pollutant behaves in this way. Examine its persistence, absorption and elimination.

Distinguish bioaccumulation from biomagnification

Comparison	Example	Reasoning
One individual over time	A fish's pollutant concentration rises with age	Intake and absorption exceed elimination and breakdown over time
Different trophic levels in one food chain	A predatory fish eats many contaminated smaller fish	A persistent substance that is poorly eliminated may reach higher concentrations at higher trophic levels

Check your understanding

1. Sustainable use of a renewable resource depends on comparing its rate of use with its rate of _____.

Check answers:

1. Replenishment / renewal.

How to recognise the problem Connect the human activity to resource use, habitat change or pollutants, then to survival, reproduction or health outcomes.

Concept VI.b Pollution control

Key ideas

① A reusable bottle can hold water many times. Avoiding disposable bottles from the outset reduces the waste requiring treatment. Reduction, reuse, recycling and substitution act at different stages. Recycling still requires collection, transport and processing, so compare actual resource use rather than a single word on packaging.

② Screens remove large solids from sewage, while sedimentation separates particles that settle readily. These physical steps do not automatically remove all dissolved organic matter. Biological treatment uses microorganisms to break down

organic pollutants, reducing the oxygen demand of material subsequently discharged. Aerobic treatment supplies oxygen so that the relevant microorganisms can continue their metabolism.

③ In systems such as activated sludge, microbial material can be separated by subsequent sedimentation. Some sludge is returned to maintain the microbial population; excess sludge requires separate treatment. Other treatments target nitrogen, phosphorus or pathogens. Sedimentation, microbial decomposition and disinfection are distinct processes.

④ A lower biochemical oxygen demand after treatment supports a reduction in the load of biodegradable organic matter. It does not directly count every pathogen or show that every chemical pollutant has disappeared. Select indicators appropriate to the pollutants of concern when evaluating effluent quality.

Different sewage-treatment stages remove different materials

Stage	Main function	What it does not establish alone
Screening	Retains larger solids	Removal of all dissolved substances
Sedimentation	Separates settleable particles	Absence of pathogens
Microbial treatment	Breaks down biodegradable organic matter and reduces oxygen demand	Removal of every chemical pollutant
Suitable disinfection	Reduces risks from relevant pathogens	Compliance with every drinking-water requirement

Check your understanding

1. Screens mainly remove large solids; dissolved organic matter can be biodegraded by _____.

Check answers:

1. Microorganisms.

How to recognise the problem Identify whether the problem involves solids, organic matter, nutrients or pathogens, then assess whether the treatment targets that problem.

Concept VI.c Conservation of species and habitats

Key ideas

① Conserving a wetland bird requires more than keeping a few birds alive. They need feeding sites, sufficient food, breeding space and safe stopping places during migration. If the environment no longer supplies these conditions, increasing individual numbers alone may not sustain the wild population.

② Biodiversity includes species diversity, genetic differences within species and the variety of ecosystems. Organisms participate in pollination, decomposition and nutrient cycling, and provide food, materials for medical research and culturally valued landscapes. Conservation can have ecological, economic, aesthetic and ethical reasons. State what each reason protects in practical terms.

③ In situ conservation maintains populations and their relationships in their original environment, for example in reserves, country parks, marine parks and wetlands. Ex situ conservation keeps organisms or genetic material in other managed settings, such as seed banks and suitable breeding programmes. Ex situ measures can reduce immediate extinction risk but cannot fully replace wild habitats and ecological interactions.

④ Habitat restoration must address the original causes of degradation, such as pollution, erosion or invasive species, and restore conditions suitable for native species. Planting many trees of one species does not by itself restore the original diversity and food web. Tree height, timber yield and species diversity are different criteria when comparing forest-management options.

⑤ Governments can establish protection and land-management measures, researchers provide monitoring evidence, and the public can reduce habitat damage and illegal wildlife consumption. Conservation also involves land-use and economic trade-offs. Use comparable evidence to explain costs and benefits rather than simply stating that environmental protection is important.

Check your understanding

1. Conservation in the original habitat is _____ conservation; a seed bank is an example of _____ conservation.

Check answers:

1. In situ; ex situ.

How to recognise the problem Identify the threat, conservation target and scale, then explain how the measure improves survival or reproduction, or retains genetic diversity.

Concept VI.d Global environmental issues

Key ideas

① Rain can wash fertiliser from farmland into a pond. Increased nitrogen and phosphorus promote algal growth, changing the light available to algae and other plants. After organisms die, microorganisms decompose their organic matter. Decomposer respiration consumes dissolved oxygen, potentially depriving fish and other animals of oxygen. An explanation of eutrophication should connect nutrient enrichment, algal growth, death and decomposition, and oxygen consumption. 'Algae use up all the oxygen' is incomplete.

② Global warming involves changes in the Earth's energy balance. Increased greenhouse gases such as carbon dioxide and methane absorb and re-emit some outgoing infrared radiation, altering heat loss and warming the climate system. Fossil-fuel combustion and some land-use changes increase greenhouse-gas concentrations. Vegetation and oceans also participate in carbon uptake and exchange. The greenhouse effect and ozone depletion are different processes.

③ Temperature changes can affect development rate, the number of reproductive cycles, distribution and species interactions. Within a suitable range, an insect that develops faster may complete more generations per year; temperatures beyond its suitable range may be harmful. When inferring ecological effects from temperature data, retain the species and conditions. Warming does not make every organism more abundant.

④ Some sulphur and nitrogen oxides form acidic substances in the atmosphere, increasing rainwater acidity. Acid deposition can alter water and soil pH, ion availability and survival. Its causes and main pathway differ from eutrophication. Emission controls and reductions in relevant fuel use act at the source.

⑤ Sustainable development considers present needs alongside the preservation of resources and environmental conditions for the future. Fisheries can retain breeding individuals, reduce overfishing and protect habitats so that use is compatible with population replenishment. Agriculture can improve fertiliser and pest management, conserve soil and use water efficiently. Compare yield, resource renewal, biodiversity and cost rather than selecting the greatest short-term yield alone.

⑥ Acid rain also changes the movement of soil ions. Some toxic metals dissolve more readily under acidic conditions, increasing roots' exposure to toxic ions. Useful mineral nutrients may be leached out with water. Both involve movement of substances, but one increases toxic exposure while the other reduces soil fertility.

Check your understanding

1. After eutrophication, decomposition of large amounts of organic matter can make dissolved oxygen _____.

Check answers:

1. Decrease.

How to recognise the problem Break an issue into its source, environmental variable, biological process and consequence. A management measure should act on a specific step in that causal sequence.

Practise global warming, acid rain and nutrient cycling in the later topic 'Pollution control and global environmental issues'. The 2015 Paper 2 Q2 example develops causal explanations of climate and aquatic changes. Practice Paper 2 Q2 applies the same approach to migratory-bird distribution and forest restoration. The coral heat-tolerance example appears under 'Conservation of species and habitats'.

Microorganisms and humans

Chapter 5

Concept VII.a Microbiology

Key ideas

① Baker's yeast consists of single cells, but each has a nucleus and yeast belongs to the fungi. Bacteria lack a membrane-bound nucleus, while some protists have different structures for movement and feeding. 'Microorganism' refers to small size; it does not place every member in one evolutionary group.

② Viruses lack a complete cellular structure. They infect living cells and use host materials and synthesis systems to replicate their genetic material, make viral components and assemble new viruses. Outside suitable host cells, they cannot multiply independently in nutrient solution as yeast can. Viral replication differs from bacterial binary fission.

③ Microbial growth depends on temperature, pH, carbon and nitrogen sources, water and oxygen availability. Requirements vary between species. Some require oxygen; others use alternative metabolic pathways without oxygen. Aerobic conditions are not optimal for every microorganism.

④ A closed culture with limited nutrients commonly shows lag, exponential, stationary and decline phases. During the lag phase, cells adapt and synthesise required materials without an obvious increase in number. In the exponential phase, numbers can increase by repeated multiplication. When resources become limiting and metabolic products accumulate, cell production and death may approximately balance. Viable numbers may subsequently fall. Interpret each phase using the actual measured variable.

⑤ Optical density measures turbidity, which often correlates with cell amount, but dead cells also scatter light. Direct counts may include dead cells; biomass measures the total mass of material; colony counts estimate viable units capable of forming colonies. A cluster of cells may produce one colony, so a colony-forming unit need not represent one cell.

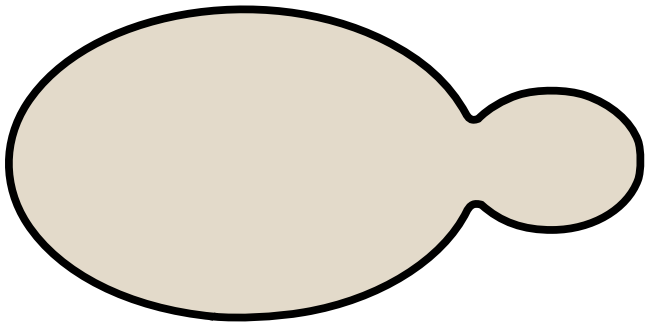
⑥ Suppose 0.10 mL of a sample diluted to one thousandth of its original concentration produces 40 countable colonies. The estimated original concentration is:

⑦ original concentration = $40 \div 0.10 \times 1000 = 400000$ CFU mL^{-1}.

⑧ This calculation corrects for plated volume and dilution. Reliable results also require suitable culture and measurement conditions. Aseptic technique aims to prevent contamination, protect the operator and prevent dispersal of material. It involves appropriate handling of equipment, limited exposure and proper disposal of used material.

Identify the measurement before interpreting a growth curve

Method	What is measured	Main limitation
Direct cell count	Observed number of cells	May include dead cells
Optical density	Turbidity / light scattering	Dead cells may affect readings
Biomass	Mass of material in the sample	Cell size may vary, so number and mass are not always proportional
Colony-forming units	Viable units forming colonies under the chosen conditions	A cluster may form only one colony

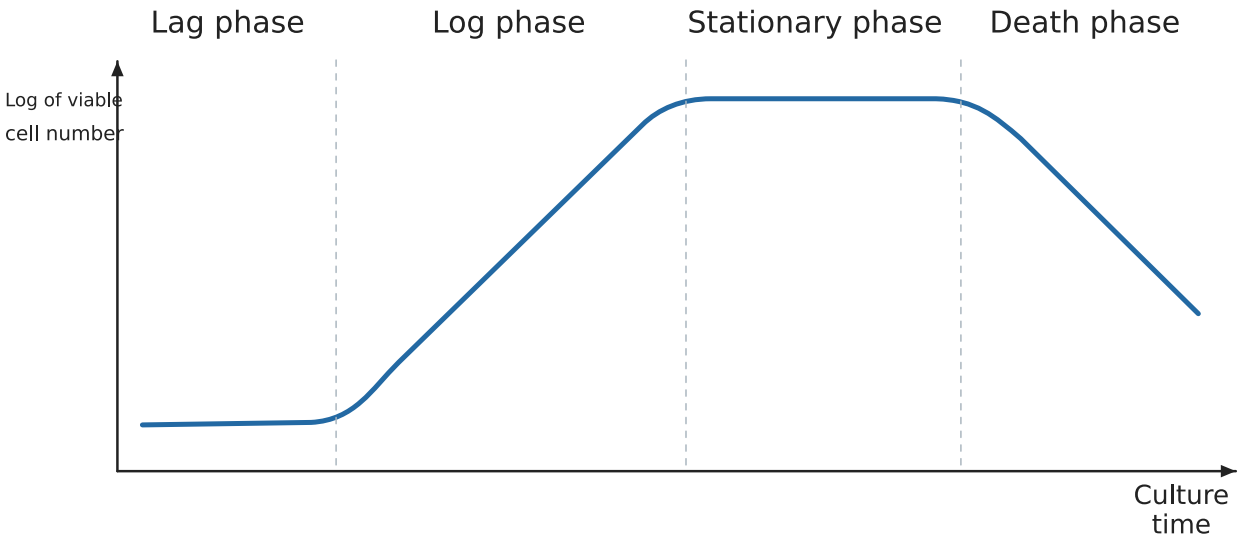


Budding yeast: a smaller bud develops from the parent cell. Yeast is a fungus with a nucleus; this outline does not show its internal structures.



A rod-shaped bacterium with flagella. Bacterial forms and appendages vary; this illustration does not imply that all bacteria have flagella.

Closed batch culture: four phases in the viable cell population



Phase	Explanation in terms of cell numbers and processes
Lag phase	Cells adapt and make needed substances; viable numbers show little increase.
Log phase	Favourable conditions allow an approximately constant proportional increase: a near-straight line on a log plot.
Stationary phase	New cells roughly balance deaths; this does not mean that all cells stop metabolising.
Death phase	Deaths exceed new cells; nutrient supply and metabolic wastes become limiting.

Total counts may include dead cells. Viable cell number and turbidity are not interchangeable in every growth phase.

The vertical axis is the logarithm of viable cell number; the horizontal axis is culture time. A stationary phase means an approximately stable net viable count, not that every cell has stopped metabolism.

Check your understanding

1. Optical density measures turbidity and cannot directly distinguish _____ cells from _____ cells.

Check answers:

1. Living; dead.

How to recognise the problem Identify the microbial group and measurement method before interpreting growth. For colony calculations, correct for sample volume, then dilution.

Concept VII.b Uses of microorganisms

Key ideas

① Yeast uses sugar in dough and produces carbon dioxide, which makes the dough rise. In some alcoholic drinks, the useful product is ethanol. Similar metabolism can therefore support different applications that retain different products.

Lactic acid bacteria convert sugars into organic acids, lowering milk pH and changing its proteins; this is used in some fermented dairy products.

② Industrial cultures must match product requirements with microbial growth conditions. Temperature and pH affect enzymes; oxygen supply influences metabolic pathways; nutrients and water support growth. Stirring helps mixing and heat transfer. The conditions favouring maximum cell multiplication are not necessarily those favouring maximum product formation at every stage. Check whether the measured variable is cell amount or product amount.

③ Some microorganisms produce antibiotics and industrial enzymes. Proteases, lipases and amylases in detergents act on different stains. Pectinase breaks down pectin in plant tissue, increasing juice release and improving clarification. Enzyme specificity and suitable conditions explain these uses; no enzyme works on every stain under every condition.

④ Vaccines use relevant pathogen antigens or antigens produced in a suitable system to stimulate immune responses. Vaccine production, antibiotic inhibition of bacteria and administration of ready-made antibodies have different mechanisms. Their links to microorganisms or disease prevention do not make them interchangeable.

⑤ Biological sewage treatment uses microorganisms to decompose organic matter. Biogas production uses microbial communities under anaerobic conditions to convert organic material step by step into products including methane-containing gas. The former mainly reduces pollution load; the latter can also recover some energy. Distinguish the conditions and final products of each system.

Related metabolism can serve different purposes

Context	Process used	Useful outcome
Bread dough	Yeast metabolises sugar	Carbon dioxide raises the dough
Some alcoholic drinks	Yeast metabolises sugar	Ethanol is retained
Juice extraction	Pectinase breaks down pectin	More juice is released and clarified
Biological sewage treatment	Microorganisms decompose organic matter	Lower oxygen demand after discharge
Biogas system	Anaerobic microbial conversion of organic matter	Methane-containing gas is obtained

Check your understanding

1. Pectinase acts mainly on _____, not on every food molecule.

Check answers:

1. Pectin.

How to recognise the problem Identify the microorganism or enzyme, the material transformed and the product obtained, then explain the necessary metabolic conditions.

Concept VII.c Microbial genetics

Key ideas

① Bacteria do not naturally make human insulin. Introducing suitable DNA that can be expressed in a host allows its protein-synthesis system to produce the desired substance. The key is usable genetic information, not injecting ready-made insulin and expecting bacteria to multiply it.

② Microorganisms reproduce quickly, and some are relatively easy to modify genetically and culture in large quantities. They can therefore serve as hosts for producing medicines or enzymes. The host must recognise the relevant regulatory signals, and the protein produced must have a suitable structure and function. DNA insertion does not guarantee correct expression.

③ Assess the benefits of modified microorganisms through yield, purity, cost and reliable supply. Possible concerns include survival outside the intended environment, transfer of genetic information, unwanted products and contamination. Production therefore requires appropriate containment, monitoring and purification. Link each concern to a biological mechanism rather than claiming that genetic modification is always dangerous.

Check your understanding

1. After a gene enters a host, successful _____ is still required to produce the desired protein.

Check answers:

1. Expression.

How to recognise the problem Check gene entry, expression, product usability and possible effects outside the production system separately.

Concept VII.d Harmful effects and control of microorganisms

Key ideas

① Food that turns sour, develops an odour or grows mould may be undergoing microbial decomposition and formation of new metabolic products. Microorganisms also spoil or degrade other materials. An unchanged appearance does not guarantee safety: some pathogens and toxins cause no readily noticeable change.

② Food-borne infection involves ingesting microorganisms that cause infection in the body. Some food poisoning mainly results from toxins already formed in food. Killing cells does not necessarily destroy existing toxins, because toxin stability varies. Reheating is therefore not a universal way to remove every food-related risk.

③ Preservation methods change different growth conditions. Low temperature usually slows enzyme activity and multiplication; drying reduces available water; concentrated sugar or salt lowers water potential; acidification changes pH. These methods need not kill every microorganism immediately. Heat treatment, disinfection and sterilisation have different purposes and ranges of effect; distinguish them by conditions and intended outcomes.

④ Contamination control also involves sources and transmission: separating untreated materials from ready-to-eat food, maintaining clean equipment and surroundings, and storing food appropriately. To evaluate control methods experimentally, compare the same organism and measurement. Slower growth, fewer viable cells and no detectable viable cells are different outcomes.

Ask which condition each method changes

Method	Principle	What does not follow automatically
Refrigeration	Slows relevant enzyme reactions and multiplication	All microorganisms die
Drying	Reduces available water	All toxins are destroyed
High sugar or salt concentration	Lowers external water potential and limits some microorganisms	No microorganism can grow
Suitable heat treatment	Damages relevant structures or molecules	Every level of heating achieves sterilisation

Check your understanding

1. Refrigeration usually _____ microbial multiplication; it does not kill every microorganism.

Check answers:

1. Slows.

How to recognise the problem Decide whether harm arises from infection, toxins or spoilage, then explain which condition for growth or survival the control method changes.

Biotechnology

Chapter 6

Concept VIII.a Modern biotechnology

Key ideas

① To make microorganisms produce human insulin, the relevant genetic information must be introduced into a usable expression system. Restriction enzymes cut DNA at specific sequences. A desired fragment and vector can have compatible ends, which DNA ligase joins to form recombinant DNA. Plasmids can act as vectors in some bacteria. After introduction into a host, successful transformants must be identified and expression of the desired gene confirmed.

② Choosing a restriction enzyme requires more than finding a name common to two diagrams. Locate the complete desired gene and regulatory region, then check that cutting does not disrupt required sequences, the ends are compatible, and the insertion does not damage essential functions. Eukaryotic genes may contain introns that some hosts cannot splice in the same way. Suitable coding sequences and regulatory elements are needed for correct production.

③ PCR amplifies a particular DNA region. Heating separates the strands, primers bind to complementary sequences, and a thermostable DNA polymerase extends from the primers. Repeated cycles increase the amount of target DNA. Primers determine the boundaries and specificity of amplification. An abundant DNA sample does not mean that arbitrary primers will amplify the target.

④ In an ideal model with doubling each cycle, one starting copy gives 1, 2, 4 and 8 copies over three cycles:

⑤ target copies = $1 \times 2 \times 2 \times 2 = 8$.

⑥ Actual efficiency and limited resources can cause departures from ideal doubling. To distinguish transgenic lines, primers can be chosen across specific insertion junctions or different flanking sequences, linking the product to the target's identity.

⑦ Gel electrophoresis moves DNA fragments in an electric field. DNA is usually negatively charged and moves towards the positive electrode. Under the same gel conditions, shorter fragments usually travel farther. Compare bands against the same reference and a size marker. A darker band does not directly mean a longer fragment.

⑧ DNA fingerprinting examines regions that vary between individuals. In parentage analysis, a child's relevant markers should be explainable by parental contributions. Identification compares actual samples across several markers. A few matching bands are not conclusive identification in every setting and do not establish identical whole genomes.

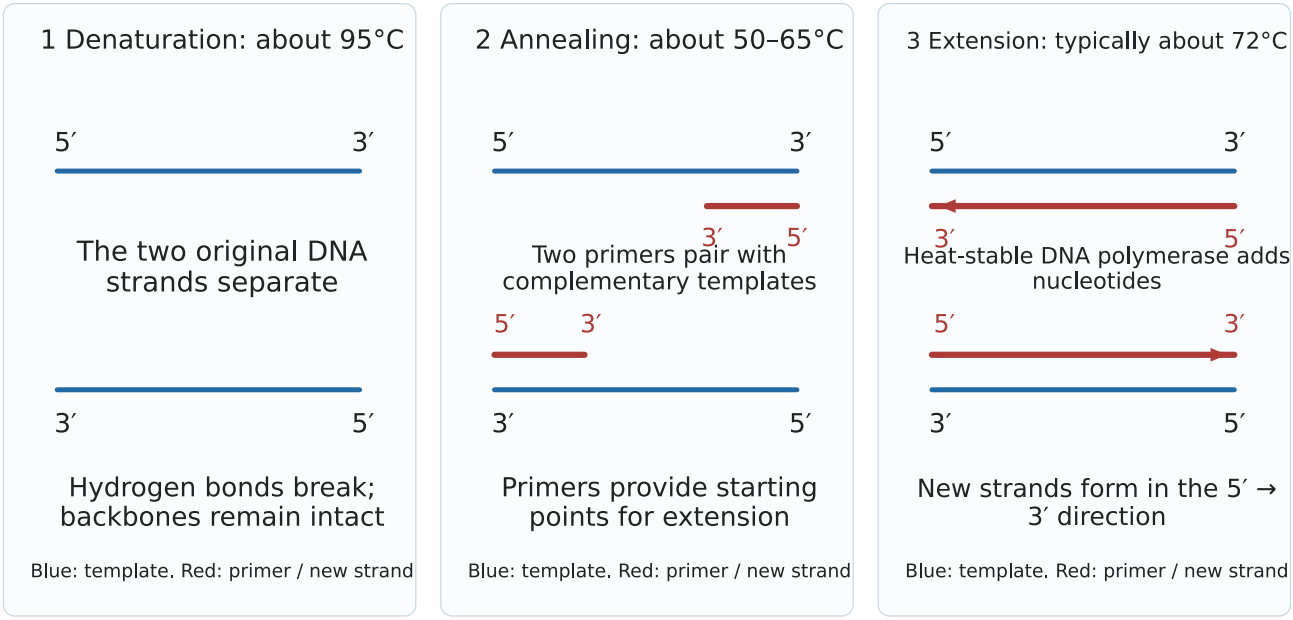
⑨ Somatic-cell nuclear transfer places a somatic-cell nucleus into an enucleated egg, stimulates development, and transfers a suitable early embryo into a surrogate. Nuclear DNA mainly comes from the nucleus donor; mitochondrial DNA mainly comes from the egg donor. The surrogate provides the developmental environment and does not become the nuclear-DNA donor simply by carrying the pregnancy. Development and environment can produce phenotypic differences even with very similar nuclear DNA.

⑩ Plant tissue culture uses the ability of some plant cells to regenerate a whole plant. Suitable tissue is grown under appropriate conditions that avoid contamination. Culture conditions are then adjusted to promote root and shoot formation, followed by growth into plants. This can rapidly propagate useful varieties, but contamination, genetic changes and shared vulnerability among many similar plants remain considerations.

Track the three sources in nuclear transfer

Source	Main contribution	What does not follow
Somatic nucleus donor	Nuclear DNA	Identical environments and phenotypes
Enucleated egg donor	Cytoplasm and most mitochondrial DNA	Retention of the original intact nucleus
Surrogate	Environment for embryonic and fetal development	Becoming the nuclear-DNA donor by carrying the pregnancy

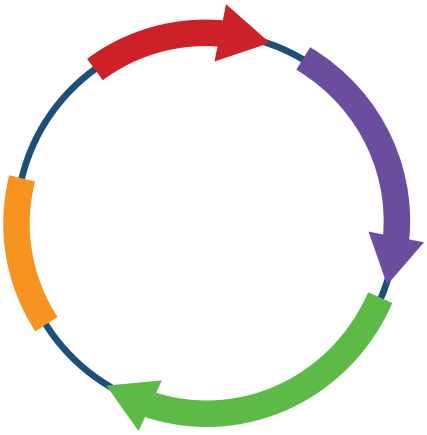
One PCR cycle: separate templates, anneal primers, extend from their 3′ ends



Repeat the three steps. Ideally, double-stranded DNA doubles each cycle: 1 → 2 → 4 → 8 copies.

Temperatures depend on the enzyme and primers. Arrows show extension direction; DNA polymerase cannot start synthesis at an arbitrary point.

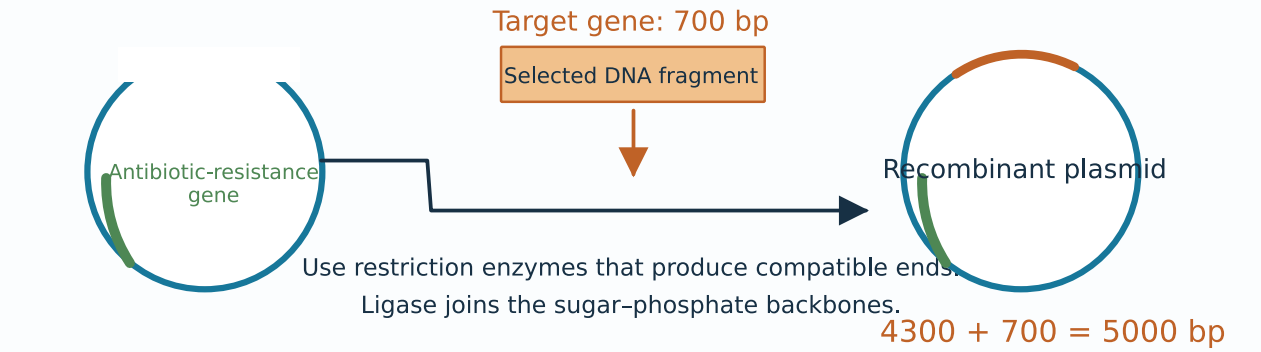
Each cycle comprises denaturation, primer annealing and extension. Thermostable DNA polymerase extends from a primer's 3′ end. Temperatures are illustrative and depend on the enzyme and primers.



A schematic circular plasmid. Colours distinguish regions only; determine vector functions from the labels and positions in the actual question.

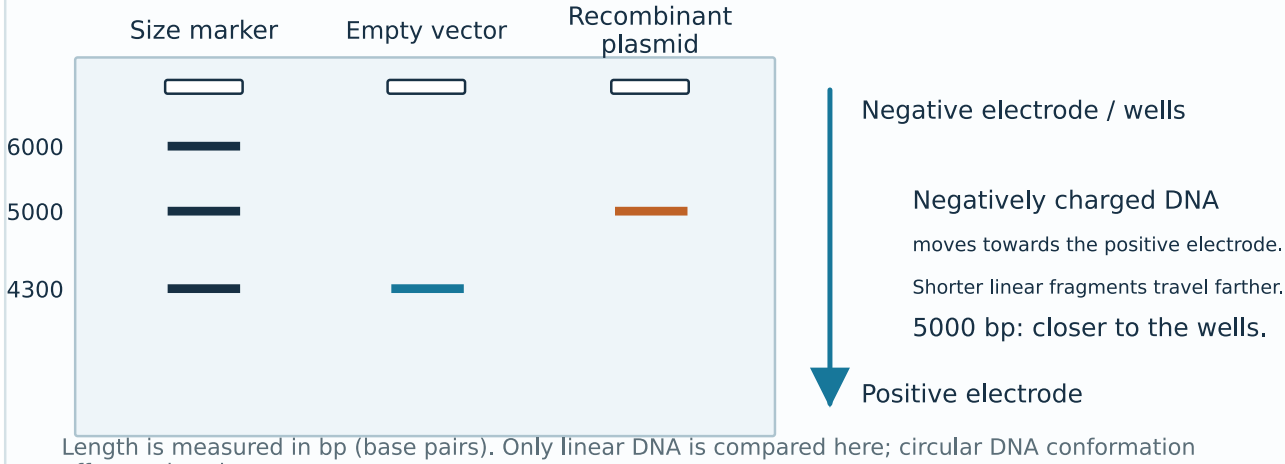
Recombinant DNA and electrophoresis: work out the fragment lengths

Create compatible ends, then join them with DNA ligase



Transform a suitable host and screen it. Resistance shows that the marker is present; it does not prove that the correct insert is present.

Linearise each plasmid at a single restriction site before comparing bands



Length is measured in bp (base pairs). Only linear DNA is compared here; circular DNA conformation affects migration.

The insert is 700 bp. Add the vector and insert lengths to obtain the length of the complete recombinant plasmid.

Use fragment lengths to check total length, then predict electrophoresis bands from the sizes of linear fragments.

Check your understanding

1. The enzyme joining DNA fragments is _____ materials that define PCR target boundaries are _____.
2. Under the same electrophoresis conditions, shorter DNA fragments usually travel _____.

Check answers:

1. DNA ligase; primers. 2. Farther.

How to recognise the problem Separate fragment isolation, vector ligation, host introduction and expression checks. For PCR, inspect primer boundaries; for electrophoresis, direction and length; for nuclear transfer, track nucleus and cytoplasm separately.

Concept VIII.b Applications of biotechnology

Key ideas

① Suitable hosts can produce particular human proteins, such as insulin or growth hormone, for medicines. Microorganisms are only one part of the production system. The product still requires separation, purification and quality checks; untreated culture fluid is not equivalent to a medicine. Recombinant technology can also produce antigens for some vaccines.

② Monoclonal antibodies originate from one cell clone and recognise the same specific antigenic site. They can be used in detection or some treatments. If binding produces a visible signal, the meaning of a positive result depends on the antigen recognised. Any colour change cannot be interpreted as a positive result for every disease.

③ Somatic gene therapy delivers genetic information to some of a patient's body cells to supplement, regulate or alter disease-related functions. Its effect depends on delivery to sufficient target cells, appropriate expression and persistence. Changes confined to somatic cells usually do not pass through gametes to offspring. This differs from changing the germ line.

④ Stem cells can self-renew and differentiate into certain cell types under suitable conditions. Cells from different sources have different developmental potential. Tissue repair requires control of differentiation, survival after transplantation and tissue function, with consideration of immune rejection and abnormal proliferation. The ability to differentiate does not mean that cells automatically repair any organ into which they are placed.

⑤ Transgenic organisms can help investigate gene function or introduce agricultural traits, such as resistance to particular pests. Evaluate the added trait, actual yield, resource inputs and effects on other organisms. Greater seed mass, higher gene expression and higher germination rate are different indicators; none represents all aspects of performance.

Identify what each application changes

Application	Main target or action	What to assess
Recombinant protein production	A host makes protein from introduced information	Correct expression, function and product quality
Monoclonal-antibody detection	Recognition of a specific antigenic site	Whether the signal corresponds to the target antigen
Somatic gene therapy	Changes functions in some patient body cells	Delivery, expression and persistence
Stem-cell applications	Self-renewal and differentiation potential	Suitable differentiation, function after transplantation and risks

Check your understanding

1. Somatic gene therapy usually does not transmit the change to offspring through _____.

Check answers:

1. Gametes.

How to recognise the problem Identify whether the intervention changes a gene, a cell or a protein product, then connect its mechanism to the intended use and specific limitations.

Concept VIII.c Bioethics

Key ideas

① A transgenic crop that reduces losses from a particular pest offers a potential agricultural benefit. Effects on non-target organisms, selection for pest resistance and seed-access costs are separate questions requiring evidence. Link each benefit or concern to a specific mechanism. 'Natural is better' and 'technology is always better' are insufficient evaluations.

② Animal cloning can preserve certain genetic features or support research, but raises questions about embryo success rates and the health and welfare of surrogates and offspring. Large-scale plant cloning rapidly supplies similar-quality plants but may reduce genetic diversity in a crop population, allowing one threat to affect many plants. The ethical and ecological issues are not identical for the two forms of cloning.

③ Genomic information can support research and risk assessment, but may reveal sensitive genetic information about individuals and their relatives. Access, informed consent and prevention of unfair use are significant social and legal considerations. An association between a variant and a disease does not mean that every carrier will develop the disease. Communicate the uncertainty.

④ Debates about stem cells and gene therapy include material sources, expected benefits, risks, fair access and the wishes of affected people. Scientific evidence helps estimate consequences, while accepting particular trade-offs also involves values. An answer can state a position, support it with biology, affected groups and feasible management measures, and address a reasonable limitation.

Evaluating pest-resistant transgenic crops requires specific reasons

Aspect	Possible argument	Evidence still needed
Agricultural benefit	Reduced yield loss from a particular pest	Yield under comparable conditions, costs and use
Ecological effects	Possible effects on non-target organisms or food webs	Actual exposure, non-target effects and field monitoring
Evolutionary effects	Persistent selection may increase pest resistance	Resistance data across generations
Social and economic effects	Seed costs and access conditions affect farmers	Costs, returns and information about affected groups

Check your understanding

1. After identifying benefits, an evaluation should use evidence to analyse _____ and the people affected.

Check answers:

1. Risks / limitations.

How to recognise the problem Define the technology and intended use, compare benefits, risks and affected groups using consistent criteria, then give a reasoned judgement.

Apply this approach to the relevant subparts of HKDSE Biology 2016 Paper 2 Q4 (VIII.b, Type 1, Example 2), HKDSE Biology 2012 Paper 2 Q4 (VIII.b, Type 1, Exercise 3), and HKDSE Biology 2014 Paper 2 Q4 (VIII.b, Type 1, Exercise 1). Identify who benefits and who bears the risks, then support your judgement with the question's evidence.

Chapter 7

Human physiology: question types and practice

V.a Osmoregulation: from blood to urine

Read the explanations and diagrams in Concept V.a first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Explain osmoregulation by tracking substances through the nephron

Degree of integration ★★★★☆

Key ideas

① Suppose 100 units of glucose enter glomerular filtrate over a period and all return to the blood. None of those 100 units remains in urine. This involves ultrafiltration followed by reabsorption; it does not mean that glucose never left the blood. Reabsorbing water can raise urea concentration in the tubular lumen, so a higher concentration does not show that new urea was made there.

② Water loss lowers blood water potential, increases ADH release and increases collecting-duct permeability to water. More water returns to the blood and urine volume falls. When reading reabsorption curves, also check whether transport capacity has reached its maximum.

How to recognise the problem. Treat blood, filtrate and urine as three distinct locations and track water, glucose and urea separately. For hormonal regulation, follow deviation → ADH → permeability → reabsorption → urine.

Example 1 [HKDSE Biology 2014 Paper 2 Q1]

(a) The table below shows the amounts of different substances handled by the kidneys:

	Amount filtered into the kidney tubules	Amount reabsorbed into blood capillaries	Amount excreted along with urine
Glucose (g day^{-1})	180	180	0
Hydrogencarbonate (mEq day^{-1})	4 320	4 318	2
Sodium (mEq day^{-1})	25 560	25 410	150
Chloride (mEq day^{-1})	19 440	19 260	180
Potassium (mEq day^{-1})	756	644	92
Urea (g day^{-1})	47	23.5	23.5

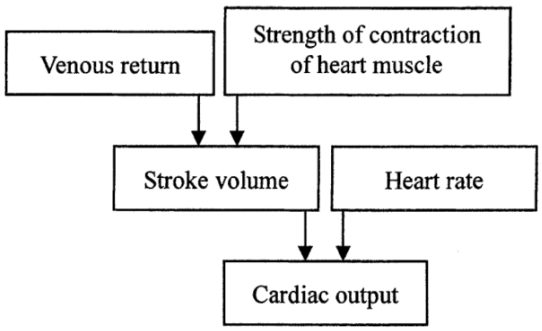
(i) With reference to the mechanisms involved in reabsorption, account for the differences in the amount of glucose and urea handled by the kidneys. (4 marks)

(ii) Of the total amount of water reabsorbed by the kidneys, only a small amount is regulated by a hormone.

(1) Using the information provided in the table, explain how most of the water is reabsorbed regardless of hormonal control. (3 marks)

(2) State the hormone which is responsible for regulating the reabsorption of water and state how it works. (2 marks)

1(b) Cardiac output refers to the blood volume supplying to the systemic circulation. The diagram below shows some factors that affect cardiac output:



(i) The contraction of which heart chamber determines the stroke volume? (1 mark)

(ii) Explain how the stroke volume is affected by the venous return and strength of the contraction of heart muscle. (2 marks)

(iii) Explain ***one*** way in which the venous return is increased when a person is doing exercise. (3 marks)

(iv) The average running speed in a marathon is usually much lower than that in a 100 m race. Explain why it is not possible for marathon runners to run at the same average speed as a 100 m runner throughout a marathon. (5 marks)

Solution a(i); original allocation: 4 mark(s)

All 180 g day^{-1} of glucose is reabsorbed because active transport in the renal tubules moves it from the filtrate back into the blood. Only 23.5 g day^{-1} of the 47 g day^{-1} of urea, or half, is reabsorbed. Urea mainly diffuses back down its concentration gradient; it is not actively recovered in its entirety.

Quote the figures to distinguish complete from half reabsorption, then associate these with active transport and diffusion respectively.

Method: Separate blood, filtrate and urine; track each substance.

a(ii)(1); original allocation: 3 mark(s)

The reabsorption of glucose and most inorganic ions shown in the table lowers the water potential of the surrounding blood/tissue fluid relative to the filtrate. Water therefore moves from the renal tubular filtrate into the blood by osmosis.

Water follows the water-potential gradient established by solute reabsorption. Not every section requires direct hormonal control, and water itself is not actively pumped out.

a(ii)(2); original allocation: 2 mark(s)

Antidiuretic hormone (ADH) increases the permeability of the collecting ducts to water, allowing more water to be reabsorbed down its water-potential gradient.

The hormone changes the water permeability of the duct wall; the actual movement of water is still driven by osmosis.

b(i); original allocation: 1 mark(s)

The left ventricle.

The question defines cardiac output as the volume of blood entering the systemic circulation, so use the blood delivered into the aorta by each contraction of the left ventricle.

b(ii); original allocation: 2 mark(s)

Increased venous return usually increases ventricular filling during diastole, leaving more blood available for the next beat. Stronger cardiac-muscle contraction expels more blood in the same contraction, increasing stroke volume.

One factor affects filling and the other affects ejection; both affect the volume delivered per beat.

b(iii); original allocation: 3 mark(s)

During exercise, alternating contractions of the skeletal muscles in the limbs compress veins and push blood towards the heart. Venous valves prevent backflow, so more blood returns to the heart through the major veins.

An alternative complete three-step explanation is that deeper breathing makes thoracic pressure more negative and promotes venous return. Do not describe veins as pumping strongly by themselves like the ventricles.

b(iv); original allocation: 5 mark(s)

A marathon requires prolonged, sustained muscular contraction. Running throughout at 100-metre sprint speed would create an ATP demand exceeding the aerobic energy supply that can be sustained over a long period. Oxygen supply cannot meet demand, so there is substantial reliance on anaerobic pathways and greater lactate production. Such high-intensity energy provision can last only briefly. Fatigue and changes in the internal environment prevent the muscles from maintaining the same contraction intensity for long, so running speed must fall.

The official scheme awards credit for five linked points: prolonged contraction, inadequate oxygen supply, anaerobic respiration and lactic acid, lactic acid accumulation, and fatigue. Modern explanations recognise fatigue as multifactorial; do not memorise lactic acid as its sole direct toxic cause. The central point is that the limit to maximal aerobic energy supply prevents sprint speed from being sustained for a long period.

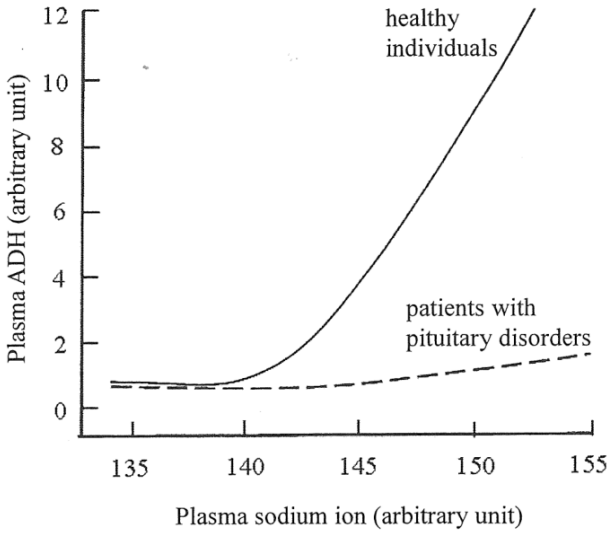
Examiner's comment (source page(s) 4)

Explain the transport mechanisms underlying the difference between glucose and urea reabsorption; saying that glucose is useful and therefore retained is insufficient. Venous return is the volume of blood returning to the right atrium, not the heart rate. Explanations of water reabsorption must address water potential and permeability.

Marking reference: HKDSE Biology 2014 Paper 2 Q1, official marking scheme, source page(s) 7.

Example 2 [HKDSE Biology 2020 Paper 2 Q1]

(a) In a study, the plasma antidiuretic hormone (ADH) level and plasma sodium ion level of two groups of individuals were compared. They were healthy individuals and patients with disorders of the pituitary gland. The graph below shows the relationship between the levels of the two substances in their bodies:



(i) With reference to the above graph, describe the change in the plasma ADH level in these two groups of individuals. (3 marks)

(ii) According to your answer in (a) (i), explain how the volume and concentration of urine produced by the patients would be different from that produced by healthy individuals. (3 marks)

(iii) Explain how the increase in plasma sodium ion level leads to changes in plasma ADH level in healthy individuals. (3 marks)

(iv) Some female patients with pituitary disorders do not menstruate. Based on your knowledge of the function of the pituitary gland, propose a possible explanation for this phenomenon. (3 marks)

1(b) In an investigation, a volunteer stayed at rest and breathed in air containing different concentrations of carbon dioxide. Some parameters related to breathing were measured. The results are shown in the table below:

Concentration of carbon dioxide in the inspired air (%)	Breathing rate (min^{-1})	Breathing depth (L)	Ventilation rate ($L\ min^{-1}$)
0.04	14	0.5	7.0
0.80	14	0.6	8.4
1.50	14	0.8	11.2
2.70	14	1.2	16.8
3.50	15	1.6	24.0
4.30	17	1.7	28.9
5.60	24	1.7	40.8
6.50	29	1.7	49.3

(i) State the parameter which contributed most to the change in ventilation rate in different ranges of carbon dioxide concentrations. (2 marks)

(ii) Suggest why the breathing depth remained steady as the carbon dioxide concentration exceeded 4.3%. (1 mark)

(iii) Describe the effect of an increase in carbon dioxide concentration on the volunteer's ventilation rate. (1 mark)

(iv) Explain how the increased concentration of carbon dioxide led to a change in the ventilation rate. (4 marks)

Method: Separate blood, filtrate and urine; track each substance.

Solution a(i); original allocation: 3 mark(s)

Below a sodium-ion level of approximately 140, ADH remains at similarly low levels in both groups. As sodium-ion levels rise further, ADH increases markedly in healthy individuals but only slightly in patients with pituitary disease.

The horizontal axis represents sodium-ion level, not time, so describing healthy individuals as secreting ADH "faster" does not express the relationship between the two variables. First describe the similarity at low sodium-ion levels, then the difference at higher levels. The patients' curve is not completely horizontal: it rises slightly, but much less than the healthy individuals' curve.

a(ii); original allocation: 3 mark(s)

Patients produce a larger volume of more dilute urine. Their lower ADH levels make the collecting ducts less permeable to water, reducing water reabsorption.

Compare the groups at the same sodium-ion level. Patients have less ADH and cannot increase collecting-duct permeability to water to the same extent as healthy individuals. Less water returns from the filtrate to the blood and more remains in the urine, increasing urine volume and reducing its concentration. State both volume and concentration, and specify permeability to water rather than suggesting that all solutes pass through more easily.

a(iii); original allocation: 3 mark(s)

An increase in sodium-ion level lowers blood water potential. Osmoreceptors in the hypothalamus detect the change and stimulate the posterior pituitary to release more ADH into the blood.

The receptors detect a change in osmotic conditions, rather than simply "knowing that sodium is high". ADH is synthesised in the hypothalamus and stored and released by the posterior pituitary. This response subsequently increases renal water reabsorption, helping to reduce the original deviation. Do not incorrectly place synthesis, detection and release in the kidneys.

a(iv); original allocation: 3 mark(s)

Insufficient FSH secretion by the pituitary fails to stimulate adequate follicle development. Oestrogen remains low, so the uterine lining cannot thicken normally or undergo normal cyclic changes, and menstruation may be absent.

The question requires a possible mechanism linking pituitary disease to the outcome. FSH is secreted by the anterior pituitary and acts on the ovaries. Ovarian follicles are an important source of oestrogen, which then affects the uterine lining. Do not state that the pituitary directly produces oestrogen or progesterone, or treat the ADH mentioned earlier as the hormone controlling menstruation.

b(i); original allocation: 2 mark(s)

At the lower carbon dioxide concentrations of 0.04%–2.70%, increased ventilation mainly results from greater breathing depth. At the higher concentrations of approximately 3.50%–6.50%, it mainly results from a higher breathing rate.

First use the relationship:

Ventilation per minute = breaths per minute × depth per breath.

In the lower range, breathing rate remains at 14 min^{-1} while depth increases from 0.5 L to 1.2 L, so depth accounts for the change. In the higher range, depth approaches a plateau of 1.6–1.7 L while rate increases from 15 to 29 min^{-1}, so frequency is the main contributor. Do not treat the "rates" in different columns as the same quantity.

b(ii); original allocation: 1 mark(s)

Lung expansion is approaching its volume limit under these conditions, so breathing depth cannot increase appreciably further.

Depth is the volume of air inhaled or exhaled per breath. The lungs and thorax cannot expand indefinitely. Beyond 4.3%, depth remains at 1.7 L, so further increases in ventilation

per minute mainly require more frequent breaths. Do not confuse this lung-volume limitation with the bladder or an unrelated structure.

b(iii); original allocation: 1 mark(s)

The higher the carbon dioxide concentration in inhaled air, the greater the ventilation rate. Ventilation per minute rises overall from 7.0 L/min to 49.3 L/min. This part asks only for a description of the results; the mechanism belongs in the next part. Do not interpret increased ventilation rate as evidence that breathing depth increases continuously throughout every range.

b(iv); original allocation: 4 mark(s)

An increase in blood carbon dioxide lowers pH. The associated chemoreceptor mechanisms increase output from the respiratory centre in the medulla oblongata. More nerve impulses reach the diaphragm and intercostal muscles, making their contractions more frequent or stronger and increasing ventilation.

Write the sequence as stimulus → detection and control centre → nervous output → respiratory muscles. Changes in carbon dioxide and acid–base conditions provide the stimulus. The centre controls respiratory-muscle movements, and the resulting changes in lung volume cause airflow. Simply stating that "negative feedback increases breathing" omits the intermediate steps. This question is not asking about heart-rate control.

Marking reference: HKDSE Biology 2020 Paper 2 Q1, official marking scheme, source page(s) 9.

Example 3 [HKDSE Biology 2016 Paper 2 Q1]

(a) The table below shows how the relative percentages of energy contribution from anaerobic and aerobic respiration change with different durations of vigorous exercise:

Duration of vigorous exercise	Relative percentage of energy contribution	
	Anaerobic respiration	Aerobic respiration
10 seconds	95	5
30 seconds	85	15
1 minute	70	30
2 minutes	50	50
8 minutes	30	70
12 minutes	15	85
30 minutes	5	95

(i) What is the relationship between the duration of vigorous exercise and the energy contribution from the two respiratory pathways? (1 mark)

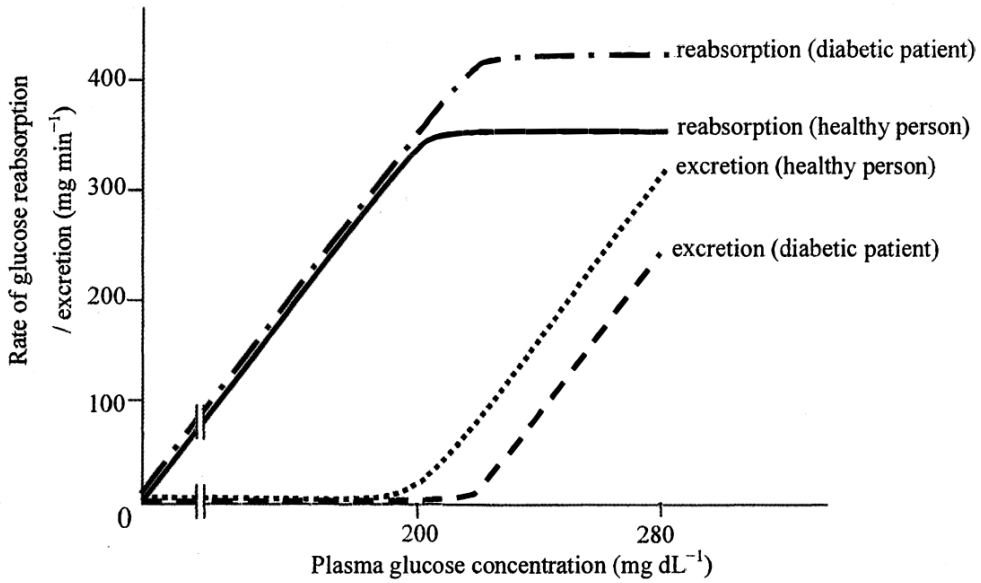
(ii) For vigorous exercise of short duration (10-30 seconds), energy mainly comes from the breakdown of the food store in muscles.

(1) Use a flowchart to show the two major steps in converting this food store to a usable form of energy through the anaerobic pathway. (2 marks)
(Note: Details of the intermediates in the pathway are not required.)

(2) Even though energy mainly comes from the anaerobic pathway, athletes who have just performed vigorous exercise for short duration still need to breathe fast for a while. Explain the importance of this phenomenon. (2 marks)

(iii) During exercise, our sympathetic nervous system is stimulated. Explain how this will bring about physiological changes to sustain the exercise. (4 marks)

1(b) The graph below shows the renal glucose handling capability (reabsorption and excretion) in a healthy person and in a patient suffering from non-insulin-dependent (type II) diabetes:



(i) Describe the change in the renal handling of glucose in the healthy person if the plasma glucose concentration increases from 0 to 280 mg dL^{-1}. (4 marks)

(ii) It is noted that there is an increased expression of a gene coding for membrane glucose carriers in the kidney tubules of type II diabetic patients.

(1) In which region of the kidney tubules are these membrane glucose carriers located? (1 mark)

(2) Suggest why the type II diabetic patient has a higher glucose reabsorption capability. (2 marks)

(iii) If the diabetic condition of the patient is not properly managed, the plasma glucose concentration can rise to a level beyond 300 mg dL^{-1}. Explain why diabetic patients urinate more frequently than healthy persons. (4 marks)

Solution (a)(i); original allocation: 1 mark(s)

Method: Separate blood, filtrate and urine; track each substance.

As vigorous exercise continues for longer, the relative percentage of energy supplied by aerobic respiration increases, while the percentage supplied by anaerobic respiration decreases.

For example, aerobic respiration supplies 5% at 10 seconds and 95% at 30 minutes. The data are percentages, so they do not establish that the absolute amount of energy supplied anaerobically necessarily decreases with time.

(a)(ii)(1); original allocation: 2 mark(s)

Glycogen → glucose → lactic acid/lactate + ATP.

First, stored muscle glycogen is broken down to provide sugars for glycolysis. Anaerobic metabolism then produces lactate and a small amount of ATP by substrate-level phosphorylation. Show the two principal steps requested; listing every intermediate is unnecessary.

(a)(ii)(2); original allocation: 2 mark(s)

Continued rapid breathing supplies extra oxygen to process lactate accumulated during exercise and restore energy reserves.

Stopping vigorous muscular activity does not immediately return metabolism to its resting level. Some lactate can be oxidised or converted back into glucose by the liver. These recovery processes require energy and oxygen, so rapid breathing may continue for a time.

(a)(iii); original allocation: 4 mark(s)

Increased sympathetic stimulation of the sinoatrial node and cardiac muscle raises heart rate and contraction force. Cardiac output increases, delivering more oxygenated blood to active muscles.

Link the nervous signal to energy demand: more frequent impulses from the sinoatrial node → higher heart rate; stronger cardiac-muscle contraction → potentially greater stroke volume. Together these increase the blood delivered to muscles per minute, providing oxygen and respiratory substrates for sustained contraction. Skeletal respiratory muscles themselves are controlled by motor nerves; there is no need to claim that sympathetic nerves directly innervate the diaphragm.

(b)(i); original allocation: 4 mark(s)

At blood glucose concentrations of approximately 0–200 mg dL^{-1}, reabsorption increases as blood glucose increases, and almost no glucose appears in the urine. Above approximately 200 mg dL^{-1}, reabsorption levels off and glucose excretion begins to increase with blood glucose.

Divide the curves into regions below and above the renal threshold, describing both reabsorption and excretion in each. The maximum reabsorption rate in healthy individuals is approximately 350 mg min^{-1}. As transport capacity approaches saturation, excess filtered glucose remains in the urine. The curved transition in the original graph makes the threshold approximate; the official scheme accepts approximately 200±20.

(b)(ii)(1); original allocation: 1 mark(s)

The proximal convoluted tubule (first convoluted tubule).

Glucose is normally selectively reabsorbed in large amounts in the proximal convoluted tubule. Do not identify the collecting duct, which mainly regulates water balance, as the main site of glucose reabsorption.

(b)(ii)(2); original allocation: 2 mark(s)

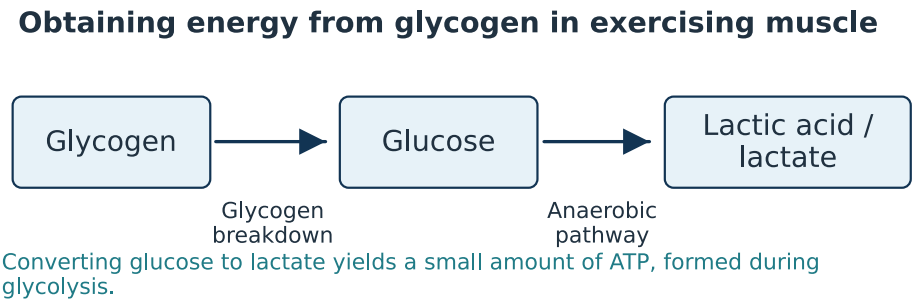
Increased expression of the relevant genes produces more glucose transport proteins in the membrane, increasing reabsorptive capacity per unit time.

Gene expression produces the corresponding proteins. More transport proteins allow more transport pathways to operate simultaneously, explaining the higher reabsorption plateau in patients with diabetes. This does not necessarily mean that every carrier becomes faster.

(b)(iii); original allocation: 4 mark(s)

Excess glucose cannot be completely reabsorbed and lowers the water potential of tubular fluid. A smaller proportion of water is reabsorbed, increasing urine volume and hence the frequency of urination.

When blood glucose exceeds transport capacity, glucose remains in the filtrate, increasing solute concentration and reducing the water-potential difference favouring movement from the tubules/collecting ducts into the blood. More water is consequently lost in urine. Link glucose in urine to water potential and urine volume; stating only that high blood glucose increases drinking is insufficient.



Graphical answer to the original question

Examiner's comment (source page(s) 4)

Do not arbitrarily describe the relationship between exercise duration and energy supply as direct proportionality. Glucose appears in urine when the amount filtered exceeds reabsorptive capacity. Its direct contribution to water loss is through lowering the water potential of the filtrate.

Marking reference: HKDSE Biology 2016 Paper 2 Q1, official marking scheme, source page(s) 8.

V.b Regulation of body temperature

Read the explanations and diagrams in Concept V.b first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Connect core temperature, skin responses and water balance

Degree of integration ★★★★☆

Key ideas

① Ice absorbs heat as it melts and warms inside the body. A measured change in skin temperature also depends on skin blood flow; the two locations need not change in the same direction. After exercise, distinguish heat production, blood flow to the skin, sweat secretion and evaporation.

② The same amount of sweat can remove different amounts of heat at different humidities. Different water losses can also trigger differences in ADH and urine volume. Read each curve first, then connect the causal relationships between organs.

How to recognise the problem Check the measurement site and treatment conditions. A warmer skin does not necessarily mean a higher core temperature, and more sweating does not necessarily mean more efficient evaporative cooling.

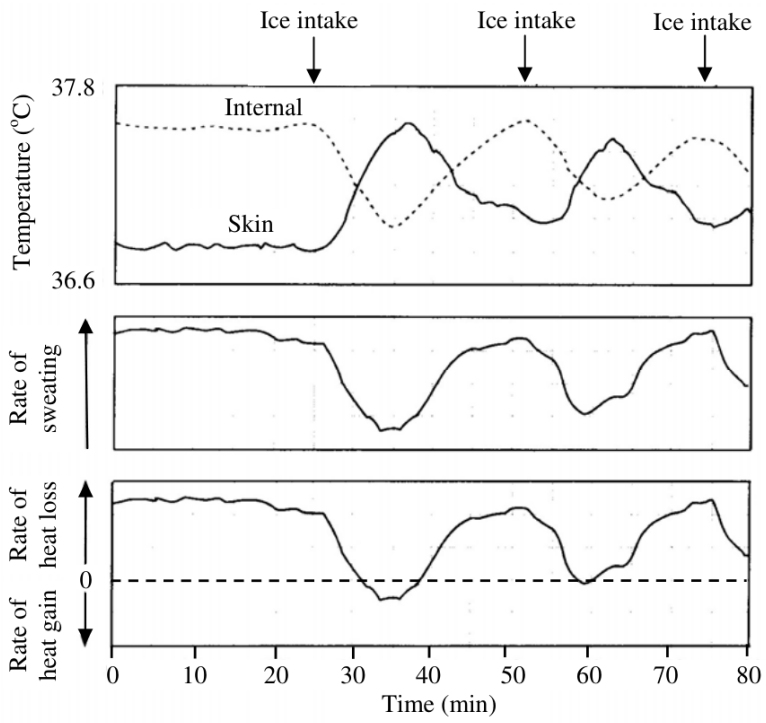
Example 1 [HKDSE Biology Sample Paper Paper 2 Q1]

1.(a) In an experiment conducted in a room with an air temperature of 45℃, a healthy man was asked to consume a large quantity of crushed ice (about 500 g) from time to time. Measurement of the following parameters were taken from the man during the course of the experiment:

- internal body temperature (taken at the eardrum which indicates the temperature of the blood supplying the hypothalamus)
- skin temperature
- rate of sweating
- rate of body heat loss / gain

(N.B. It was noted that the metabolic rate of this person ***remained unchanged*** throughout the experiment.)

The data are presented in the following graphs:



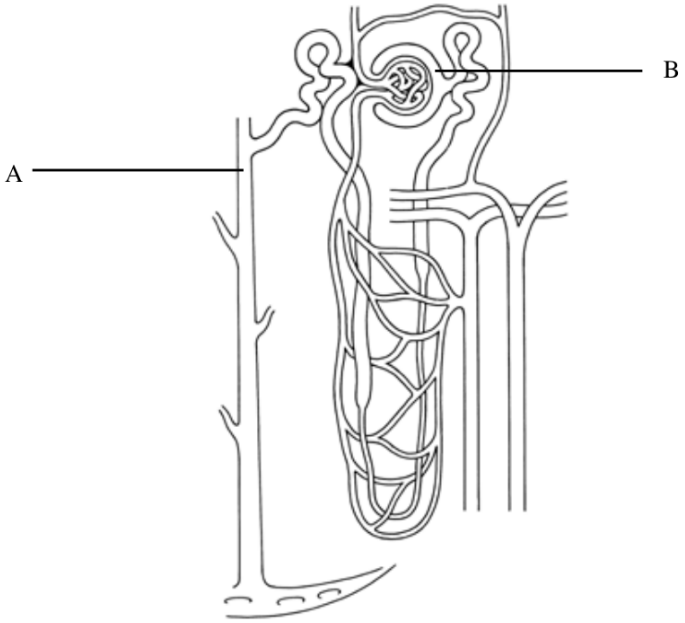
(i) Describe the changes in the man's internal body temperature and the skin temperature within the five minutes after the crushed ice was consumed. (2 marks)

(ii) Explain how the consumption of crushed ice had brought about the change in the internal body temperature you stated in (i). (2 marks)

(iii) The change in skin temperature within five minutes after the consumption of crushed ice was the result of homeostatic response. Explain the mechanisms involved in bringing about this change in skin temperature with reference to the graphs. (4 marks)

(iv) According to the findings of this experiment, the following deduction can be made: environmental temperature plays a less dominant role in causing sweating when compared to internal body temperature.
What evidence supports this deduction? (3 marks)

1.(b) The diagram below shows the structure of a nephron:



(i) Explain why the fluid in A has a lower percentage of glucose but a higher percentage of urea than that in B. (2 marks)

(ii) Explain the change in the concentration of the fluid in A after heavy sweating. (5 marks)

(iii) Patients suffering from kidney failure may need to receive treatments involving a dialysis machine which works on similar biological principles as the kidney. State ***one*** similarity and ***one*** difference between the functioning of the artificial membrane in the dialysis machine and that of the wall of nephrons. (2 marks)

Solution a(i); original allocation: 2 mark(s)

Method: Check the measurement site, then connect blood flow, sweat and heat loss.

Core temperature decreases, while skin temperature increases.

Starting at each arrow marking ice ingestion, consider only the following five minutes. The broken line for core temperature falls, whereas the solid line for skin temperature rises. This part asks for a description, so state the change in each curve. "The body becomes colder" omits the observation that the skin becomes warmer.

a(ii); original allocation: 2 mark(s)

As ice melts and warms inside the body, it absorbs heat and cools the blood flowing through the digestive tract. This cooler blood reaches the hypothalamus, indicating a fall in core temperature.

The initial effect is heat transfer: the body transfers heat to the ice, melting and warming it, so nearby blood loses heat. Circulation carries this temperature change to the hypothalamus. The ice does not directly contact the brain, and heat production does not stop after eating; the question specifies an unchanged metabolic rate.

a(iii); original allocation: 4 mark(s)

After detecting the fall in core temperature, the hypothalamus sends nervous signals that reduce sweat-gland secretion. Less heat is removed by sweat evaporation, so skin temperature rises.

Begin the complete chain with detection. Cooler blood supplying the hypothalamus triggers negative feedback that conserves body heat. Reduced stimulation of sweat glands lowers sweating rate, as shown by the curve, and reduces evaporative heat loss from the skin. The room remains at 45°C, so the skin can still gain heat from the hotter environment. When heat loss no longer offsets heat gain, skin temperature rises. This explains the sweating, heat-exchange and skin-temperature data together. Do not attribute warming to increased metabolic heat production when the question fixes metabolic rate.

a(iv); original allocation: 3 mark(s)

Room temperature remains at 45°C, yet each fall in core temperature is accompanied by a marked decrease in sweating rate. Sweating subsequently increases as core temperature recovers, showing that it mainly follows changes in core temperature.

If high environmental temperature alone dominated this response, continuous exposure to 45°C should sustain heavy sweating. Instead, with the environment unchanged and the skin even becoming warmer, sweating decreases as core temperature falls. This supports a greater influence of internal temperature information. It is an inference about relative effects under these experimental conditions, not evidence that environmental temperature never affects sweating.

b(i); original allocation: 2 mark(s)

As fluid passes from B through the renal tubules to A, glucose is reabsorbed, so its percentage is lower at A. A larger proportion of water than urea is reabsorbed, increasing the percentage of urea remaining.

A is in the collecting duct and receives fluid already processed by the renal tubules. Normally, almost all filtered glucose returns to the blood in the proximal convoluted tubule, leaving little or none at A. Much water is also recovered, leaving a relatively greater proportion of urea. A higher urea concentration does not mean that the renal tubule produces urea along this route or that the total amount of urea necessarily increases.

b(ii); original allocation: 5 mark(s)

Heavy sweating lowers blood water potential through water loss, stimulating the posterior pituitary to release more ADH. ADH increases the water permeability of the distal convoluted tubules and collecting ducts. More water is reabsorbed into the blood, making the urine at A more concentrated.

Sweat removes water, first changing blood concentration and water potential. The hypothalamus detects this deviation and increases renal water conservation through ADH. As more water returns from the ducts to the blood by osmosis, the remaining urea and other solutes are contained in less water. Urine volume falls and concentration rises. ADH is produced in the hypothalamus and released by the posterior pituitary. Greater permeability does not mean that water is more readily lost from the body.

b(iii); original allocation: 2 mark(s)

Similarity: both are selectively permeable, allowing some small molecules to pass while retaining blood cells and large protein molecules. Difference: living renal-tubule cells can actively and selectively reabsorb useful substances; an artificial dialysis membrane lacks this metabolic function.

Compare the actual exchange mechanisms. A dialysis membrane allows small molecules such as urea to move down concentration gradients, but does not independently determine how much glucose the body needs and use ATP to transport it back. Nephrons contain living cells whose transport proteins and energy supply enable such regulation. Dialysis fluid therefore needs an appropriate composition to prevent excessive loss of essential substances. Do not treat the dialysis membrane as an actively functioning renal cell.

Marking reference: HKDSE Biology Sample Paper Paper 2 Q1, official marking scheme, source page(s) 7.

V.c Regulation of blood gas levels

Read the explanations and diagrams in Concept V.c first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Explain exercise regulation using cardiac output and blood gases

Degree of integration ★★★☆☆

Key ideas

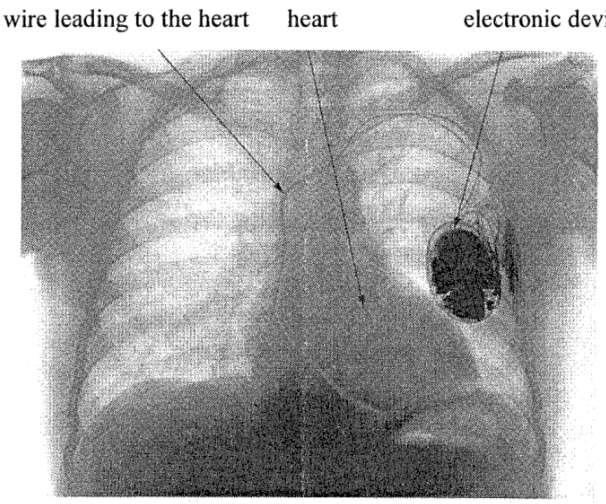
① If person A has a stroke volume of 70 mL and heart rate of 75 beats per minute, cardiac output is 5250 mL per minute. Person B, with 90 mL per beat and 60 beats per minute, has an output of 5400 mL per minute. B has a lower heart rate but higher cardiac output. Training effects therefore cannot be judged from a heart-rate curve alone.

② Increased respiration in active tissues produces carbon dioxide and associated acid–base changes that contribute to regulation of ventilation and cardiovascular activity. If the whole question also concerns water intake or hormones, retain the separate stimuli and effectors of each regulatory pathway.

How to recognise the problem Calculate stroke volume × heart rate before comparing oxygen supply and metabolic demand. Distinguish the pacemaker activity initiating a heartbeat from nervous and hormonal regulation that alters heart rate.

Example 1 [HKDSE Biology 2013 Paper 2 Q1]

(a) The X-ray photograph below shows a small electronic device that has been surgically implanted into the chest cavity of a patient suffering from heart disease. This device maintains the proper rhythm of the patient's heart.



(i) Which structure of the heart does the device replace functionally? (1 mark)

(ii) The structure mentioned in (i) triggers a series of events that lead to the proper functioning of the heart. Describe these events. (4 marks)

(iii) During the cardiac cycle, there is a period of time in which both atria and ventricles are in a relaxed state. Briefly describe the pathway of blood flow returning from the lungs to the heart chambers during this period. (3 marks)

(iv) With reference to a hormone, describe how it can bring about an increase in cardiac output. (3 marks)

1(b) Kathy has been married for two years. In the first year, she took a progesterone-containing contraceptive pill. Then she stopped taking it and got pregnant. Three months later she noticed bleeding from her vagina. Her doctor gave her progesterone to stop the bleeding.

(i) Explain how progesterone in the contraceptive pill prevented Kathy from becoming pregnant. (5 marks)

(ii) (1) What was the possible cause and consequence of the bleeding from Kathy's vagina? (2 marks)

(2) How did the progesterone prescribed by the doctor help Kathy in this case? (2 marks)

Solution a(i); original allocation: 1 mark(s)

The sinoatrial node, the heart's natural pacemaker.

The implanted electronic device generates rhythmic electrical stimuli, replacing or supporting the normal initiation of the heartbeat.

Method: Calculate cardiac output, then relate it to oxygen supply and regulation.

a(ii); original allocation: 4 mark(s)

Electrical impulses from the sinoatrial node spread through both atrial walls, causing the atria to contract simultaneously. The impulses reach the atrioventricular node and then travel through conducting tissue to both ventricular walls, causing the ventricles to contract after the atria.

Describe initiation, spread through and contraction of the atria, the atrioventricular node, and subsequent ventricular contraction in order. This sequence allows blood to enter the ventricles before being pumped out of the heart.

a(iii); original allocation: 3 mark(s)

Blood returns from the lungs through the pulmonary veins to the left atrium, then flows through the open bicuspid valve into the left ventricle.

When both atria and ventricles relax, venous return can still fill the chambers. Blood does not have to wait for active atrial contraction before any can enter.

a(iv); original allocation: 3 mark(s)

Adrenaline makes cardiac muscle contract faster and more strongly. Heart rate and stroke volume increase, so cardiac output per minute increases.

Cardiac output = heart rate × stroke volume.

Explain how the hormone changes these two determinants rather than merely saying that it "stimulates the heart".

b(i); original allocation: 5 mark(s)

The contraceptive pill maintains a relatively high progesterone level, exerting negative feedback on the pituitary and inhibiting FSH and LH secretion. Low FSH prevents adequate follicle development, while low LH suppresses ovulation. Without release of an ovum, fertilisation cannot occur.

Explain the progesterone-related hormonal feedback described in the question. The key is suppression of gonadotrophins, interrupting follicle development and ovulation, not direct killing of sperm by progesterone.

b(ii)(1); original allocation: 2 mark(s)

In the situation described, bleeding may indicate that the uterine lining is beginning to break down. Destabilisation of the lining supporting pregnancy may result in miscarriage.

The original question uses progesterone treatment in this scenario and asks about the relationship between the lining and attachment of the embryo. This is a physiological explanation of the stated situation; bleeding in an actual individual does not by itself establish its cause.

b(ii)(2); original allocation: 2 mark(s)

Progesterone maintains the thickness and blood supply of the uterine lining, supporting stable attachment of the placenta/embryo and continuation of pregnancy.

Here, progesterone maintains the lining after pregnancy has begun. Distinguish this from the earlier use of high progesterone to suppress ovulation.

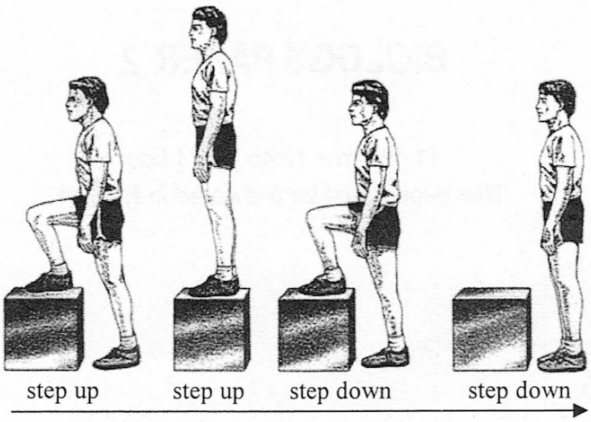
Examiner's comment (source page(s) 4)

Do not describe cardiac electrical impulses as nerve impulses arriving from neurones. Venous return during diastole does not depend on atrial contraction. Cardiac output depends on both heart rate and stroke volume.

Marking reference: HKDSE Biology 2013 Paper 2 Q1, official marking scheme, source page(s) 7.

Example 2 [HKDSE Biology 2021 Paper 2 Q1]

(a) Two young students, Alice and Billy, carried out a series of step-up exercises of increasing intensity. Each exercise lasted for three minutes by stepping up and down on a wooden box at a fixed speed (i.e. 2, 6 or 10 step-ups per 10 seconds) as shown in the diagram below:



Their heart rates and blood lactate concentrations were measured at rest and immediately after each exercise. Before the start of the next exercise, the students were allowed to rest until their heart rate and blood lactate concentration returned to their levels at rest. The results are shown in the table below:

Intensity of exercise (No. of step-ups per 10s)	Heart rate (beat min^{-1})		Blood lactate concentration (mmol L^{-1})	
	Alice	Billy	Alice	Billy
0 (at rest)	58	80	0.42	0.43
2	64	91	0.63	0.82
6	94	132	0.82	2.40
10	130	178	1.20	5.80

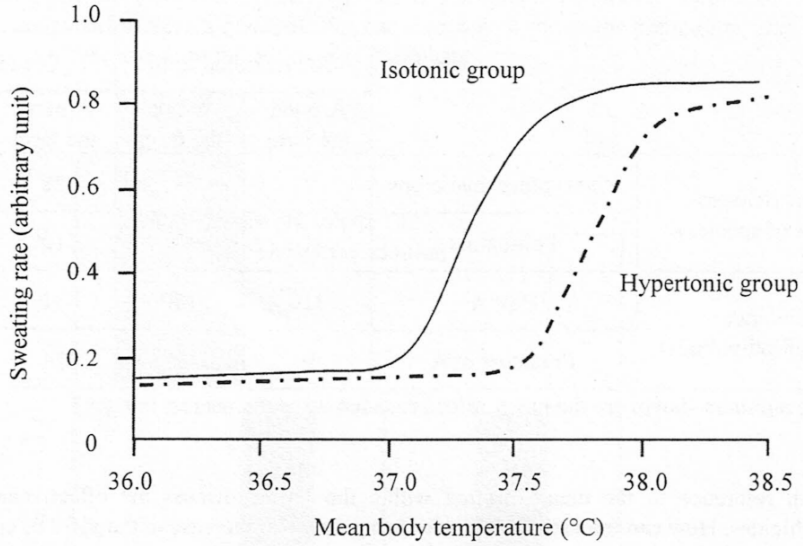
(i) Describe the overall effects of increasing the intensity of exercise on the heart rate and blood lactate concentration. (1 mark)

(ii) According to your answers in (i), explain the change in blood lactate concentration during the exercise. (3 marks)

(iii) According to your answer in (i), describe how the nervous system brings about the change in heart rate during the exercise. (4 marks)

(iv) Alice is a trained athlete. Give ***two*** pieces of supporting evidence from the data. (2 marks)

1(b) An investigation was carried out to study the effect of the water content of blood on the thermoregulatory response. Volunteers were divided into two groups with the blood water content of one group maintained at isotonic condition while the other group at hypertonic condition. They were asked to immerse their bodies in hot water to mid-chest level for 30 minutes. The rate of sweating at the forehead and the body temperature of the volunteers were continuously monitored throughout the experiment. The relationship between these two parameters of the groups is shown in the graph below:



(i) State the receptor and effector involved in the thermoregulatory response of the isotonic group. (2 marks)

(ii) With reference to the thermoregulatory response of the isotonic group shown in the above graph, explain why the change brought about by this response is regarded as an example of negative feedback. (4 marks)
(Note: The nervous coordination of the response is ***not*** required.)

(iii) With reference to the above graph, deduce the effect of the hypertonic condition of blood on the negative feedback mechanism of the thermoregulation of the volunteers. (2 marks)

(iv) The effect described in (iii) is beneficial in regulating water balance in the hypertonic group of volunteers. Explain why. (2 marks)

Solution a(i); original allocation: 1 mark(s)

Overall, higher exercise intensity is associated with higher heart rate and blood lactate concentration.

For both students, values in both columns increase from rest to faster stepping. Mention both dependent variables.

a(ii); original allocation: 3 mark(s)

The muscles' rate of energy demand exceeds the rate supplied by aerobic respiration. Additional anaerobic respiration produces lactate, which accumulates when production exceeds removal.

During more vigorous exercise, oxygen supply may not fully meet the rate of muscular energy demand, so aerobic and anaerobic energy provision can occur together. Do not state that all aerobic respiration stops. Rising blood lactate indicates net accumulation from the balance between production and removal.

Method: Calculate cardiac output, then relate it to oxygen supply and regulation.

a(iii); original allocation: 4 mark(s)

Chemoreceptors in the medulla oblongata detect blood acidification. The cardiovascular centre increases sympathetic signals to the sinoatrial node, raising heart rate.

The question uses the fall in blood pH accompanying increased lactate as the stimulus. The medullary cardiovascular centre sends more nerve impulses to the pacemaker/sinoatrial node. The node generates electrical signals more frequently, and these spread through cardiac muscle, causing faster contractions. Connect the stimulus, detection, centre, sinoatrial node and muscle response; "sympathetic nerves speed up the heartbeat" alone is incomplete.

a(iv); original allocation: 2 mark(s)

Alice has a lower resting heart rate and a lower lactate concentration at the same exercise intensity.

At rest, Alice's heart rate is 58 beats/minute, compared with Billy's 80 beats/minute. At the highest stepping intensity, their lactate concentrations are 1.20 mmol/L and 5.80 mmol/L respectively. Lower heart rate and lactate accumulation at the same workload are consistent with better cardiorespiratory fitness. Compare equal exercise intensities, not values from different rows.

b(i); original allocation: 2 mark(s)

The receptors are thermoreceptors in the hypothalamus; the effectors are sweat glands.

The figure concerns core temperature and sweating, so connect detection of internal temperature with the secretory response of sweat glands.

b(ii); original allocation: 4 mark(s)

A rise above the normal body temperature of approximately 37°C triggers sweating. Sweat evaporation removes heat, lowering body temperature and counteracting the original deviation.

Negative feedback involves a normal value, a deviation that triggers a response, and a response that reduces the deviation. Sweat must evaporate to absorb heat effectively. Merely saying that hot sweat is excreted omits the main cooling process.

b(iii); original allocation: 2 mark(s)

The hypertonic group begins substantial sweating at a higher threshold. Its curve shifts to the right, with the set point moving to approximately 37.5°C.

Compare the horizontal-axis positions where the curves begin to rise markedly. The isotonic group begins near 37.0°C, whereas the hypertonic group begins later. Comparing sweating at only one temperature misses the threshold change.

b(iv); original allocation: 2 mark(s)

Delayed sweating reduces water loss and helps the hypertonic group conserve water, at the cost of delaying heat loss.

The hypertonic group's body fluids have a lower water potential, creating a need to conserve water. Raising the sweating threshold reduces water loss at the same temperature and reflects a trade-off between water balance and cooling.

Examiner's comment (source page(s) 8, 9)

Link high breathing rate after exercise to the oxygen required to process lactate. Describe the nervous pathway from receptors through the cardiac control centre to the heart. A rightward shift of the whole sweating curve indicates a changed thermoregulatory set point, not merely a different reading at one temperature.

Marking reference: HKDSE Biology 2021 Paper 2 Q1, official marking scheme, source page(s) 7, 8.

V.d Hormonal control of the reproductive cycle

Read the explanations and diagrams in Concept V.d first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Infer ovulation, contraception and exercise responses from hormone curves

Degree of integration ★★★☆☆

Key ideas

① Falling oestrogen can reduce pituitary inhibition and allow FSH to rise. Before ovulation, sustained high oestrogen can instead promote positive feedback and an LH surge. Identify the cycle stage; an increase in any hormone is not automatically negative feedback.

② Supplied hormones that suppress follicle maturation and the LH surge can reduce ovulation by modifying reproductive regulation. Compare normal, affected and treated curves at the same times before proposing mechanisms.

How to recognise the problem Separate pituitary, ovary and uterus. Match each hormone to its source, target and timing. If other subparts involve exercise, give the complete water-balance or blood-flow pathway separately.

Example 1 [HKDSE Biology 2023 Paper 2 Q1]

(a) Susan is a healthy young woman who got an abdominal injury in a traffic accident months ago. Since then, her menstrual flow lasted much longer than her usual period. Her doctor arranged blood tests to check her hormone levels during a menstrual cycle. The results are shown below:

Name: Susan Sex: Female Age: 27

28-day menstrual cycle	Hormone	Level	Normal Range
Days 1-11	Oestrogen	34.3	125.9-661.2
	Follicle stimulating hormone (FSH)	31.7	4.3-7.1
	Progesterone	0.6	0.6-1.0
Days 12-16	Oestrogen	138.1	320.6-914.8
	Follicle stimulating hormone (FSH)	38.5	5.3-11.5
	Progesterone	1.5	2.6-5.1
Days 17-28	Oestrogen	92.3	151.2-552.8
	Follicle stimulating hormone (FSH)	22.3	2.4-6.2
	Progesterone	6.8	9.5-40.3

* All values are expressed in arbitrary units.

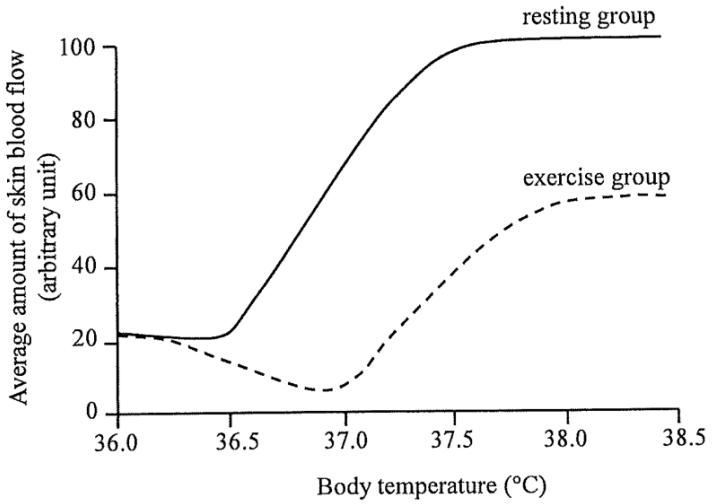
(i) With reference to the secretion of oestrogen, which part of Susan's reproductive system could have been affected after the abdominal injury? Explain how this would lead to the oestrogen levels shown in her blood test results. (3 marks)

(ii) With reference to the interaction between oestrogen and FSH, explain the overall result of the FSH levels in Susan's blood tests. (3 marks)

(iii) Before the accident, Susan's menstrual flow lasted for only four to five days. After the accident, her menstrual flow lasted much longer. With reference to the above results, suggest an explanation to account for this phenomenon. (2 marks)

(iv) To check if there is ovulation in Susan's menstrual cycle, the level of which hormone should be measured? What is the expected change for the level of this hormone if there is ovulation? (2 marks)

1(b) In a study of the effect of exercise on thermoregulatory response in a hot environment, two groups of healthy volunteers stayed in a room at 38 °C and 50% relative humidity. One group was kept at rest while the other group was asked to run on treadmills set at a constant speed of 5 km hr^{-1} for 45 minutes. The skin blood flow in the legs and body temperature of the volunteers were continuously monitored throughout the experiment. The relationship between these two parameters is shown in the graph below:



(i) Explain why there was an increase in blood flow to the skin of the resting group. (4 marks)

(ii) (1) With reference to the curve of the exercise group, deduce the condition of the arterioles leading to the skin surface when the body temperature increased from 36°C to 37°C. (2 marks)

(2) What is the significance of such change to the exercise group? (2 marks)

(iii) Apart from the change in (ii), state ***two*** other differences in the thermoregulatory response of skin blood flow between the two groups. (2 marks)

Solution a(i); original allocation: 3 mark(s)

Damage to the ovaries or developing follicles may reduce oestrogen secretion, keeping oestrogen below the normal range throughout the cycle.

Oestrogen is mainly secreted by developing ovarian follicles. Injury to this tissue reduces its capacity to produce the hormone. The values 34.3, 138.1 and 92.3 are all below the lower limits of their respective normal ranges, consistent with this explanation.

a(ii); original allocation: 3 mark(s)

Low oestrogen weakens negative feedback on pituitary FSH secretion, so the pituitary releases more FSH.

Within the appropriate concentration range, oestrogen normally inhibits FSH secretion. Inadequate oestrogen throughout the cycle reduces this inhibitory signal, explaining why FSH exceeds the normal upper limit in all three intervals. High FSH does not guarantee an ovarian response because the ovary itself may be damaged.

a(iii); original allocation: 2 mark(s)

Insufficient progesterone makes the uterine lining harder to maintain. It begins shedding earlier, prolonging bleeding.

The table shows progesterone below the normal range particularly on days 12–16 and 17–28. Progesterone normally maintains the thickness and stability of the lining. When levels are too low, shedding may start early and persist, producing a longer menstrual period.

a(iv); original allocation: 2 mark(s)

Measure luteinising hormone (LH). A marked LH surge should normally occur before ovulation.

Method: Align each hormone's source, target and timing.

A sharp rise in LH triggers ovulation. Monitoring for a brief LH surge therefore corresponds more directly to the hormonal event preceding ovulation than a single oestrogen reading.

b(i); original allocation: 4 mark(s)

High temperature causes heat accumulation in the body. Thermoreceptors send information to the hypothalamus, whose heat-loss centre sends nervous signals that dilate skin arterioles and increase skin blood flow.

The room temperature of 38°C exceeds the initial body temperature, while metabolism continues producing heat, so body temperature rises. Thermoreceptors in the skin and hypothalamus detect the change. Through nervous regulation of arteriolar smooth muscle, the hypothalamus increases vessel diameter. More blood carries internal heat to the surface, assisting heat loss with sweat evaporation. When the surroundings are hotter than the skin, outward conduction or radiation alone cannot explain heat loss.

b(ii)(1); original allocation: 2 mark(s)

The arterioles constrict, as skin blood flow in the exercise group falls from approximately 20 to approximately 5 between 36°C and 37°C.

With other factors similar, reduced skin blood flow indicates narrowing of the supplying arterioles and increased resistance. State the falling trend in the graph before linking it to vessel condition.

b(ii)(2); original allocation: 2 mark(s)

Blood is redistributed to exercising muscles, supplying more oxygen and respiratory substrates to support contraction.

Active leg muscles have increased ATP demand. Reducing blood flow to the skin prioritises muscle perfusion, increasing oxygen and glucose delivery and metabolic-waste removal.

b(iii); original allocation: 2 mark(s)

The exercise group begins to increase skin blood flow markedly only at a higher body temperature. Its maximum plateau blood flow is also lower.

The resting group's flow starts rising after approximately 36.4°C and approaches 100 by approximately 37.6°C. The exercise group's flow starts recovering at approximately 36.9°C, approaches a plateau only near 38°C and reaches only approximately 60. These compare the temperature at response onset and the maximum response magnitude respectively.

Examiner's comment (source page(s) 9, 10)

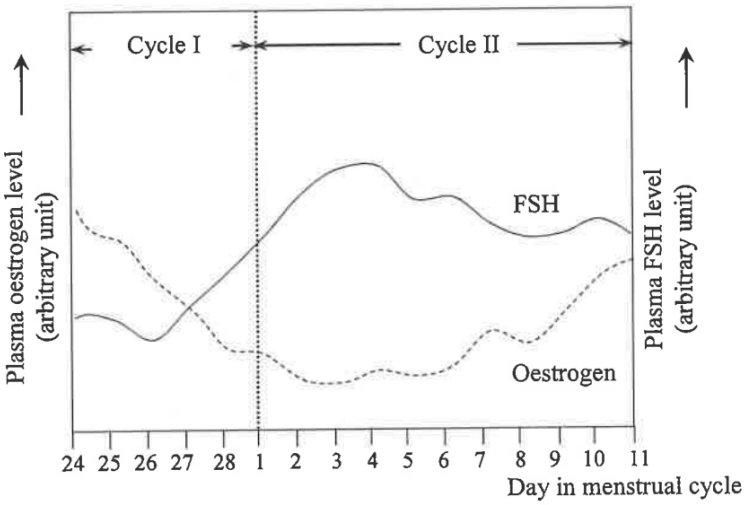
Low oestrogen removes negative feedback on pituitary FSH secretion; it does not directly stimulate FSH molecules. The horizontal axis of the skin-blood-flow graph is body temperature, not time. The effectors for vasodilation and vasoconstriction are arterioles; capillaries lack the same smooth-muscle regulation.

Marking reference: HKDSE Biology 2023 Paper 2 Q1, official marking scheme, source page(s) 9, 10.

Example 2 [HKDSE Biology 2019 Paper 2 Q1]

(a) To investigate the interaction between oestrogen and follicle stimulating hormone (FSH), the plasma levels of the two hormones in a group of females were measured over a period of time. Graph 1 below shows the change in the plasma levels of the two hormones throughout the course of the study:

Graph 1



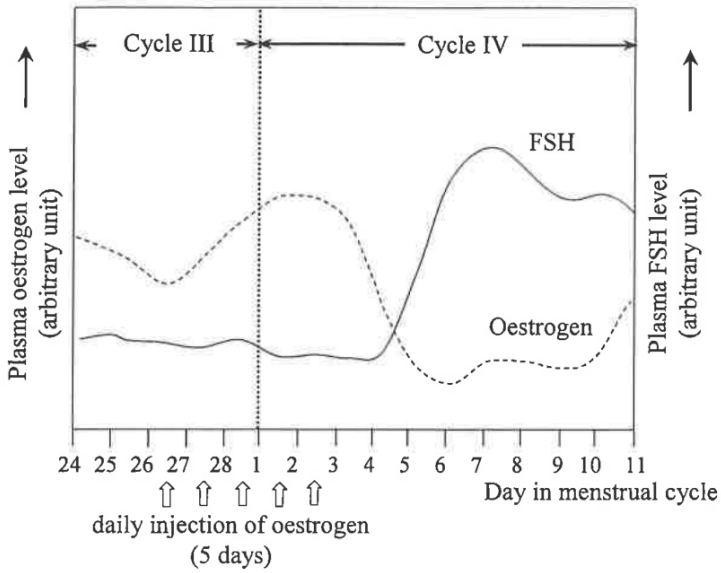
(i) With reference to the events occurring in the ovaries, explain the changes in plasma oestrogen level during the following periods of time:

(1) from day 24 of cycle I to day 3 of cycle II (2 marks)

(2) from day 5 to day 11 of cycle II (2 marks)

(ii) The same group of females received an additional daily oestrogen injection from day 26 of cycle III for 5 consecutive days. Graph 2 below shows the changes in the plasma levels of the two hormones over the same period of time:

Graph 2



(1) The pattern of the change in plasma FSH level in Graph 2 was modified by the injection of additional oestrogen. With reference to graphs 1 and 2, deduce the effect of oestrogen on FSH. Explain your answer. (4 marks)

(2) Contraceptive pills usually contain oestrogen and progesterone. With reference to your answer in (1), explain the function of oestrogen in these contraceptive pills. (2 marks)

1(b) To investigate the effects of dehydration on cardiovascular functions, professional cyclists were divided into two groups (dehydrated group and hydrated group) and asked to ride a cycling machine set at the same resistance for 120 minutes. The dehydrated group did not drink any fluid throughout the course of the experiment, while the hydrated group drank an isotonic fluid to compensate for the water loss during cycling. The table below shows the changes in their cardiovascular functions:

Duration of exercise (min)	Heart rate (beat min^{-1})		Stroke volume (L)		Cardiac output (L min^{-1})	
	Dehydrated group	Hydrated group	**Dehydrated group**	Hydrated group	**Dehydrated group**	Hydrated group
10	**138**	139	**0.138**	0.136	**19.0**	18.9
60	**155**	148	**0.120**	0.129	**18.6**	19.1
110	**168**	150	**0.093**	0.128	**15.7**	19.2

(i) Over the course of the experiment, the cycling speed of the hydrated group was constant while that of the dehydrated group dropped slowly. With reference to the data on cardiac output, explain the performance of the two groups of cyclists in terms of the cycling speed. (5 marks)

(ii) (1) With reference to the data on heart rate and stroke volume in the dehydrated group, which parameter led to the change in their cardiac output? (1 mark)

(2) With reference to water balance in the dehydrated group, explain the change in the parameter stated in (1) during the course of the experiment. (2 marks)

(iii) Suggest why there was a greater increase in heart rate in the dehydrated group as compared to the hydrated group. (2 marks)

Solution a(i)(1); original allocation: 2 mark(s)

Method: Align each hormone's source, target and timing.

Degeneration of the ovarian corpus luteum reduces oestrogen secretion, causing oestrogen to fall from day 24 of cycle I to day 3 of cycle II.

This interval spans the end of one cycle and the beginning of the next. Link the hormonal decline to degeneration of the previously secreting corpus luteum, not to loss of oestrogen in menstrual blood.

a(i)(2); original allocation: 2 mark(s)

Ovarian follicles gradually develop and mature, secreting increasing amounts of oestrogen. Days 5–11 of cycle II are within the follicular-development phase. The new follicles increase oestrogen production, corresponding to the rising broken curve. Do not attribute secretion at this stage to a new post-ovulatory corpus luteum.

a(ii)(1); original allocation: 4 mark(s)

Oestrogen exerts negative feedback on/inhibits FSH secretion.

In Figure 1, FSH rises when oestrogen falls near the end of cycle I. In Figure 2, FSH remains low during the five days of oestrogen injections in the corresponding interval. After injections stop, oestrogen falls and FSH rises again. Together, the normal pattern, injection period and post-injection pattern support inhibition of FSH by higher oestrogen. Different curve shapes alone are insufficient evidence.

a(ii)(2); original allocation: 2 mark(s)

Oestrogen in the contraceptive pill suppresses FSH, preventing normal follicle development and leaving no mature ovum available for ovulation and fertilisation.

The question asks about oestrogen in this negative-feedback mechanism. Start with FSH suppression, then connect it to follicles and ovulation. Do not give only "prevents pregnancy" or replace the entire mechanism with progesterone action.

b(i); original allocation: 5 mark(s)

Cardiac output in the hydrated group stays near 19 L min^{-1}, whereas that of the dehydrated group falls from 19.0 to 15.7 L min^{-1}. Reduced oxygen and nutrient delivery to active muscles lowers ATP production, slowing cycling speed.

Cardiac output is blood volume delivered per minute. The hydrated group remains approximately stable from 18.9 to 19.2 L min^{-1}, maintaining oxygen and substrate delivery for muscular respiration. Reduced perfusion in the dehydrated group lowers sustainable energy release and ATP supply, preventing contractions from maintaining the original speed. Connect the blood-flow data to respiration, energy and muscular contraction.

b(ii)(1); original allocation: 1 mark(s)

Stroke volume.

Heart rate in the dehydrated group rises from 138 to 168 beats min^{-1}, which would tend to raise cardiac output. However, stroke volume falls from 0.138 to 0.093 L, sufficiently to reduce total output. Since cardiac output = heart rate × stroke volume, the key factor is falling stroke volume.

b(ii)(2); original allocation: 2 mark(s)

The dehydrated group continues losing water through sweating and breathing without replacement. Blood volume decreases, reducing stroke volume.

Reduced circulating blood volume lowers venous return and ventricular filling, leaving less blood available for the next contraction. Stroke volume is not the number of heartbeats per minute; these are different parameters.

b(iii); original allocation: 2 mark(s)

The larger increase in heart rate compensates for the dehydrated group's lower volume per beat, attempting to maintain adequate cardiac output.

A faster heart rate partly offsets the reduction in stroke volume. The data show incomplete compensation, however, because cardiac output and cycling speed still fall. A compensatory response does not establish complete restoration of function.

Examiner's comment (source page(s) 8, 9)

Link oestrogen changes to corpus-luteum degeneration or follicle development, then use the data before and after injection to support negative feedback. Compare both exercise groups: cardiac output is approximately stable with hydration, while stroke volume falls with dehydration and increased heart rate only partly compensates. Do not replace this comparison with a generic account of nervous heart-rate regulation.

Marking reference: HKDSE Biology 2019 Paper 2 Q1, official marking scheme, source page(s) 7.

Practice V.a Osmoregulation: from blood to urine

Related type 1

Explain osmoregulation by tracking substances through the nephron

Degree of integration ★★★★☆

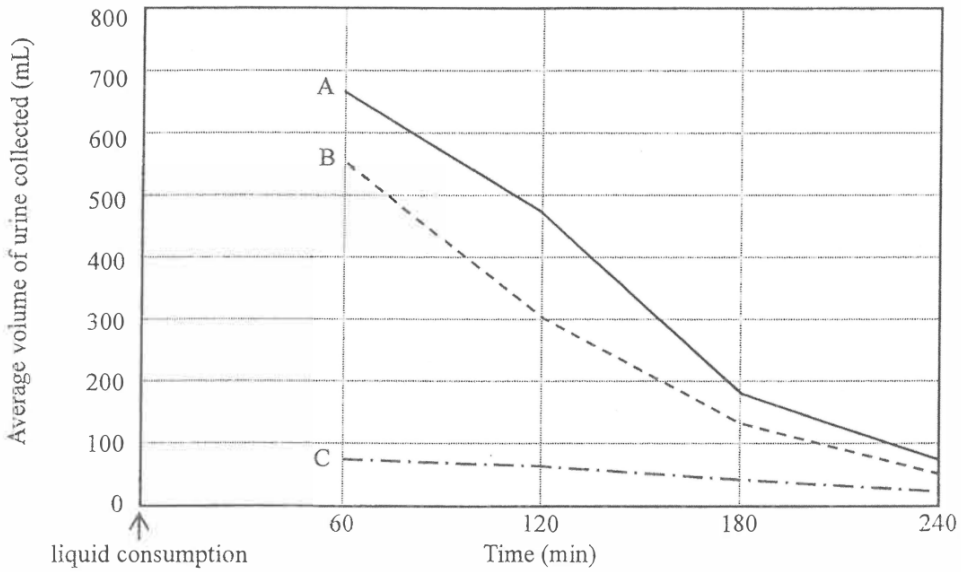
Practice

1. [HKDSE Biology 2018 Paper 2 Q1]

Hint: Separate blood, filtrate and urine; track each substance.

1(a) In a study of the effect of consuming different liquids on urine production, three groups of healthy persons were asked to follow the same pattern of physical activity and dietary intake. After that, Group A consumed a 1500 mL alcoholic beverage, Group B consumed 1500 mL water and Group C did not consume any liquid. Their urine was collected and its volume measured at 60-minute intervals over a period of 240 minutes. The results are shown in the graph below:

Key: ——— Group A: each person consumed a 1500 mL alcoholic beverage
- - - - - - Group B: each person consumed 1500 mL water
—·—·— Group C: did not consume any liquid



(i) With reference to the hormonal control of osmoregulation, explain why Group C had a much lower average volume of urine produced than Group A and Group B. (5 marks)

(ii) (1) With reference to the results of Group A and Group B, what is the overall effect of consuming alcoholic beverages on urine production? (1 mark)

(2) Based on (ii) (1), deduce ***one*** possible effect of alcohol on the hormonal control of osmoregulation. (1 mark)

(iii) Explain why the participants should avoid doing vigorous physical activity during the study. (2 marks)

1(b) Nowadays, many weather forecast apps list 'real feel' temperature in addition to air temperature. The real feel temperature is the temperature which takes into account multiple factors influencing the effectiveness of heat loss from the human body. The higher the effectiveness, the lower is the real feel temperature. The table below shows the real feel temperatures at different air temperatures and relative humidities (other environmental conditions remain the same):

		Air temperature (°C)			
		24	*28*	*32*	*36*
Relative humidity (%)	*40*	24	29	34	39
	50	24	29	35	41
	60	25	30	36	41
	70	26	31	37	42
	80	26	32	37	44
	90	27	32	38	45
	100	27	33	39	46

Note: Real feel temperatures are highlighted in grey.

(i) Describe the effect of relative humidity on the real feel temperature. Explain this phenomenon with reference to the effectiveness of heat loss from the body. (3 marks)

(ii) How does the effect described in (i) change at higher air temperatures? Explain this phenomenon with reference to the effectiveness of heat loss from the body. (3 marks)

(iii) Other than relative humidity and air temperature, suggest ***two*** environmental factors that may affect the real feel temperature. Explain your answer. (4 marks)

(iv) People who are engaged in outdoor work or activities are advised to drink plenty of water when the Hong Kong Observatory issues the Very Hot Weather Warning. Based on your biological knowledge, suggest an explanation for this advice. (1 mark)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 5 mark(s)**

Group C drinks no water and has a lower blood water potential than groups A and B, so more ADH is released. More water is reabsorbed in the collecting ducts, producing less urine.

First compare the drinking conditions: both A and B drink 1,500 mL of fluid, whereas C receives no additional water. The relatively lower blood water potential in C is detected by hypothalamic osmoreceptors, stimulating the posterior pituitary to release more antidiuretic hormone (ADH). ADH increases the permeability of collecting-duct walls to water, so more water returns from the filtrate to the blood and less remains in the ducts to form urine. This completes the causal chain: blood water potential → receptors → hormone → renal response → urine volume. ADH is produced in the hypothalamus and released by the posterior pituitary, not the medulla oblongata. Increased duct-wall permeability does not mean that more water flows out of the body.

a(ii)(1); original allocation: 1 mark(s)

Alcohol increases urine production.

Both A and B drink 1,500 mL, with alcohol in A being the principal difference. A produces more urine than B during the first few collection intervals, supporting a diuretic effect of alcohol. Compare A with B, which receives the same fluid volume. Comparing A only with C, which drinks nothing, cannot exclude the influence of fluid intake.

a(ii)(2); original allocation: 1 mark(s)

Alcohol may inhibit ADH release.

With less ADH, the collecting ducts are less permeable to water and reabsorb less water, increasing urine volume as observed in group A. This is an inferred hormonal mechanism based on urine output. The graph does not directly measure blood ADH, so the result cannot be described as a direct observation of hormonal change.

a(iii); original allocation: 2 mark(s)

Vigorous exercise increases water loss through sweating, subsequently reducing urine volume and interfering with the effect of the drinks on urine production.

For example, after both drinking 1,500 mL, a resting person and a runner who sweats heavily lose different amounts of water through the skin. The exerciser's lower blood water potential can stimulate ADH release and renal water

conservation. Without controlling exercise, differences in urine output cannot be attributed specifically to the drinks rather than sweating. Vigorous exercise should therefore be avoided during the experiment.

b(i); original allocation: 3 mark(s)

Higher relative humidity produces a higher apparent temperature because sweat evaporates less readily, removing less heat.

Read across a row at a fixed actual air temperature. For example, at 32°C, increasing relative humidity from 40% to 100% raises apparent temperature from 34°C to 39°C. Humid air already contains more water vapour, reducing the water-vapour difference between the moist skin surface and the air and slowing sweat evaporation. Evaporation absorbs heat, so less evaporation reduces cooling and makes the conditions feel hotter. The key is sweat evaporation, not simply "more sweating" or an assertion that humidity directly increases radiation.

b(ii); original allocation: 3 mark(s)

At higher air temperatures, the temperature difference between the skin and surrounding air is smaller. Heat loss by conduction, convection and radiation decreases, making the body more dependent on sweat evaporation. The restriction imposed by humidity on evaporation therefore has a greater effect.

Compare the same humidity change in the table. From 40% to 100% humidity, apparent temperature rises only from 24°C to 27°C in the 24°C row, but from 39°C to 46°C in the 36°C row. High air temperature reduces heat loss driven by a temperature difference. If high humidity also restricts evaporation, the fall in total heat loss is greater. The 46°C value is apparent temperature, not actual air temperature; it does not show that heat flows from air into the body in every condition in the table.

b(iii); original allocation: 4 mark(s)

Increasing wind speed promotes convection and sweat evaporation, lowering apparent temperature. Stronger sunshine increases the radiant heat absorbed by the body, raising apparent temperature.

Wind removes the warm, moist air next to the skin and replaces it with drier air, maintaining the evaporation gradient and increasing heat loss. For the second factor, compare shade with direct sunlight at the same air temperature and humidity: direct sunlight increases absorbed solar radiation and therefore feels hotter. For each factor, specify the direction of change and explain its effect on heat gain or loss. Listing "wind" and "sunlight" alone is incomplete.

b(iv); original allocation: 1 mark(s)

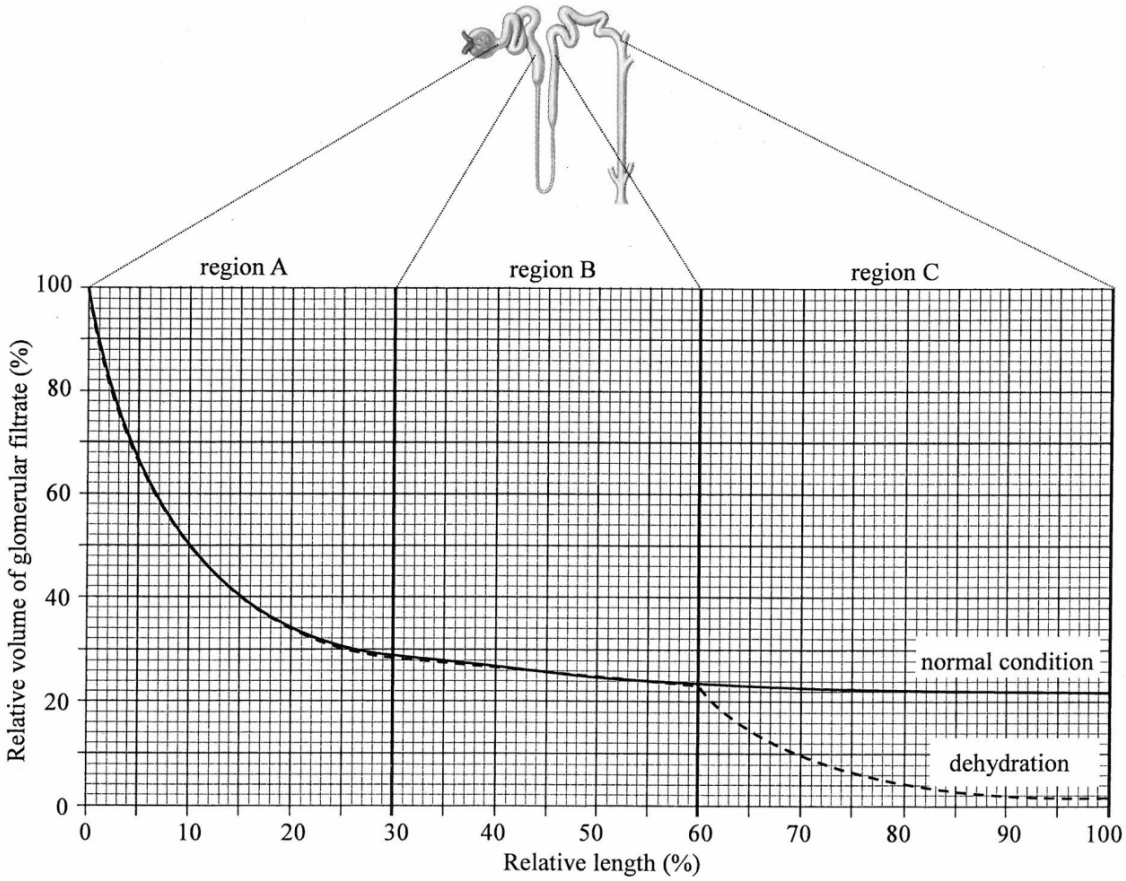
Replace water lost in sweat to maintain sufficient water for temperature regulation.

In hot conditions, sweat evaporation removes heat while using body water. Drinking replaces this loss, allowing circulation and cooling by sweating to continue. The emphasis is on replenishing water to support cooling, not on assuming that drinking more must increase urination.

Marking reference: HKDSE Biology 2018 Paper 2 Q1, official marking scheme, source page(s) 6.

2. [HKDSE Biology 2022 Paper 2 Q1]
Hint: Separate blood, filtrate and urine; track each substance.

(a) The graph below shows the change in volume of the glomerular filtrate along different regions of the kidney tubule under normal and dehydrated conditions:



(i) Describe how the glomerular filtrate is formed in the Bowman's capsule. (2 marks)

(ii) With reference to the above graph, state the region of the kidney tubule in which most water is reabsorbed. (1 mark)

(iii) If the average volume of the glomerular filtrate formed each day is 180 L, what is the volume of water reabsorbed daily in the region stated in (ii)? (1 mark)

(iv) Explain how water is reabsorbed into the blood from the glomerular filtrate within the region stated in (ii). (3 marks)

(v) With reference to the hormonal control of osmoregulation, explain the difference in the relative volume of the glomerular filtrate at the end of region C of the kidney tubule under the two different conditions mentioned above. (4 marks)

1(b) An experiment was conducted to investigate the respiratory responses during exercise in healthy persons and in patients with hardening of lung tissue. They were asked to ride a cycling machine set at the same resistance, holding a slow constant speed for ten minutes. Their breathing rates, breathing depths and plasma gas levels were continuously monitored throughout the experiment. The results are shown in the table below:

Time (min)	Breathing rate (breath min^{-1})		Breathing depth (L)		Plasma oxygen level (arbitrary unit)		Plasma carbon dioxide level (arbitrary unit)	
	Healthy persons	Patients	Healthy persons	Patients	Healthy persons	Patients	Healthy persons	Patients
0	14	20	0.9	0.7	82.5	75.8	42.5	42.0
2	16	29	1.8	1.0	83.9	70.2	43.2	42.4
4	18	35	2.2	1.1	84.0	67.1	43.3	43.5
6	19	37	2.3	1.2	84.3	63.2	43.8	43.6
8	20	40	2.4	1.2	84.3	62.0	43.9	43.7
10	20	44	2.4	1.2	84.3	60.5	43.9	43.7

(i) State how the breathing depth of the patients was different from that of the healthy persons. (2 marks)

(ii) Based on the condition of the patients, suggest an explanation for the difference stated in (i). (2 marks)

(iii) With reference to the data shown, deduce with reasons which parameter, plasma oxygen level or plasma carbon dioxide level, was more significant in bringing about changes in the breathing rate of the patients after six minutes. (3 marks)

(iv) With reference to the plasma gas levels, explain why patients would feel dizzy if the experiment continued for another ten minutes. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 2 mark(s)**

High blood pressure in the glomerulus filters water and small molecules from the blood into Bowman's capsule. Blood cells and most proteins remain in the blood.

High hydrostatic pressure drives plasma components through the filtration barrier of the glomerular capillaries and Bowman's capsule. Water, glucose, urea and ions that pass through form the glomerular filtrate. Blood cells and large proteins cannot pass because of their size.

a(ii); original allocation: 1 mark(s)

Region A.

In A, the curve falls from 100% to approximately 29%, meaning that about 71% of the filtered water has been reabsorbed. The decrease is smaller in each other individual region. Do not identify the site with the smallest remaining volume as necessarily reabsorbing the most water.

a(iii); original allocation: 1 mark(s)

Approximately 127.8 L.

Fraction reabsorbed in region A = 100% − 29% = 71%.

Volume = 180 L × 0.71 = 127.8 L.

Allowing for graph-reading uncertainty, approximately 126–129.6 L is acceptable. Include a volume unit.

a(iv); original allocation: 3 mark(s)

Useful solutes are actively reabsorbed from the filtrate into the blood, lowering blood water potential. Water moves into the blood by osmosis down its water-potential gradient.

Region A corresponds to the proximal convoluted tubule, where large amounts of glucose and ions are reabsorbed. Solute transfer gives the nearby blood a lower water potential than the tubular filtrate, so water moves passively across membranes towards the blood. Osmosis itself does not require ATP to drive water directly.

a(v); original allocation: 4 mark(s)

During dehydration, less filtrate remains at the end of C. The hypothalamus detects the lower blood water potential, and the pituitary releases more ADH. This increases the permeability of the wall of C to water and increases water reabsorption.

Dehydration makes the blood more concentrated and lowers its water potential. Hypothalamic osmoreceptors initiate ADH regulation. More ADH makes terminal regions, including the collecting ducts, more permeable to water, allowing more water to return to the blood. The broken curve therefore ends below the normal solid curve, representing a smaller volume of more concentrated urine. This negative feedback conserves body water.

b(i); original allocation: 2 mark(s)

The patient has a lower breathing depth at every time point and reaches the maximum earlier.

For example, at minute 6 the patient's depth is 1.2 L, compared with 2.3 L in the healthy person. The patient remains at 1.2 L from minute 6, whereas the healthy person reaches 2.4 L only at minute 8. Compare both the level and the time taken to reach a plateau.

b(ii); original allocation: 2 mark(s)

Stiffening of lung tissue reduces its elasticity and ability to expand, limiting the volume of air entering with each inhalation.

During normal inhalation, the lungs can expand fully as the thoracic cavity enlarges. Stiffened tissue cannot expand to the same extent, giving a smaller depth per breath and causing the expansion limit to be reached earlier.

b(iii); original allocation: 3 mark(s)

The plasma oxygen level is the more likely stimulus for the continuing rise in breathing rate during this interval.

From minutes 6–10, the patient's blood oxygen level falls from 63.2 to 60.5 while breathing rate rises from 37 to 44 breaths/minute. In the healthy person, blood oxygen stays at approximately 84.3 and breathing rate at approximately 19–20. Carbon dioxide is approximately stable in both groups during this interval, changing only from 43.6 to 43.7 in the patient. It therefore poorly explains the patient's specific continuing increase, so the data better support a low-oxygen stimulus.

b(iv); original allocation: 2 mark(s)

The patient's blood oxygen level may continue to fall, reducing oxygen supply to the brain and causing dizziness.

Even with more frequent breaths, stiff lungs limit effective ventilation. Falling blood oxygen reduces oxygen delivery to the brain and restricts aerobic respiration in brain cells, potentially causing dizziness.

Examiner's comment (source page(s) 8, 9)

Ultrafiltration is driven by blood pressure; not all plasma components enter Bowman's capsule by osmosis. Explain how solute transport establishes the gradient for water reabsorption. Judgements about respiratory stimuli must use the patient's data rather than simply reciting that carbon dioxide is usually the main stimulus.

Marking reference: HKDSE Biology 2022 Paper 2 Q1, official marking scheme, source page(s) 9, 10.

Practice V.b Regulation of body temperature

Related type 1

Connect core temperature, skin responses and water balance

Degree of integration ★★★★☆

Practice

1. [HKDSE Biology 2012 Paper 2 Q1]

Hint: Check the measurement site, then connect blood flow, sweat and heat loss.

1.(a) In a study about the replenishment of water after exercise, participants performed exercise until they lost 1 500 mL water. They were then divided into 3 groups and asked to consume a 2 000 mL sports drink containing 0, 50 and 100 mmol / L sodium respectively. Urine samples were collected at 1-hour intervals for 6 hours and the net body fluid balance throughout the course of the experiment was determined. The results are respectively shown in the Figures 1A and 1B below:

Key:

0 mmol / L sodium

50 mmol / L sodium

100 mmol / L sodium

Figure 1A

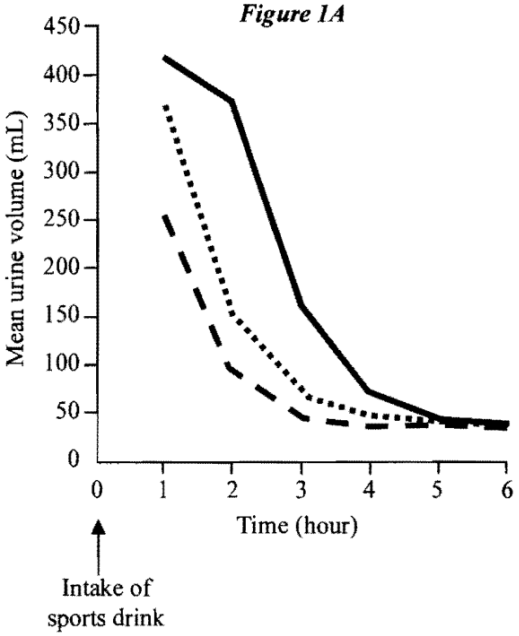
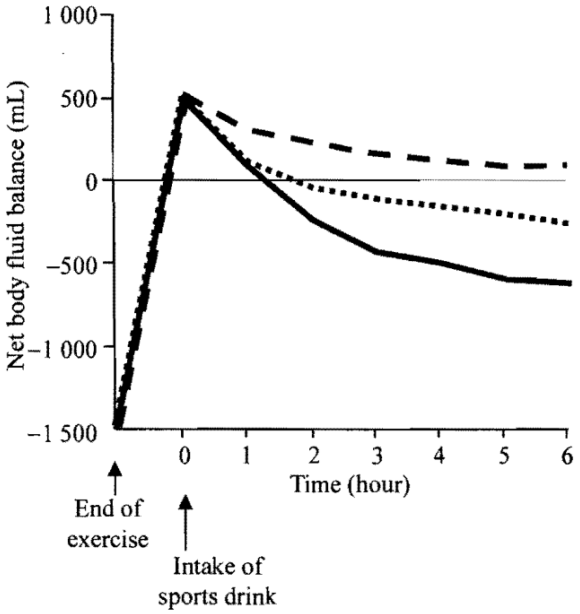


Figure 1B



(i) Describe the general patterns of the urine output after consuming drinks with different sodium concentrations. (4 marks)

(ii) Account for the high urine output of the participants who consumed sports drink with 0 mmol / L sodium. (4 marks)

(iii) In terms of the replenishment of water, which sports drink would you recommend for athletes to consume after exercise? Explain your answer. (3 marks)

1.(b) In the study described in (a), the participants performed exercise in a room maintained at 34°C and 60-70% relative humidity.

(i) State ***two*** major ways in which participants lost water during exercise. (2 marks)

(ii) If the temperature and relative humidity in the room had been set higher, it would have been dangerous to the participants. Explain why this is so. (4 marks)

(iii) After the exercise, the breathing rate of the participants remained at a fairly high level. Explain the significance of this. (3 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 4 mark(s)**

All three groups have their highest urine output in the first hour after drinking. Output then decreases continuously, becoming approximately stable and similar between groups by about the fifth hour. Higher sodium concentrations in the drinks produce lower urine outputs during the first few hours.

Describe the shared time trend separately from the differences between groups. For example, in the first hour, the 0 mmol/L group produces approximately 420 mL and the 100 mmol/L group approximately 250 mL. By hours five and six, all groups are near 40 mL. Do not restrict the comparison to one time point.

a(ii); original allocation: 4 mark(s)

Drinking the sodium-free drink raises blood water potential. The hypothalamus detects this change, and the posterior pituitary releases less antidiuretic hormone. The collecting ducts become less permeable to water, less water is reabsorbed, and more urine is produced.

Keep the directions consistent: higher water potential → less ADH → lower permeability → less reabsorption → more urine. The hypothalamus detects the change and the posterior pituitary releases ADH; do not confuse their roles.

a(iii); original allocation: 3 mark(s)

Based on the rehydration results, choose the drink containing 100 mmol/L sodium. This group produces less urine and retains more water. Figure 1B shows that its net fluid balance remains above zero throughout the observation period after drinking.

The question asks for a judgement based on the study, so use both the output in Figure 1A and the net retention in Figure 1B. This answer applies to these experimental conditions; a concentration from one study is not a universal drinking recommendation.

b(i); original allocation: 2 mark(s)

Water is lost in sweat and as water vapour in exhaled air.

Exercise increases heat production and ventilation, increasing water loss through both sweating and breathing.

b(ii); original allocation: 4 mark(s)

Exercise continuously produces heat. Hotter, more humid conditions hinder heat loss and may cause overheating. High humidity reduces sweat evaporation and hence evaporative heat loss. If environmental temperature approaches or exceeds body temperature, heat loss by convection and radiation decreases, and the body may even gain heat from its surroundings.

Do not assume that more sweating must produce greater cooling. Evaporation removes substantial heat; high humidity may cause sweat to run off without evaporating effectively.

b(iii); original allocation: 3 mark(s)

Maintaining a higher breathing rate supplies more oxygen to process lactate accumulated during exercise, for example by oxidising lactate or supplying energy for the liver to convert it back into glycogen, and helps restore normal blood pH.

Stopping exercise does not immediately complete all metabolic recovery. Greater oxygen supply supports recovery after exercise; connect faster breathing with this metabolic need.

Examiner's comment (source page(s) 2)

Use net fluid balance to support the rehydration recommendation. Link faster breathing after exercise to the restoration of blood pH as well.

Marking reference: HKDSE Biology 2012 Paper 2 Q1, official marking scheme, source page(s) 6.

2. [HKDSE Biology 2017 Paper 2 Q1]

Hint: Check the measurement site, then connect blood flow, sweat and heat loss.

(a) Ellen went swimming with her friends on a windy day. They competed to hold their breath under water for the longest time. Ellen finally won the competition by holding her breath for 2 minutes.

(i) When Ellen was holding her breath, which part of her brain was controlling the breathing actions? (1 mark)

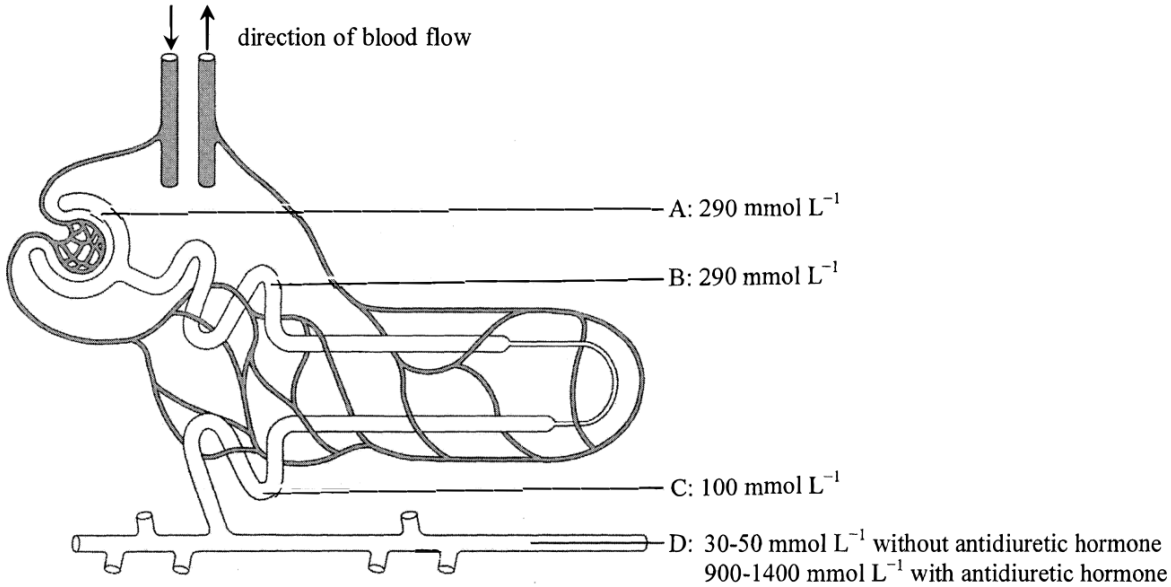
(ii) When Ellen left the water, she breathed rapidly and heavily.

(1) What was the stimulus leading to this response? How was this stimulus brought about when Ellen was holding her breath under water? (3 marks)

(2) Describe the nervous coordination leading to this response. (4 marks)

(iii) After Ellen had left the water, she felt cold in the wind. Give ***one*** physiological response her body would exhibit to help her regulate the body temperature. State the significance of this response. (3 marks)

1(b) The diagram below shows a nephron and its associated structures. The solute concentration of the fluid at different positions in the tubules are indicated:



(i) With reference to the physiological processes involved, explain why there is no change in the solute concentration of the fluid inside the tubule as the fluid flows from point A to point B. (4 marks)

(ii) Account for the difference in the solute concentration of fluid in point D with or without antidiuretic hormone (ADH). (3 marks)

(iii) If protein is present in the fluid in point D, which part of the nephron is most likely damaged? Explain your answer. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation (a)(i); original allocation: 1 mark(s)

The motor area of the cerebrum.

Holding the breath is conscious control of breathing movements and involves the cerebral cortex. Automatic regulation of the normal breathing rhythm principally involves the respiratory centre in the medulla oblongata. Since the question specifically concerns breath-holding, distinguish voluntary control from automatic responses.

(a)(ii)(1); original allocation: 3 mark(s)

Blood carbon dioxide concentration rises. During breath-holding, cells continue to respire and produce CO_2, but it cannot be removed by exhalation and therefore accumulates in the blood.

Include the stimulus, its source and the failure to remove it. Being underwater does not cause large amounts of dissolved carbon dioxide to enter the blood directly; the principal source is the person's own cellular metabolism.

(a)(ii)(2); original allocation: 4 mark(s)

Chemoreceptor mechanisms in locations including the medulla oblongata and carotid bodies detect CO_2/associated pH changes and stimulate the medullary respiratory centre. The centre increases nerve impulses to the respiratory muscles, making their contractions faster and stronger.

Organise the response as receptors → centre → motor nerves → effectors. More frequent and stronger contractions of the intercostal muscles and diaphragm make breathing faster and deeper, helping remove accumulated CO_2. The effectors are skeletal muscles; do not replace this pathway with a vague claim that hormones cause the lungs to contract by themselves.

(a)(iii); original allocation: 3 mark(s)

Skin arterioles constrict, reducing blood flow near the skin surface and heat loss to the environment, thereby maintaining core temperature.

Colder skin does not indicate failed regulation. Retaining more heat in deeper tissues is a heat-conserving response to cold. Alternatively, explain shivering: repeated muscular contractions increase respiratory metabolism and heat production. Develop one mechanism completely.

(b)(i); original allocation: 4 mark(s)

The proximal convoluted tubule reabsorbs useful solutes, and water follows by osmosis down a water-potential gradient. If water and solutes decrease in roughly the same proportion, the filtrate concentration can remain at 290 mmol L^{-1}.

An unchanged concentration does not imply an absence of reabsorption. Glucose, amino acids and sodium leave the tubular fluid, lowering the water potential of surrounding fluid so that water returns to the blood. Solute and water quantities decrease together, like removing a portion of solution with the same composition; the remaining concentration can therefore stay unchanged.

(b)(ii); original allocation: 3 mark(s)

With ADH present, the distal convoluted tubules and collecting ducts become more permeable to water. More water is reabsorbed, so solute concentration at D is higher. Without ADH, more dilute urine forms.

In the original figure, D is at 900–1400 mmol L^{-1} with ADH but only 30–50 without it. ADH mainly changes water permeability. Water is removed while most urinary solutes remain, increasing concentration; ADH does not add large amounts of protein to the urine.

(b)(iii); original allocation: 2 mark(s)

The glomerular filtration barrier may be damaged.

Normal plasma proteins are relatively large, and most are retained in the blood by the glomerular filtration barrier. Appreciable protein in the urine at D may indicate damage allowing proteins to leak into the filtrate. Identify the glomerulus as the most likely nephron region rather than naming only the whole kidney.

Examiner's comment (source page(s) 5)

Breath-holding causes carbon dioxide accumulation; do not first assume an increase in lactate. Explain respiratory regulation through receptors, the medullary centre and respiratory muscles, including changes in both contraction frequency and strength.

Marking reference: HKDSE Biology 2017 Paper 2 Q1, official marking scheme, source page(s) 8.

Practice V.c Regulation of blood gas levels

Related type 1

Explain exercise regulation using cardiac output and blood gases

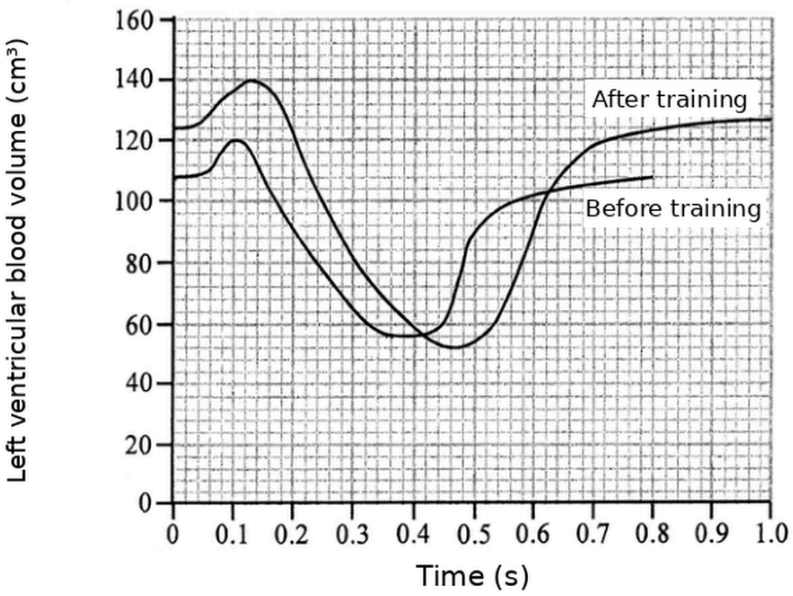
Degree of integration ★★★☆☆

Practice

1. [HKDSE Biology 2025 Paper 2 Q1 (English translation)]

⟵ Hint: Calculate cardiac output, then relate it to oxygen supply and regulation.

(a) A young man participated in a 12-month aerobic exercise training programme. Before the programme began and after it was completed, researchers measured the changes in the volume of blood in his left ventricle during one cardiac cycle while he was at rest. The results are shown below:

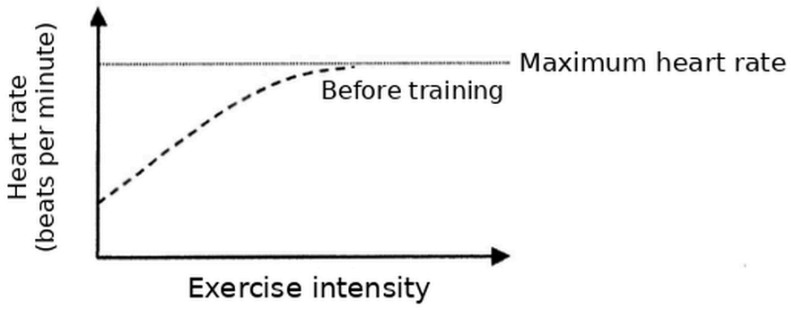


(i) Calculate the young man's stroke volume, heart rate and cardiac output before and after training. Copy the following table into your answer book and complete it. (3 marks)

	Before training	After training
Stroke volume (cm^3)		
Heart rate (beats per minute)		
Cardiac output ($cm^3\ min^{-1}$)		

(ii) Based on your answers in (i), suggest one change in heart structure that could explain the change in the young man's resting stroke volume after training. Explain your answer. (3 marks)

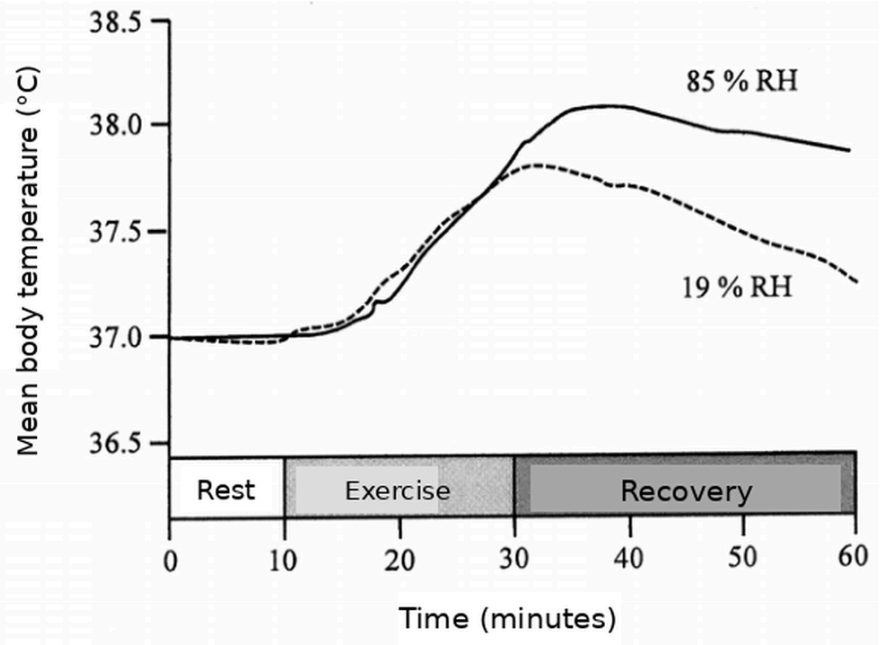
(iii) The graph below shows the young man's heart rate at different exercise intensities before training:



Copy the above graph into your answer book, including the dotted line, and sketch a curve to show the young man's heart rate at different exercise intensities after training. (2 marks)

(iv) Explain the change in resting cardiac output before and after training. (3 marks)

(b) To investigate the effects of relative humidity (RH) on thermoregulation during exercise and recovery in a hot environment (30 °C), one group of volunteers was exposed to high humidity (85% RH) and another to low humidity (19% RH). After resting for 10 minutes, the volunteers ran on a treadmill at a fixed speed for 20 minutes. They stood during the subsequent recovery period. The changes in their body temperature were recorded as shown below:



(i) (1) With reference to the graph, state two differences in mean body temperature between the two groups of volunteers during recovery. (2 marks)

(2) Based on your answer in (1), deduce the main route of heat loss in the volunteers exposed to 19% RH during recovery. (3 marks)

(ii) With reference to the graph, predict which group of volunteers had a higher level of ADH in the blood during recovery. Explain your answer. (4 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **(a)(i); original allocation: 3 mark(s)**

Before training: stroke volume 64 cm³, heart rate 75 beats/minute, cardiac output 4800 cm³/minute. After training: stroke volume 88 cm³, heart rate 60 beats/minute, cardiac output 5280 cm³/minute.

First read the maximum and minimum ventricular volumes within one cycle:

Before training: 120 − 56 = 64 cm³.

After training: 140 − 52 = 88 cm³.

Then read the duration of one cardiac cycle. Before training it is approximately 0.8 s, so 60 ÷ 0.8 = 75 beats/minute. After training it is approximately 1.0 s, so 60 ÷ 1.0 = 60 beats/minute.

Finally, cardiac output = stroke volume × heart rate:

64 × 75 = 4800 cm³/minute.

88 × 60 = 5280 cm³/minute.

(a)(ii); original allocation: 3 mark(s)

Stroke volume increases after training. One possible change is an enlarged ventricular cavity, holding more blood before ejection. Another is a thicker cardiac-muscle wall, allowing contraction to eject more blood.

Give one structural change and its effect. “The heart becomes stronger” does not identify a structure, while “the heart becomes larger” does not distinguish cavity capacity from muscle-wall thickness. Choose one explanation and follow structure → function → increased stroke volume.

(a)(iii); original allocation: 2 mark(s)

After training, the curve is lower at the same submaximal exercise intensity and reaches the same maximum heart rate only at a higher exercise intensity. See the answer graph below.

Retain the original horizontal level for maximum heart rate. Start the new curve lower, draw it rising below the original curve, and make it approach the same maximum further to the right. Do not lower the maximum itself.

(a)(iv); original allocation: 3 mark(s)

Resting cardiac output increases from 4800 to 5280 cm^3/minute. Although heart rate falls, the increase in stroke volume has the larger effect on their product, so cardiac output still rises.

Use the calculated results to acknowledge that this individual’s cardiac output increases, then explain the product. Stroke volume rises by 37.5% and heart rate falls by 20%, giving:

$1.375 \times 0.8 = 1.10$.

Cardiac output therefore increases by 10%. The general observation that athletes have lower resting heart rates does not imply that cardiac output must decrease in this question.

(b)(i)(1); original allocation: 2 mark(s)

During recovery, mean body temperature in the 19% RH group begins to decrease earlier and falls faster. Its mean temperature is also subsequently lower than that of the 85% RH group.

Choose two differences in the overall trends. Locate the start of recovery, then compare the onset of cooling, slopes or overall levels. A temperature difference at one time point cannot be counted as two independent differences.

(b)(i)(2); original allocation: 3 mark(s)

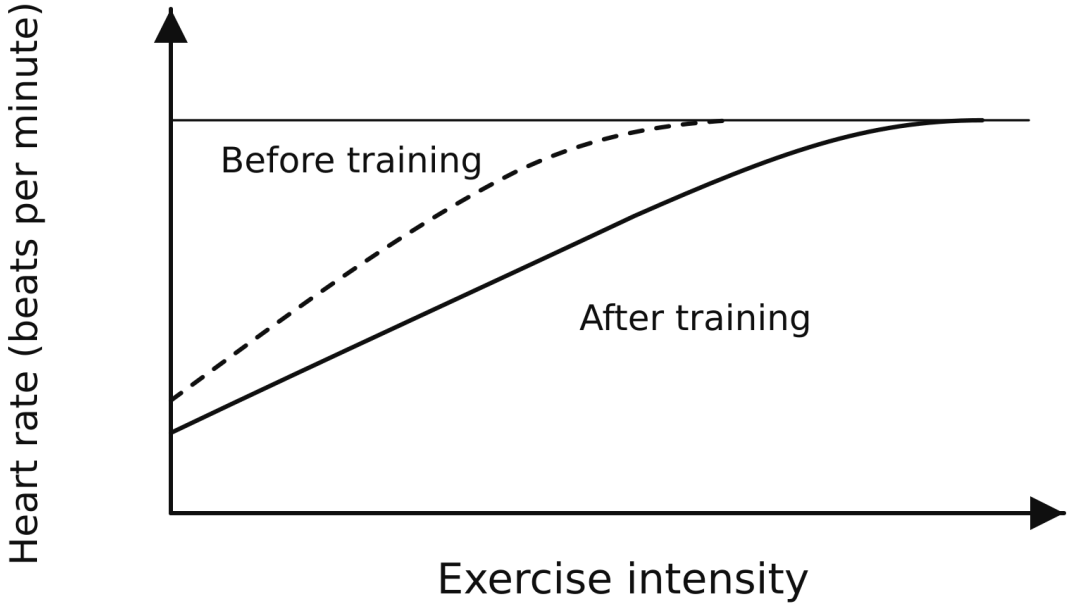
The 19% RH group mainly loses heat through sweat evaporation. Its faster fall in body temperature supports more effective heat loss. Lower relative humidity favours sweat evaporation, which absorbs heat from the body.

Environmental temperature is the same for both groups; relative humidity is the main variable. Connect lower humidity → faster evaporation → more effective heat loss. “Sweating” alone does not identify the process that actually removes heat.

(b)(ii); original allocation: 4 mark(s)

According to the graph and marking reference, predict a higher ADH level in the 85% RH group. Its higher temperature during recovery triggers a stronger sweating response and greater water loss, lowering blood water potential and stimulating more ADH release.

Distinguish sweat production from evaporation efficiency. At high humidity, sweat evaporates less readily and cooling is less effective. The higher body temperature may nevertheless cause more sweat to be secreted. Here, water loss and ADH are inferred from the temperature response; sweat volume and hormone concentration were not measured directly in the graph. Corresponding measurements would still be needed to test the inference.



Answer graph from the official marking reference: the curve after training is lower and reaches the same maximum heart rate at a higher exercise intensity.

Examiner's comment (source page(s) 75, 76)

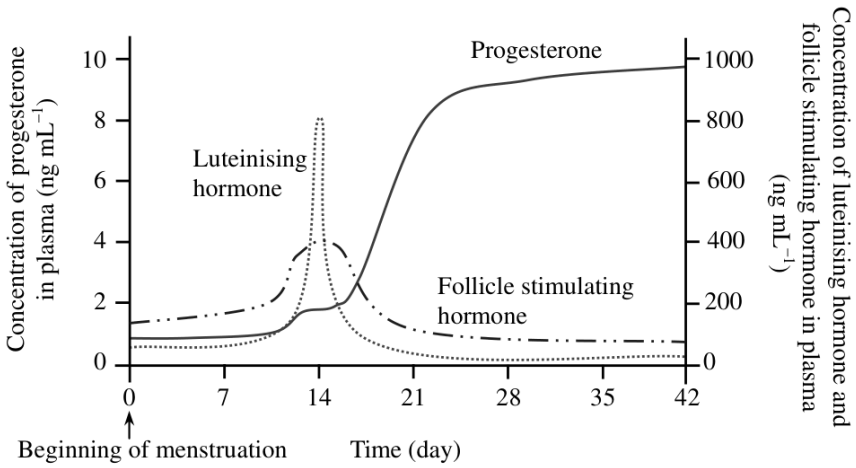
Read stroke volume and cycle duration from the graph before calculating. The answer curve must reach the same maximum heart rate at a higher exercise intensity. In humidity questions, distinguish sweating from sweat evaporation; dry conditions alone do not establish greater water loss.

Marking reference: HKDSE Biology 2025 Paper 2 Q1, official marking scheme, source page(s) 61, 62.

2. [HKDSE Biology Practice Paper Paper 2 Q1]

Hint: Calculate cardiac output, then relate it to oxygen supply and regulation.

(a) The following graph shows the plasma concentrations of progesterone, luteinising hormone and follicle stimulating hormone of a woman over a period of time:



(i) Name the process that took place in the ovary on day 14. (1 mark)

(ii) Account for the change in the plasma concentration of progesterone from day 14 to day 42. (3 marks)

(iii) What would happen to the woman if the progesterone level dropped significantly on day 35? (1 mark)

(iv) With reference to the changes in the concentration of the hormones shown in the above graph, explain why progesterone can be used as a drug for contraception. (3 marks)

1. (b) The table below shows the data of some parameters of the cardiovascular and respiratory systems of a healthy untrained person at rest, during light exercise and during vigorous exercise:

	At rest	Light exercise	Vigorous exercise
Heart rate (beats / min)	75	145	190
Stroke volume (dm^3)	0.07	0.09	0.11
Breathing rate (breaths / min)	14	24	40
Tidal volume (dm^3)	0.86	1.67	2.50

(i) Using the data provided, calculate the cardiac output (dm^3 / min) and ventilation rate (dm^3 / min) of this person when he is at rest, when he is doing light exercise and when he is doing vigorous exercise respectively. (2 marks)

(ii) State the changes in this person's cardiac output and ventilation rate with the increasing level of exercise. What is the importance of these changes? (4 marks)

(iii) Describe how the person's sympathetic nerve brings about the change in the cardiac output during exercise. (2 marks)

(iv) Explain why the volume of urine produced by this person after vigorous exercise is less than usual. (4 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 1 mark(s)**

Ovulation.

The LH peak on day 14 corresponds to ovulation.

a(ii); original allocation: 3 mark(s)

The LH surge promotes formation of the corpus luteum from follicular cells remaining after ovulation. The corpus luteum secretes progesterone, causing its rise after day 14. Continued high progesterone after day 28 supports the conclusion that the woman is pregnant. In early pregnancy, the corpus luteum continues secreting progesterone to maintain the uterine lining.

Identify the hormone's source and explain why it does not fall as in a cycle without conception; simply describing successive high and low levels is insufficient.

a(iii); original allocation: 1 mark(s)

The uterine lining may be shed, potentially causing miscarriage.

Progesterone maintains the lining in early pregnancy. A sudden large fall in the stated situation reduces this support.

a(iv); original allocation: 3 mark(s)

Sustained high progesterone exerts negative feedback on the hypothalamic–pituitary axis, keeping FSH and LH relatively low. Follicle development and the LH surge are suppressed, making ovulation unlikely.

Use the graph to relate high progesterone to low FSH/LH, then connect this to the absence of mature follicles and ovulation. Progesterone does not directly kill ova.

b(i); original allocation: 2 mark(s)

Cardiac output = heart rate × stroke volume.

At rest: $75 \times 0.07 = 5.25$ dm^3 min^{-1}.

Light exercise: $145 \times 0.09 = 13.05$ dm^3 min^{-1}.

Vigorous exercise: $190 \times 0.11 = 20.9$ dm^3 min^{-1}.

Ventilation rate = breathing frequency × tidal volume.

$14 \times 0.86 = 12.04$ dm^3 min^{-1}.

$24 \times 1.67 = 40.08$ dm^3 min^{-1}.

$40 \times 2.50 = 100$ dm^3 min^{-1}.

Each set of three correct results earns 1 mark. Both formulae multiply volume per event by events per minute, giving volume per minute.

b(ii); original allocation: 4 mark(s)

As exercise intensity increases, both cardiac output and ventilation rate increase. Faster blood transport and pulmonary gas exchange supply working muscles with more oxygen and glucose, supporting increased respiration and ATP supply. Carbon dioxide and other metabolites are also removed faster, helping maintain the internal environment.

Link the data trend to supply, energy production and removal rather than simply stating that breathing and heartbeat become faster.

b(iii); original allocation: 2 mark(s)

Increased sympathetic action on the heart makes the sinoatrial node generate impulses more frequently and strengthens cardiac-muscle contraction. Heart rate and stroke volume increase, raising cardiac output.

Sympathetic nerve endings act on the heart mainly through noradrenaline. Adrenaline secreted by the adrenal medulla can also enhance the response; do not confuse these two sources.

b(iv); original allocation: 4 mark(s)

Water loss through sweating during vigorous exercise lowers blood water potential. The hypothalamus detects the change and stimulates the posterior pituitary to release more ADH. ADH increases collecting-duct permeability to water, allowing greater reabsorption by osmosis. Urine volume falls and urine becomes more concentrated.

Connect water loss, water potential, ADH, permeability and reabsorption continuously. The kidneys do not simply detect heat and stop producing urine.

Examiner's comment (source page(s) 3, 4)

A good answer shows formulae, substitutions and units, linking the data trends to muscular oxygen demand and metabolism. Explain the corpus luteum and maintenance of pregnancy in the progesterone question rather than only describing the curves.

Marking reference: HKDSE Biology Practice Paper Paper 2 Q1, official marking scheme, source page(s) 11.

Practice V.d Hormonal control of the reproductive cycle

Related type 1

Infer ovulation, contraception and exercise responses from hormone curves

Degree of integration ★★★☆☆

Practice

1. [HKDSE Biology 2015 Paper 2 Q1]
Hint: Align each hormone's source, target and timing.

(a) A young couple, John and Judy, wanted to have a baby. After a year of trying, Judy had still not become pregnant. They consulted their family doctor on this issue. The doctor suggested that both of them should undergo some tests to check their fertility.

(i) The doctor requested a semen sample from John for microscopic examination. Suggest *two* items in the sample that should be checked. (2 marks)

(ii) After collecting information about Judy's menstrual cycle, the doctor requested blood samples from Judy to monitor her levels of LH and FSH.

(1) Why should the levels of LH and FSH be checked? (3 marks)

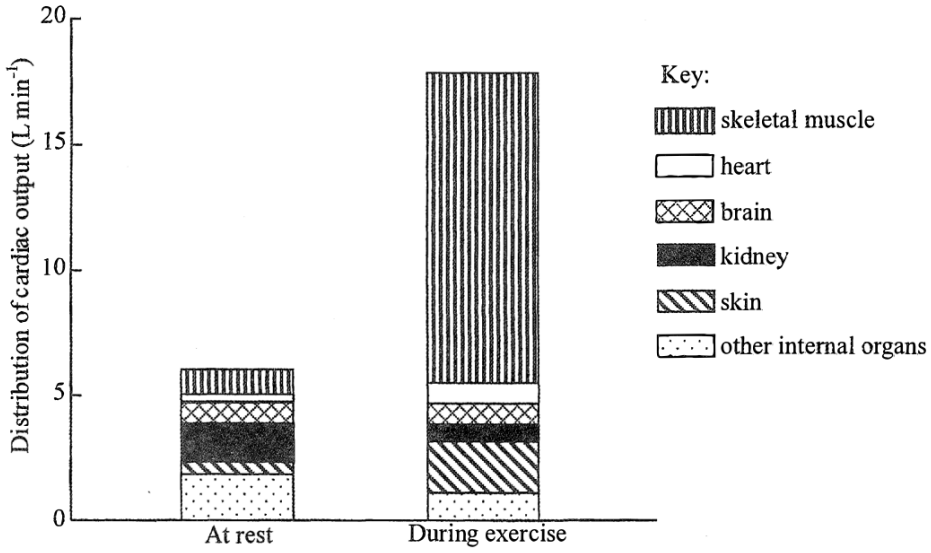
(2) The doctor also checked the condition of Judy's oviducts. What is the importance of this examination? (2 marks)

(iii) All the test results were normal. Three months later, Judy did not experience menstruation on the expected day.

(1) Explain why the absence of menstruation can be a sign of pregnancy. (2 marks)

(2) A hormone named HCG was detected in Judy's blood and urine. HCG prevents the degeneration of the yellow body. Based on the action of HCG, explain why Judy did not experience menstruation. (2 marks)

1(b) The diagram below shows the distribution of cardiac output to various parts of the human body at rest and during exercise:



(i) With reference to the control of heart beat, briefly describe *two* regulatory ways in which the cardiac output can be increased during exercise. (2 marks)

(ii) Explain the importance of skeletal muscles having the greatest increase in blood supply during exercise. (3 marks)

(iii) Explain why there is an increase in blood flow to the skin during exercise. (4 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation (a)(i); original allocation: 2 mark(s)

Check sperm count and sperm motility. Abnormal sperm morphology is another acceptable item.

Fertilisation requires enough sperm to reach the ovum, effective swimming and normal structure. The question asks for two features that can be examined in the sample, so name measurable or observable features.

(a)(ii)(1); original allocation: 3 mark(s)

Check whether FSH and LH rise normally before ovulation. FSH promotes follicle development, while the LH surge induces ovulation.

Follicles must develop before an ovum can be released at the appropriate time. FSH and LH participate in these linked steps, so their concentrations and changes across the cycle help assess fertility.

(a)(ii)(2); original allocation: 2 mark(s)

Confirm that the oviducts are not blocked, allowing sperm and ova to travel through them and meet for fertilisation.

Even with normal hormone secretion and ovulation, a blocked pathway may prevent gametes meeting. This examination assesses the transport route rather than measuring hormones again.

(a)(iii)(1); original allocation: 2 mark(s)

Absence of menstruation means the uterine lining has not been shed at the end of the previous cycle. An embryo may have implanted and enabled maintenance of the lining.

Menstruation results from shedding of the lining, so its retention may indicate pregnancy. “May” does not mean that delayed menstruation alone confirms pregnancy.

(a)(iii)(2); original allocation: 2 mark(s)

HCG prevents degeneration of the corpus luteum. Continued progesterone and oestrogen secretion maintains the uterine lining, so menstruation does not occur.

Start with the stated action of HCG and connect the corpus luteum, hormones and lining. Saying only that HCG suppresses menstruation omits the structure and hormones through which it acts.

(b)(i); original allocation: 2 mark(s)

The cardiovascular centre increases sympathetic stimulation of the pacemaker, and the adrenal glands secrete more adrenaline.

Both routes can make the heart contract faster or more strongly, increasing blood pumped per minute. One uses nervous signals and the other a blood-borne hormone; distinguish them clearly.

(b)(ii); original allocation: 3 mark(s)

More blood flows to skeletal muscles, delivering more oxygen and nutrients for respiration and ATP production to support contraction, while removing carbon dioxide faster.

The figure shows the largest blood-flow increase in skeletal muscles. Explain it through their increased energy demand during exercise. Greater supply and waste removal are two consequences of the same increase in circulatory flow.

(b)(iii); original allocation: 4 mark(s)

Muscle activity produces more heat. Thermoreceptors in the hypothalamus or skin detect the rise in body temperature. The heat-loss centre sends nervous signals causing arterioles near the skin to dilate, increasing surface blood flow for heat loss.

Build the causal sequence from heat production through receptors and the centre to the vascular response and heat loss. Increased skin blood flow is regulated; blood does not change routes arbitrarily.

Examiner’s comment (source page(s) 4)

Assess whether the expected reproductive-hormone peaks occur over the whole cycle. Low LH or FSH at one moment does not establish an abnormality. Explain exercise responses through nervous signals, organ responses, oxygen supply and ATP demand in sequence.

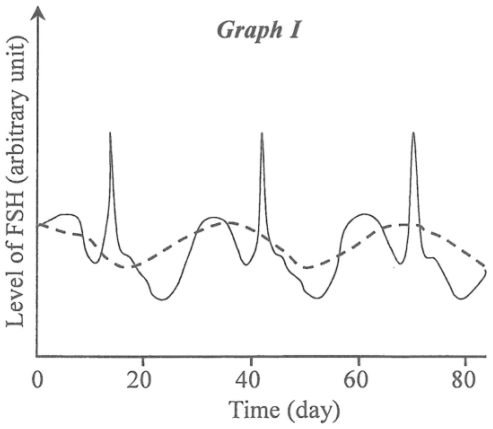
Marking reference: HKDSE Biology 2015 Paper 2 Q1, official marking scheme, source page(s) 7.

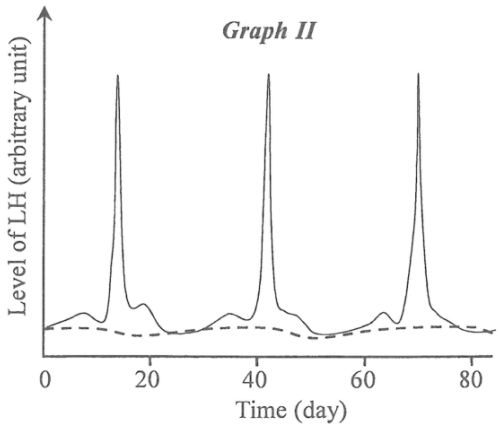
2. [HKDSE Biology 2024 Paper 2 Q1]
Hint: Align each hormone's source, target and timing.

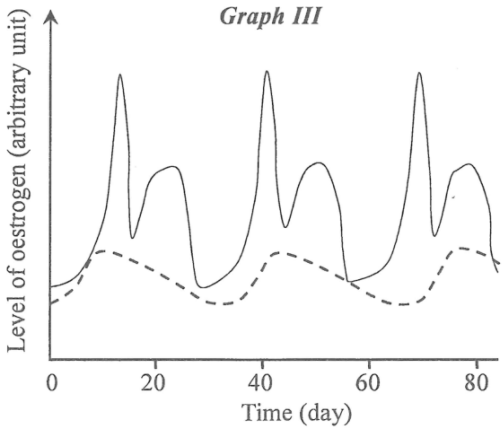
(a) A contraceptive pill contains a synthetic hormone X. The graphs below show the plasma levels of three hormones in a woman during normal menstrual cycles and those when she was taking the pill daily:

Graph I: follicle stimulating hormone (FSH)
Graph II: luteinising hormone (LH)
Graph III: oestrogen

Key:
——— hormone level during normal cycles
- - - - - hormone level when taking the pill daily







(i) With reference to Graph I and Graph II, explain how this contraceptive pill works. (4 marks)

(ii) The effect of this synthetic hormone X is similar to one of the female sex hormones. Which female sex hormone is it? Explain your answer. (2 marks)

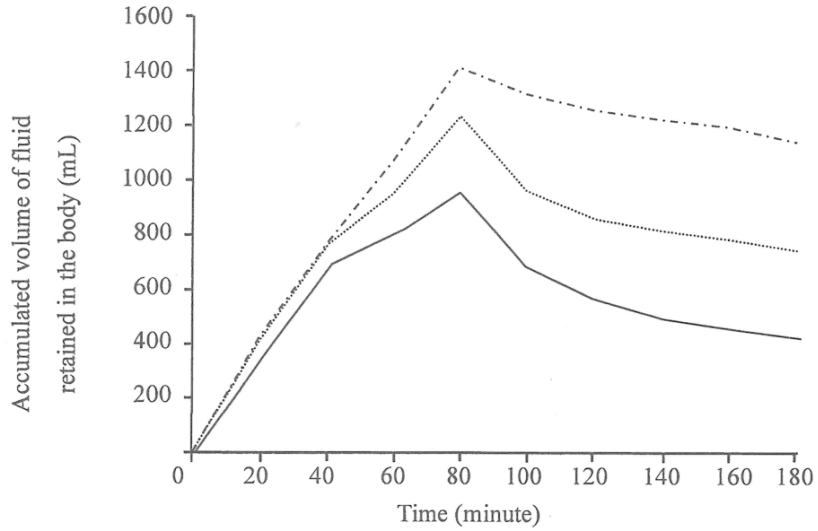
(iii) Another effect of taking this contraceptive pill is that the uterine lining becomes thinner. Explain why this phenomenon also contributes to contraception. (1 mark)

(iv) With reference to Graph III, explain why the uterine lining became thinner when she was taking the pill daily. (2 marks)

1(b) In a study to compare the ability to stay hydrated after drinking different types of drinks, healthy participants were required to consume the same diet and undergo the same physical activities on the day before the experiment.

On the day of the experiment, they emptied their bladders and then sat quietly for 20 minutes. The participants were divided into three groups. Each consumed a different drink (water, sports drink with glycerol and sports drink with salt). 500 mL of the drink was consumed at 0, 20, 40 and 60 minutes while urine was collected every 20 minutes for 180 minutes. Throughout the experiment, the participants sat quietly in the laboratory and were exposed to the same settings of temperature and humidity. The change in the accumulated volume of fluid retained in the body are shown in the graph below:

Key:
——— drinking water only
..... drinking sports drink with glycerol
-·-·-·- drinking sports drink with salt



(i) In this experiment, the ability to stay hydrated is expressed in terms of the accumulated volume of fluid retained in the body after consuming the drinks. Give a word formula to show how to calculate the accumulated volume of fluid retained from the data collected. (1 mark)

(ii) Explain the difference in the accumulated volume of fluid retained between the group which consumed water and the group which consumed the salt solution. (4 marks)

(iii) The ability to stay hydrated is crucial for marathon runners because access to water is limited. Explain why retaining more water is advantageous to marathon runners. (3 marks)

(iv) Suggest ***two*** modifications to the experiment to verify your answer in (iii). (2 marks)

(v) Suggest another advantage marathon runners have when drinking sports drinks with glycerol. (1 mark)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 4 mark(s)**

The drug keeps FSH low and removes its normal peak, preventing normal follicle maturation. LH also lacks a surge, so ovulation does not occur.

Compare each graph and link each change to its effect. FSH stimulates follicle development, so suppressed secretion reduces mature-follicle formation. The mid-cycle LH surge triggers ovulation. Removing this surge prevents release of a mature ovum for fertilisation.

a(ii); original allocation: 2 mark(s)

Progesterone.

At relatively high levels, progesterone exerts negative feedback on the hypothalamus and pituitary, inhibiting FSH and LH secretion. X produces a similar inhibitory effect, resembling progesterone action.

a(iii); original allocation: 1 mark(s)

A thinner uterine lining is less suitable for implantation.

Even if fertilisation occurs, the early embryo must implant in an appropriately developed lining to establish subsequent nutrient supply. An excessively thin lining can hinder this step.

a(iv); original allocation: 2 mark(s)

Oestrogen is generally lower during drug treatment, reducing stimulation of uterine-lining thickening.

The treatment curve in Figure III lacks the normal cycle's peaks. Oestrogen normally promotes proliferation of the lining, so suppressed levels prevent normal thickness from developing. "Because ovulation does not occur" alone does not explain this mechanism.

b(i); original allocation: 1 mark(s)

Cumulative retained fluid volume = cumulative fluid intake − cumulative collected urine volume.

Both quantities must cover the same time interval. This is the question's defined retention indicator; do not subtract urine from one 20-minute interval from total fluid intake.

b(ii); original allocation: 4 mark(s)

The saline sports-drink group retains more fluid. Relative to the water-only group, it has lower blood water potential, greater ADH release, higher collecting-duct water permeability and greater water reabsorption.

With equal fluid intake, salt increases blood solute concentration, making water potential lower than with water alone. The hypothalamus responds by stimulating the posterior pituitary to release more ADH. More water channels are inserted in collecting-duct walls, returning more filtered water to the blood and reducing urine output. Compare the relative difference between groups; it is unnecessary to claim that every drink lowers water potential below its pre-drinking value.

b(iii); original allocation: 3 mark(s)

Retaining more water provides water for sweating. Sweat evaporation removes heat generated by muscles during running. Sustained muscular contraction and metabolism increase body heat. With limited opportunities to drink, greater prior water retention can sustain sweating longer. Conversion of sweat from liquid to vapour absorbs heat, helping prevent an excessive rise in body temperature.

b(iv); original allocation: 2 mark(s)

Replace sitting at rest with running on a treadmill at a fixed speed. In addition to the original measurements, measure body temperature or sweat production.

The first change makes activity more representative of marathon exercise. The second directly tests whether greater retained water supports heat loss. Keep exercise intensity, duration and indoor conditions equal between groups to compare drink effects.

b(v); original allocation: 1 mark(s)

Glycerol can provide energy for metabolism.

Glycerol can enter cellular metabolic pathways and supply usable energy during sustained exercise. This benefit is separate from water retention.

Examiner's comment (source page(s) 74, 75)

Compare normal and treated curves in each graph to explain contraception; merely reciting FSH/LH functions is insufficient. Compare the drinks through blood water potential and ADH rather than importing a different year's post-exercise rehydration scenario. The modified experiment should have both groups run at the same speed and measure body temperature or sweat production to test cooling.

Marking reference: HKDSE Biology 2024 Paper 2 Q1, official marking scheme, source page(s) 60, 61.

Chapter 8

Applied ecology: question types and practice

VI.a Human impacts on the environment

Read the explanations and diagrams in Concept VI.a first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Evaluate management through renewal, competition and diversity

Degree of integration ★★★☆☆

Key ideas

① If a fishery doubles its number of boats, a small increase in total catch does not immediately show that the fish population has grown. Compare catch per unit effort and consider fish growth, reproduction and replenishment.

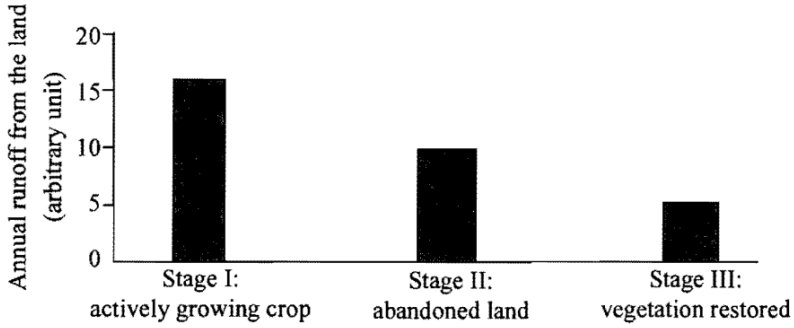
② Forest management also has several indicators. Taller remaining trees may reflect reduced competition without greater species diversity. If a table gives increases in diversity index, compare those increases; do not treat them as final index values.

How to recognise the problem Determine whether the comparison concerns totals, per-unit effort, final values or increases. Then explain management effects through resources, competition, habitat and replenishment.

Example 1 [HKDSE Biology 2012 Paper 2 Q2]

!.(a) In shifting agriculture, plots of land on hill slopes are cleared of trees so as to grow crops. The trees are then burnt and the ash is dispersed throughout the field as fertilizer. After growing crops for several years until the soil loses its fertility, the land will be abandoned. After a period of time, the land will be recolonized by natural vegetation. Figure 2A below shows the annual runoff (water run off from soil) at 3 different stages of shifting agriculture:

Figure 2A



(i) Briefly describe the process in Stage II that leads to the restoration of the natural vegetation in Stage III. (3 marks)

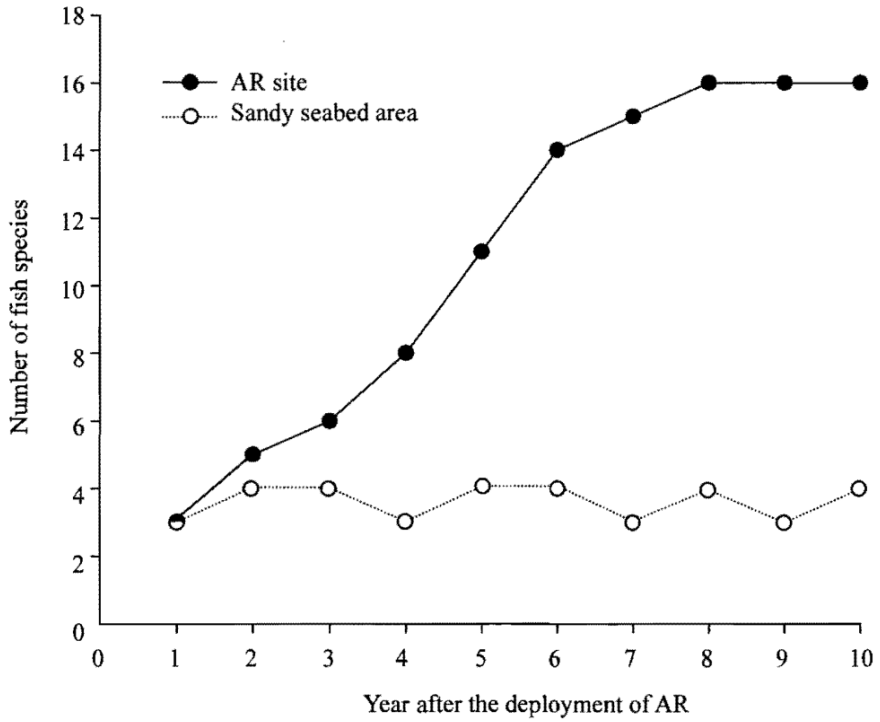
(ii) Suggest ***two*** agricultural activities which could account for the difference in the annual runoff between Stage I and Stage III. (5 marks)

(iii) As soil nutrients are depleted in Stage I, the leaves of the crops become yellow. Which soil nutrient is probably lacking? Explain your answer. (2 marks)

(iv) State ***two*** ways in which soil nutrients are lost from land. (2 marks)

2.(b) An artificial reef (AR) is a man-made structure deployed on the seabed to enhance biodiversity. A ten-year study was carried out on a sandy seabed to investigate the effect of an AR on the number of fish species. The number of fish species at the AR site (around and within AR) was compared with another similar sandy seabed area nearby. The results are shown in Figure 2B below:

Figure 2B



(i) Compare the results obtained in terms of the number of fish species at the AR site and the sandy seabed area. (3 marks)

(ii) Explain why AR has an effect on the number of fish species at the AR site. (3 marks)

(iii) Give ***two*** criteria for the selection of suitable materials for AR construction and explain the importance of these criteria. (2 marks)

Solution a(i); original allocation: 3 mark(s)

Method: Set the comparison basis, then examine resource renewal and competition.

Fast-growing plants with relatively low nutrient requirements, such as mosses and ferns, first colonise vacant land. Their death and decomposition increase soil fertility. The improved soil then supports shrubs and trees, which gradually become the dominant vegetation.

This is ecological succession. Explain how early plants modify soil to allow later plants to establish; listing "grass, shrubs, trees" alone is insufficient.

a(ii); original allocation: 5 mark(s)

Run-off is greater in stage I than in stage III. First, repeated tillage disrupts and loosens soil structure, making soil easier for rainwater to remove. Second, harvesting removes organic matter in plants, reducing its return to the soil. Less humus lowers water-holding capacity.

State the observed difference for 1 mark, then link each of the two agricultural activities to soil changes and water loss. More fertiliser is not direct evidence of greater water retention.

a(iii); original allocation: 2 mark(s)

Magnesium deficiency is possible because magnesium is required for chlorophyll production. Alternatively, nitrogen/nitrate deficiency may be responsible because nitrogen is needed to synthesise chlorophyll and other nitrogen-containing substances.

Yellowing leaves indicate insufficient green chlorophyll. Choose one element and explain its connection to chlorophyll; the vague statement "lack of fertiliser" is insufficient.

a(iv); original allocation: 2 mark(s)

Some nutrients dissolve in water and are carried away in run-off. Others are adsorbed onto soil particles and lost when those particles are eroded.

Distinguish transport in solution from transport attached to solid soil particles.

b(i); original allocation: 3 mark(s)

At the artificial reef, fish-species richness rises from approximately 3 species in year 1 to approximately 16 in year 8, then remains stable. On the sandy seabed it fluctuates only between approximately 3 and 4 species. Apart from similar initial values, species richness is therefore higher at the artificial reef.

Compare species number, not the total number of individual fish. Include the rise followed by a plateau, the absence of a marked change in the control, and the difference between sites.

b(ii); original allocation: 3 mark(s)

The reef increases habitat diversity, attracting fish that use different conditions. Crevices provide shelter from predators as well as feeding and breeding sites. As more organisms establish, other fish may be attracted to feed on them.

Explain increased species richness through specific habitat functions. “A better environment” does not identify the changed resource or interaction. The official maximum for this part is 3 marks.

b(iii); original allocation: 2 mark(s)

Choose non-toxic materials to avoid harming organisms, and corrosion-resistant materials to make the reef durable. Rough surfaces are another option, allowing larvae and other organisms to attach readily.

Choose any two features and link each to its ecological function or protective effect.

Examiner’s comment (source page(s) 2)

Soil nutrients can be lost both dissolved in water and attached to eroded soil particles. Artificial reefs can increase the range of available habitats.

Marking reference: HKDSE Biology 2012 Paper 2 Q2, official marking scheme, source page(s) 7.

Example 2 [HKDSE Biology Sample Paper Paper 2 Q2]

2.(a) The table below shows the total fish catch and the total number of days spent on catching tuna for all the fishing boats, i.e. the fishing effort, in the Atlantic Ocean from 1980 to 1987:

Year	Total fish catch (ton x 10^3)	Fishing effort (number of days x 10^3)	Fish catch per unit fishing effort (ton per day)
1980	40	5	8.00
1981	45	8	5.63
1982	44	12	3.67
1983	60	20	3.00
1984	80	30	2.67
1985	85	35	2.43
1986	90	40	*x*
1987	100	60	*y*

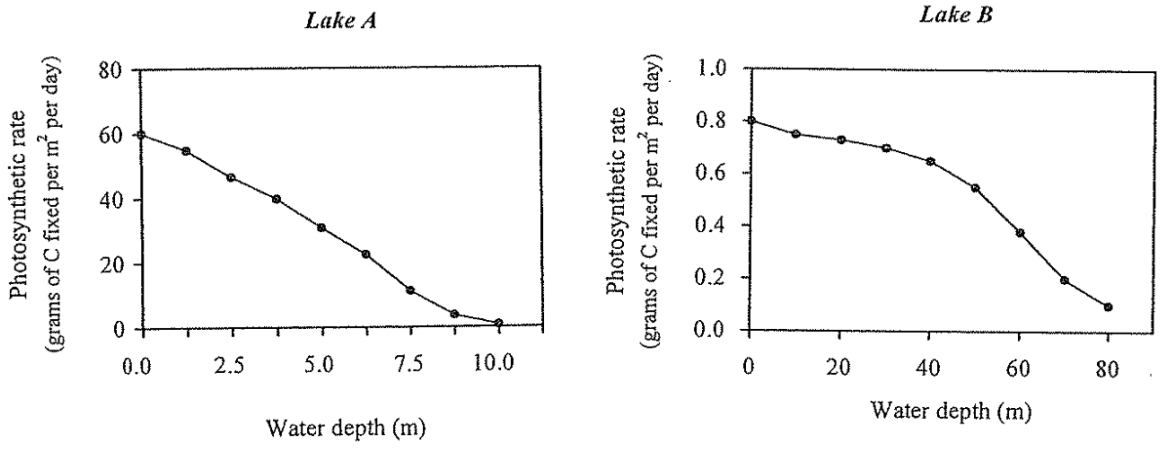
(i) Calculate the values of *x* and *y*, which represent the fish catch per unit fishing effort in the years of 1986 and 1987 respectively. (1 mark)

(ii) Plot a graph to show the fish catch per unit fishing effort from 1980 to 1987. (3 marks)

(iii) Referring to your graph and the data given above, explain the change in fish catch per unit fishing effort from 1980 to 1987. (3 marks)

(iv) Suggest ***two*** control measures for fishing and describe how each of them can help to maintain a sustainable supply of fish. (4 marks)

2.(b) The diagrams below show the changes in photosynthetic rate with water depth of two lakes, A and B. One of the lakes is polluted by chemical fertilizers leached from nearby farmlands.



(i) Explain the change in photosynthetic rate with increasing water depth in lake A. (2 marks)

(ii) Which of these lakes is polluted by chemical fertilizers? Explain your answer. (3 marks)

(iii) Which lake is more likely to experience a greater diurnal fluctuation (i.e. between day and night) of dissolved oxygen content? Explain your answer. (4 marks)

Solution a(i); original allocation: 1 mark(s)

x = 2.25, y = 1.67.

Catch per unit effort is total catch divided by total fishing days. The factors of 10^3 in both columns cancel.

x = 90 ÷ 40 = 2.25.

Method: Set the comparison basis, then examine resource renewal and competition.

$y = 100 \div 60 \approx 1.67$.

Keep two decimal places, consistent with the other years.

a(ii); original allocation: 3 mark(s)

Plot year on the horizontal axis and catch per unit fishing effort per day on the vertical axis. Plot all eight points and join them chronologically to form a declining curve.

Choose uniform scales: 1980–1987 horizontally and a vertical range accommodating 0–8.00. The successive annual values are 8.00, 5.63, 3.67, 3.00, 2.67, 2.43, 2.25 and 1.67. Label both axes, include the vertical-axis unit and add an informative title. Locate each value at its year and connect the points in order. Do not plot total catches such as 40 and 45 or draw an unsupported diagonal line without locating the actual data.

a(iii); original allocation: 3 mark(s)

Catch per unit effort declines continuously because fishing effort increases faster than total catch. This may reflect depletion of fish stocks by overfishing, requiring more effort to catch the same quantity of fish.

In 1980, 5×10^3 fishing days yielded 40×10^3 tonnes. In 1987, 100×10^3 tonnes required 60×10^3 fishing days. Total catch increased only 2.5-fold, whereas effort increased 12-fold, reducing return per unit effort. A greater total catch does not necessarily indicate a larger fish population; standardise for effort first. Overfishing is a reasonable explanation, but these data alone cannot exclude all other influences on catch efficiency.

a(iv); original allocation: 4 mark(s)

Set a minimum mesh size so juvenile fish can escape and reach reproductive age. Establish a closed fishing season during breeding to allow adults to reproduce and replenish the population.

The first measure controls the size caught: larger mesh allows some small fish through, reducing capture before reproduction. The second controls timing, reducing fishing pressure during important spawning or breeding periods and allowing recruitment. For each, state the measure and explain how it maintains replenishment; “catch fewer fish” does not specify an implementable control. Sustainable catches must not exceed the population’s recovery capacity over the long term.

b(i); original allocation: 2 mark(s)

With increasing depth, water and suspended substances absorb and scatter light, reducing its intensity. Light increasingly limits photosynthesis, so photosynthetic rate decreases with depth.

Lake A has stronger photosynthesis near the surface, declining towards zero below. Light must pass through the overlying water, leaving less available at depth and limiting light-dependent reactions and subsequent carbon fixation. “Deep water is colder” is unsupported because the figure supplies no temperature evidence.

b(ii); original allocation: 3 mark(s)

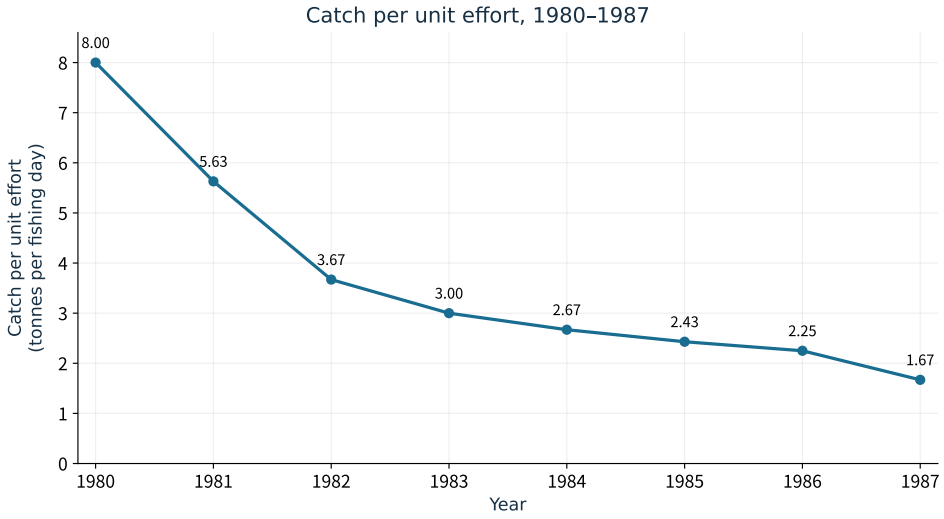
Lake A. Its near-surface photosynthetic rate is far higher than lake B’s, consistent with fertiliser nutrients promoting phytoplankton growth.

Check the vertical scales first: lake A is near 60 at the surface, whereas lake B is near 0.8. The quantities have the same dimensions, but the scale ranges differ greatly, so do not compare only the curves’ physical heights on the page. More nitrate, phosphate and other nutrients can remove limitations and support abundant producers with high surface photosynthesis. Dense phytoplankton also obstruct light penetration to deeper water. A steeper curve alone, without checking both scales, is insufficient.

b(iii); original allocation: 4 mark(s)

Lake A. During daylight, abundant phytoplankton produce oxygen by photosynthesis, raising dissolved oxygen. At night, photosynthesis stops while the greater biomass continues consuming oxygen by respiration, lowering dissolved oxygen further and producing a larger day–night fluctuation.

Compare oxygen gains and losses within one day. Lake A has more producers and a greater daytime oxygen-production capacity. After dark, photosynthesis stops, but plants, animals and decomposers continue respiring. The abundant phytoplankton also consume oxygen, increasing the difference between the daytime maximum and night-time minimum. “More algae means less oxygen” omits the differing daytime and night-time processes.



Catch per unit fishing effort, 1980–1987.

Marking reference: HKDSE Biology Sample Paper Paper 2 Q2, official marking scheme, source page(s) 8, 9.

VI.b Pollution control and global environmental issues

Read pollution control in Concept VI.b and global environmental issues in Concept VI.d, then use these examples to build causal explanations from environmental data. For forest restoration and species protection, also apply resource management from Concept VI.a and conservation from Concept VI.c.

Type 1 Explain ecological outcomes from pollution and environmental variables

Degree of integration ★★★★☆

Key ideas

① State the change caused by human activity, then follow environmental variable → biological process → ecological consequence. Microorganisms use organic matter discharged into a pond. Increased aerobic respiration by decomposers can lower dissolved oxygen, causing less oxygen-tolerant animals to die or leave.

② Biochemical oxygen demand measures the oxygen requirement of a sample under specified conditions. Field dissolved oxygen is the oxygen present in the water at that time; these are not the same reading. Downstream, organic matter may be depleted while oxygen continues to dissolve from the air, allowing dissolved oxygen to recover. Even with equal oxygen levels, flow speed, shade and food can make communities differ.

③ Track pollutant movement separately. Accumulation within one individual over time and increasing concentration along a food chain are different comparisons. Check whether the horizontal axis shows age, intake or trophic level.

④ Plants absorbing heavy metals transfer the metals into their tissues; the contaminated material still requires suitable removal or treatment. Distinguish pollutant concentration, total amount and the percentage in each location when comparing before and after treatment.

⑤ For climate questions, separate carbon movement from heat transfer. Fossil-fuel combustion increases atmospheric carbon dioxide. Carbon dioxide absorbs outgoing long-wave infrared radiation from the Earth's surface and re-emits radiation in all directions.

⑥ Before surface temperature adjusts, net heat loss to space is reduced, warming the climate system. Photosynthesis removes carbon dioxide, but whether extra substrate increases photosynthetic rate also depends on light, temperature and gas entry into leaves.

⑦ Lower stomatal density may reduce both gas-exchange capacity and transpiration. Reduced gas exchange can offset some of the photosynthetic increase. Lower transpiration supplies less water vapour to the atmosphere and may alter regional rainfall.

⑧ An explanation connecting warming to organisms needs an intermediate step.

⑨ If northern winters become more suitable for a bird species and sufficient food is available, the birds may overwinter farther north instead of migrating as far south. Additional birds may compete with resident birds for food or become prey. The outcome depends on the particular food web.

⑩ A temperature curve can fluctuate from year to year while still showing a long-term rise. Read the trend and geographical direction first, then explain food availability, habitat and species interactions.

⑪ Acid rain requires a different causal sequence.

⑫ Sulphur dioxide and nitrogen oxides from combustion can form acidic substances in the atmosphere, increasing rain-water acidity. Lower water pH can harm acid-sensitive organisms. Soil mineral nutrients may be leached more readily, while some toxic metal ions become more soluble.

⑬ These changes affect nutrient supply and root health in different ways. Acid formation does not explain greenhouse-gas absorption of infrared radiation.

⑭ Forest restoration also depends on material remaining after disturbance. If logging removes above-ground timber, compare nutrients retained above and below ground and how they support renewed plant growth. The forest with the most timber before felling does not necessarily retain the most nutrients afterwards.

⑮ Tree selection must consider local soil, climate and growing conditions. Large stands of one species can be damaged together by a specific pathogen or pest. Recovery of plant growth, recovery of biodiversity and reduced pollution are distinct outcomes requiring their own evidence.

How to recognise the problem Identify whether the comparison concerns pollutants, dissolved oxygen, pH, temperature, species number or nutrient distribution. Check units, denominators and conditions, then select the relevant mechanism. Organise each paragraph as evidence → biological or environmental process → consequence for specified organisms. For management measures, identify the step altered. Warming, acid rain and organic pollution each require their own intermediate processes.

Example 1 [HKDSE Biology 2018 Paper 2 Q2]

2(a) (i) The concentrations of copper and cadmium per unit biomass in marine phytoplankton and its consumer, filter-feeding bivalves, were determined in a study. Their concentrations are shown in the table below:

	Copper (arbitrary unit)	*Cadmium (arbitrary unit)*
Concentration in phytoplankton (Cp)	20	0.6
Concentration in bivalve (Cb)	16	1.2
Cb/Cp Ratio	0.8	?

(1) Calculate the *Cb/Cp* ratio for cadmium. (1 mark)

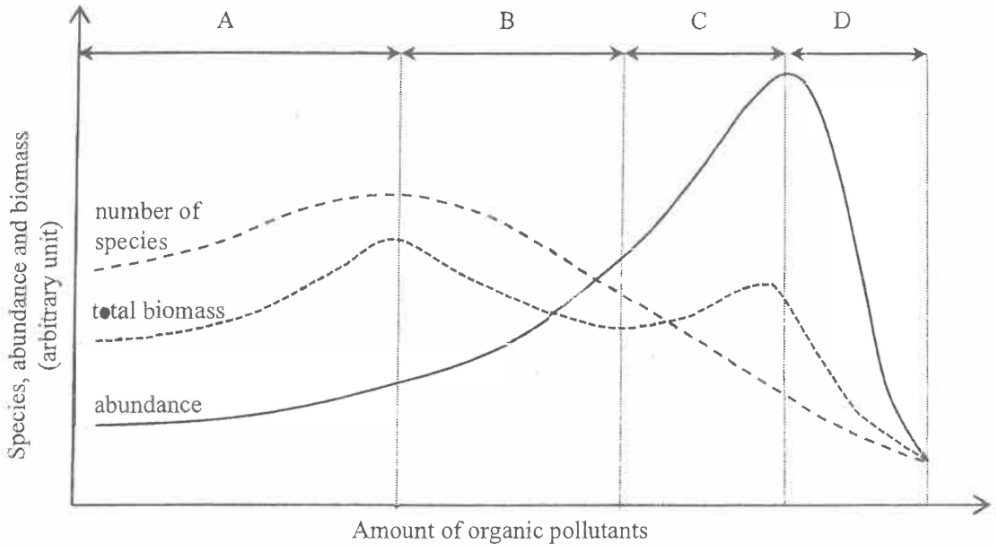
(2) What is the implication of a *Cb/Cp* ratio greater than 1? (1 mark)

(3) Compare the *Cb/Cp* ratios for copper and cadmium. List ***two*** possible reasons for the difference. (3 marks)

(ii) Briefly describe an experiment to study the effect of different concentrations of cadmium on the mortality of bivalves. (3 marks)

(iii) Cadmium in rechargeable batteries is one of the sources of cadmium pollution in the environment. Suggest ***two*** possible ways to reduce such pollution. (2 marks)

2(b) Invertebrates that live on, in and near the seabed are important components of the coastal ecosystem. These invertebrates feed on organic matter. The diagram below shows the effects of increasing amounts of organic pollutants from household sewage on the invertebrate community in a harbour in terms of the number of species, abundance (total number of organisms) and total biomass:



(i) Explain why the invertebrate community expands when the amount of organic pollutants increases at Stage A. (2 marks)

(ii) It is found that the proportion of larger invertebrates in the community declines in Stage B as the amount of organic pollutants continues to increase.

(1) With reference to the graph, give evidence to support the above statement. (3 marks)

(2) Suggest why the increase in the amount of organic pollutants will lead to a decline in the proportion of larger invertebrates in the community at Stage B. (3 marks)

(iii) Although the abundance and biomass of invertebrates continues to increase at Stage C, the community is deteriorating. State a piece of evidence from the graph to support this statement. (1 mark)

(iv) Suggest ***one*** property of the dominant species in the invertebrate community at Stage C. (1 mark)

Solution a(i)(1); original allocation: 1 mark(s)

The Cb/Cp ratio for cadmium is 2.

Cb is the concentration per unit biomass in bivalves; Cp is the corresponding concentration in phytoplankton. Do not reverse numerator and denominator.

Cb/Cp = 1.2/0.6 = 2.

a(i)(2); original allocation: 1 mark(s)

A ratio above 1 indicates a higher heavy-metal concentration in the consumer than in its food, showing concentration magnification between these two trophic levels.

For example, cadmium's ratio of 2 means that bivalves contain twice the phytoplankton concentration per equal unit biomass. It does not mean that bivalve total biomass is greater or that each animal eats exactly two phytoplankton organisms. The comparison concerns standardised concentrations.

a(i)(3); original allocation: 3 mark(s)

Cadmium's ratio of 2 exceeds copper's 0.8. Cadmium may be excreted less readily and may be harder to convert through detoxification or related metabolic processes into readily eliminated forms, causing greater retention.

First compare the actual values, then identify two physiological processes affecting retention. Slower excretion favours accumulation. Difficulty converting a metal into forms suitable for safe transport and elimination can also increase retention. These are possible

Method: Read the environmental variable, then use its mechanism to explain ecological effects.

mechanisms, and the table alone cannot establish their relative contributions. Copper and cadmium are elements and cannot be broken down until they "disappear". Detoxification or metabolism here concerns binding, chemical forms and elimination.

a(ii); original allocation: 3 mark(s)

Divide equal or similar numbers of bivalves into groups exposed to different cadmium concentrations. After the same fixed period, record deaths or percentage mortality in each group and compare concentration with toxicity.

Cadmium concentration is the independent variable and mortality the dependent variable. Use the same species and similar-sized animals, controlling temperature, salinity, food and exposure time so cadmium concentration is the main difference. If initial numbers differ slightly, percentage mortality is fairer than raw death counts.

Percentage mortality = number dead ÷ initial number × 100%.

a(iii); original allocation: 2 mark(s)

Reduce use of cadmium-containing products or replace them with suitable cadmium-free alternatives. Send cadmium-containing batteries and similar products to appropriate recycling facilities to recover their materials.

The first measure reduces cadmium entering waste at source, for example by choosing suitable cadmium-free batteries. The second collects used cadmium-containing products for recycling, preventing dispersal in rubbish or entry into water. Give concrete reduction/substitution and recycling measures. "Raise environmental awareness" does not explain how pollution is reduced.

b(i); original allocation: 2 mark(s)

In region A, increased organic matter supplies more food to these invertebrates and raises carrying capacity. Increased reproduction or immigration raises their numbers.

The question states that these animals feed on organic matter, so a small additional supply initially increases their food resources. Relaxed resource limitation can allow births to exceed deaths or attract immigrants, increasing abundance. The horizontal axis represents organic pollution, not time, so do not interpret the graph as a record from one day to another. Do not ignore the stated diet and substitute a complete algal-eutrophication account.

b(ii)(1); original allocation: 3 mark(s)

In region B, total biomass falls while the total number of individuals rises. Mean biomass per animal therefore falls, indicating a declining proportion of larger animals.

Use both curves. Falling biomass alone could mean fewer individuals of all sizes. The simultaneous rise in abundance supports smaller mean body size.

Mean biomass per individual = total community biomass ÷ number of individuals.

A smaller numerator and larger denominator necessarily give a smaller quotient. This links both observations to the conclusion about fewer large individuals proportionally.

b(ii)(2); original allocation: 3 mark(s)

Microbial decomposition of abundant organic matter consumes oxygen, making bottom waters oxygen-poor. Larger invertebrates generally have higher total oxygen requirements and are more likely to die or leave, reducing their proportion.

Begin with increased pollution, then decomposer respiration and oxygen consumption, the animals' low-oxygen conditions, and the resulting size-composition change. Do not jump to "pollution makes animals shrink" or claim food shortage when organic food supply is increasing. This is a reasonable community explanation using the stated size trend, not a claim that every large species has identical oxygen tolerance.

b(iii); original allocation: 1 mark(s)

Species number continues to fall in region C.

More individuals or greater total biomass may merely reflect proliferation of a few pollution-tolerant species. Continued species loss shows that community composition remains impaired; greater animal abundance alone does not establish ecological recovery.

b(iv); original allocation: 1 mark(s)

Species with high tolerance of oxygen shortage.

When organic pollution causes substantial oxygen consumption, species able to survive low dissolved oxygen are more likely to dominate. The requested characteristic is tolerance of oxygen shortage, directly matching the environmental selection described. "Highly adaptable" is insufficiently specific.

Marking reference: HKDSE Biology 2018 Paper 2 Q2, official marking scheme, source page(s) 7.

Example 2 [HKDSE Biology 2019 Paper 2 Q2]

(a) An artificial wetland was introduced to treat sewage in some small villages in Africa and the effluent collected was used for irrigating farms. This helped to alleviate the problems of water shortage and sewage treatment. In a study, domestic sewage after primary treatment was conveyed to either a sewage treatment plant or an artificial wetland, and the effluents were collected and compared.

(i) Briefly describe the processes involved in the primary treatment of sewage. (2 marks)

(ii) The table below shows some parameters in the influent and effluents collected from the sewage treatment plant and the artificial wetland:

	Influent (sewage after primary treatment)	Effluent from different systems	
		Sewage treatment plant	Artificial wetland
Oxygen content ($mg\ L^{-1}$)	5.0	5.3	3.5
Organic nitrogen content ($mg\ L^{-1}$)	35	3	12
Phosphate content ($mg\ L^{-1}$)	29	28	27
Magnesium content ($mg\ L^{-1}$)	42	40	41

(1) With regard to the handling of organic nitrogen in the two systems, explain the difference in the organic nitrogen content in the effluents from the sewage treatment plant and the artificial wetland. (4 marks)

(2) Using the effluent of this study to irrigate the farms is advantageous to the growth of crops. Apart from the presence of nitrates, briefly explain another advantage in terms of the synthesis of biomolecules. (2 marks)

(iii) State ***two*** advantages of adopting an artificial wetland over a sewage treatment plant for small villages. (2 marks)

2(b) Mining activities often produce large quantities of heavy metal wastes, which cause contamination of the ecosystem. The photographs below show an abandoned mining site and the ecological restoration of this mining site after 10 years:

Photograph I – Abandoned mining site



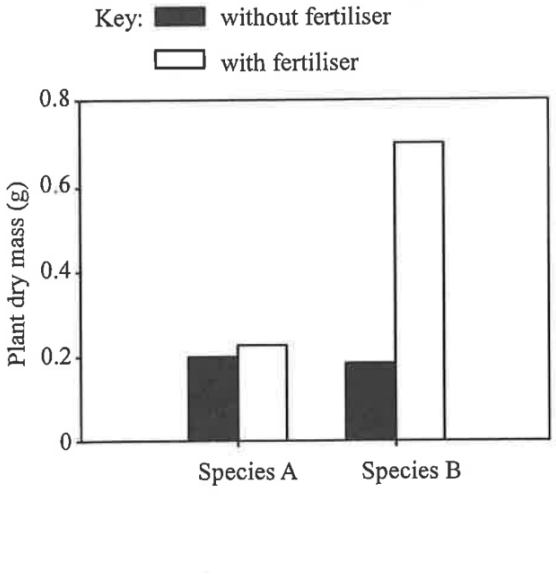
Photograph II – the mining site after ecological restoration



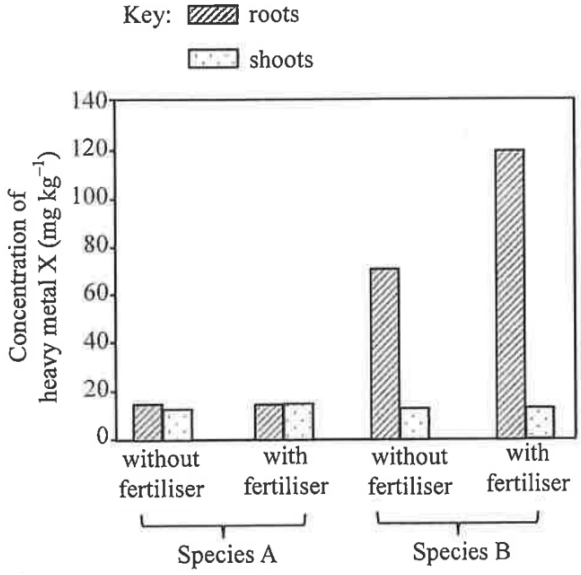
(i) Besides heavy metal pollution, state ***two*** other ecological problems of the abandoned mining site. (2 marks)

(ii) In a study, two native plant species, A and B, were tested for their suitability to restore an abandoned mining site. Their seedlings were planted in pots containing soil from the mining site with a high concentration of heavy metal X. Half of the pots of each plant species were supplied with fertiliser but not the other half. Graph 1 shows the dry masses of plants in the eighth week of the experiment and Graph 2 shows the concentrations of heavy metal X in the roots and shoots:

Graph 1



Graph 2



(1) Species B is more suitable for the restoration of the mining site in terms of the removal of heavy metal X from the soil. With reference to the above two graphs, give evidence to support this choice. (4 marks)

(2) There is another plant species C which accumulates a much higher concentration of heavy metal X in the shoots than in the roots. Which species, B or C, would you choose for the restoration of the abandoned mining site? Why? (2 marks)

(3) Give ***two*** reasons why native plant species were used in this experiment. (2 marks)

Solution a(i); original allocation: 2 mark(s)

First remove larger solids by screening/filtration. Then allow suspended solids to settle and remove the sludge at the bottom.

Primary treatment mainly uses physical separation. Screening removes large debris, while sedimentation removes settleable suspended particles. It does not remove all dissolved organic matter or pathogens, so further treatment is still required.

a(ii)(1); original allocation: 4 mark(s)

The sewage-treatment plant's effluent contains less organic nitrogen: 3 mg L^{-1} compared with 12 mg L^{-1} from the constructed wetland.

The plant actively introduces air into the sewage, providing more oxygen. Its effluent contains 5.3 mg L^{-1} oxygen, compared with the wetland's 3.5 mg L^{-1}. Better oxygen supply promotes decomposer activity and faster conversion of organic nitrogen into ammonium compounds, leaving less organic nitrogen. This conversion is not nitrification, which subsequently converts ammonium into nitrite/nitrate.

a(ii)(2); original allocation: 2 mark(s)

For example, magnesium can be used to synthesise chlorophyll, or phosphorus to make nucleic acids, ATP and phospholipids.

Link a mineral element in the table to a specific biological molecule. Magnesium is a constituent of chlorophyll, while phosphorus is incorporated into nucleic acids, ATP or phospholipids. Saying minerals help plants grow does not answer at the molecular level requested.

a(iii); original allocation: 2 mark(s)

Lower construction and infrastructure costs, and lower energy and staffing requirements during operation.

A constructed wetland uses plants and microorganisms to treat the limited sewage volume of a small village, without the same mechanical aeration and equipment operation required by a treatment plant. Distinguish construction costs from routine operating costs; the comparison concerns the small-scale village in the question.

b(i); original allocation: 2 mark(s)

For example, soil erosion/soil degradation and destruction of natural vegetation and habitats.

Figure I shows extensive bare ground. Without vegetation stabilising the soil, water can erode it more readily. Conditions supporting the original plants and animal habitats are also reduced. Heavy-metal pollution is excluded by the question, so provide two other ecological consequences.

b(ii)(1); original allocation: 4 mark(s)

Fertilisation increases B's biomass more strongly. Its root concentration of X is also higher and increases further, allowing more X to accumulate in the plant.

Figure 1: A's dry mass rises only from approximately 0.20 to 0.24 g, whereas B's rises from approximately 0.20 to 0.72 g. Figure 2: B's initial root X concentration is approximately 70 mg kg^{-1}, exceeding A's approximately 15. After fertilisation, B rises to approximately 120, while A changes little. Both growth and root concentration favour B; use both graphs.

b(ii)(2); original allocation: 2 mark(s)

C can be chosen because harvesting its shoots removes accumulated X from the contaminated site.

C stores more heavy metal in above-ground shoots, making harvesting and removal easier and meeting the aim of removing metal from the mining area. If reducing food-web exposure is the main criterion, B is also defensible because its lower shoot X concentration reduces intake by herbivores and subsequent consumers. The choice must match the explanation; the question does not accept only one letter.

Method: Read the environmental variable, then use its mechanism to explain ecological effects.

b(ii)(3); original allocation: 2 mark(s)

Native species are already adapted to local conditions. They can better restore the original community and reduce the risk of introducing invasive species.

Local adaptation improves establishment at the restoration site. Compatibility with the original community provides food and habitats needed by local animals. Highly competitive introduced plants could create another ecological pressure after the pollution is remediated.

Examiner's comment (source page(s) 9)

Primary sewage treatment is physical separation and need not include chemical treatment. Distinguish decomposition, nitrogen fixation and nitrification. Link minerals to the biological molecules requested. Evidence for B must combine dry mass and root X concentration; justify C through harvesting shoots to remove metal from the land.

Marking reference: HKDSE Biology 2019 Paper 2 Q2, official marking scheme, source page(s) 8.

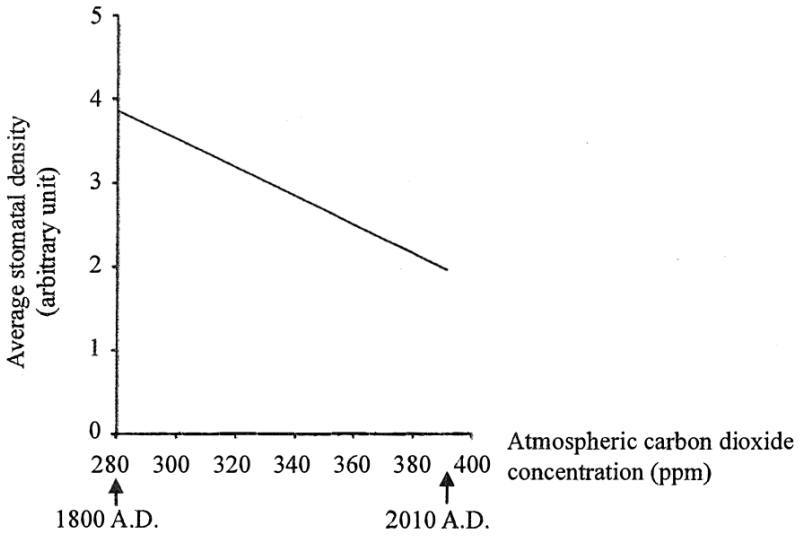
Example 3 [HKDSE Biology 2015 Paper 2 Q2]

(a) The atmospheric concentration of carbon dioxide has been increasing in the last 210 years. The increase is believed to be the major cause of global climate change.

(i) Explain why the increase in carbon dioxide concentration leads to a change in the atmospheric temperature. (2 marks)

(ii) It has been suggested that the rate of increase in carbon dioxide concentration can be slowed down by photosynthesis. What is the biological principle behind this suggestion? (3 marks)

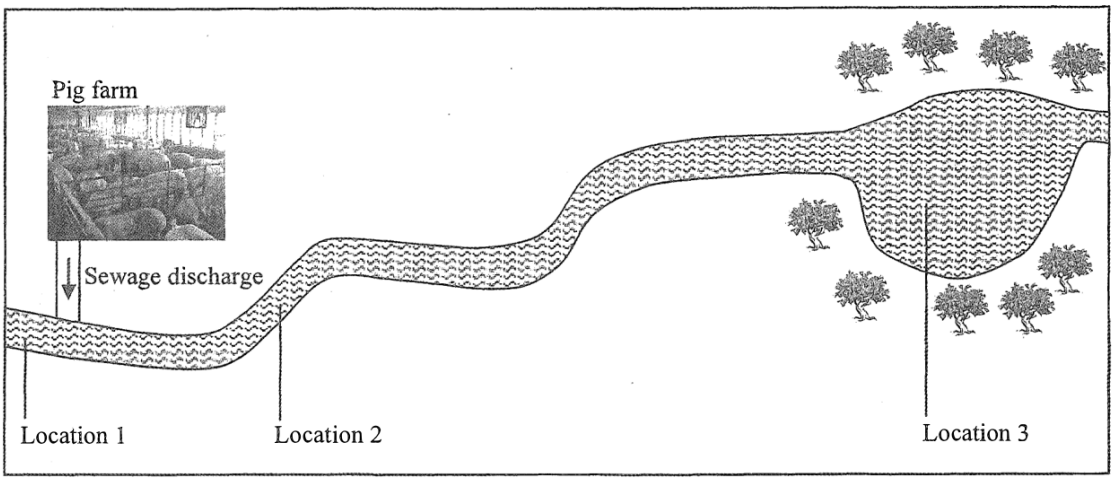
(iii) The graph below shows the changes in the average stomatal density of some plant species and the atmospheric carbon dioxide concentration over the past 210 years:



(1) How may the observation contradict the suggestion given in (ii)? (3 marks)

(2) It has been hypothesized that the decrease in the average stomatal density of plant species may further affect the regional climate. With reference to the water cycle, explain the rationale behind this hypothesis. (2 marks)

2(b) A pig farm is located in the upper course of a freshwater stream. Sewage is discharged illegally into the stream. To investigate the impact of the sewage discharge, water samples and aquatic animals were collected from upstream near to the sewage discharge (Location 1) and 50 m downstream (Location 2). The diagram below shows the position of the pig farm and the stream:



The table below shows the data obtained at locations 1 and 2:

	Location 1	Location 2
Dissolved oxygen (% saturation)	95	22
Animal community	Fish species A	Fish species C
	Fish species B	Larvae of insect species A
	Snail species A	
	Crab species A	

(i) (1) Explain the change in the level of dissolved oxygen as water flows from Location 1 to Location 2. (4 marks)

(2) With reference to the change in the level of dissolved oxygen, explain the differences in the animal species at Location 2 as compared to Location 1. (2 marks)

(ii) It was found that the level of dissolved oxygen 1 km downstream (Location 3) from the sewage discharge had been restored to 95%.

(1) Suggest how the level of dissolved oxygen was restored at Location 3. (2 marks)

(2) Although the level of dissolved oxygen had recovered, the animal community found at Location 3 was different from that at Location 1. Suggest why. (2 marks)

Solution (a)(i); original allocation: 2 mark(s)

Carbon dioxide absorbs outgoing long-wave infrared radiation from the Earth's surface, increasing atmospheric heat retention and raising temperature.

After absorbing solar energy, the ground emits thermal radiation. Greenhouse gases reduce the direct loss of this energy to space. Specify absorption of heat radiation emitted by the ground; do not simplify this to blocking all sunlight.

Method: Read the environmental variable, then use its mechanism to explain ecological effects.

(a)(ii); original allocation: 3 mark(s)

Carbon dioxide is a raw material for photosynthesis. If it was initially limiting, increased concentration can raise photosynthetic rate and plant carbon dioxide uptake, slowing the increase in atmospheric concentration.

This is a possible negative feedback. Explain increased raw-material availability, potentially increased carbon fixation, and finally partial opposition to the atmospheric increase. The feedback is limited when other factors become limiting.

(a)(iii)(1); original allocation: 3 mark(s)

The graph shows stomatal density decreasing as carbon dioxide concentration increases. Fewer stomata can reduce leaf gas exchange and carbon dioxide entry, offsetting part of the increased external supply so photosynthesis may not rise as much as expected.

The effects act in opposite directions: more external substrate may promote photosynthesis, while fewer entry channels may restrict exchange. Considering both identifies the limitation of the earlier prediction.

(a)(iii)(2); original allocation: 2 mark(s)

Lower stomatal density can reduce transpiration and water-vapour input to the atmosphere, potentially changing condensation, rainfall amount or rainfall distribution.

Connect plant water loss to atmospheric moisture and rainfall rather than equating stomata directly with rainfall. This is the regional water-cycle effect considered by the hypothesis.

(b)(i)(1); original allocation: 4 mark(s)

Dissolved oxygen falls from 95% to 22% saturation. Sewage introduces abundant organic matter, which decomposing microorganisms use while carrying out aerobic respiration. Their oxygen consumption lowers dissolved oxygen downstream.

Use both readings to establish the fall, then identify the organisms and oxygen-consuming process. Organic matter does not make oxygen vanish on entering the water; increased microbial decomposition creates greater oxygen demand.

(b)(i)(2); original allocation: 2 mark(s)

Low dissolved oxygen kills or displaces animals requiring higher oxygen levels. Low-oxygen-tolerant fish species C and insect larvae remain, producing a community different from upstream.

Animals differ in tolerance ranges. Link the species changes in the table to dissolved oxygen rather than merely copying the two species lists.

(b)(ii)(1); original allocation: 2 mark(s)

As organic matter is gradually decomposed and exhausted, microbial oxygen demand falls. Meanwhile, atmospheric oxygen continues dissolving into the water, and aquatic-plant photosynthesis can replenish oxygen.

Recovery combines reduced consumption with continued replenishment. “Downstream is cleaner” does not explain oxygen sources and losses.

(b)(ii)(2); original allocation: 2 mark(s)

Despite the same dissolved oxygen, site 3 may have slower flow, more shade or different biological interactions, so its suitable species assemblage need not match site 1.

Dissolved oxygen is only one environmental factor. Differences in channel width and bank vegetation shown in the figure support comparisons of other habitat conditions. One identical reading does not establish identical habitats.

Examiner’s comment (source page(s) 4)

Organic matter in sewage increases decomposer oxygen demand; an algal bloom need not be assumed first. Link upstream–downstream differences in flow, light, decomposers and organic matter to the data. The greenhouse effect involves infrared radiation emitted by the Earth’s surface.

Marking reference: HKDSE Biology 2015 Paper 2 Q2, official marking scheme, source page(s) 8.

VI.c Conservation of species and habitats

Read the explanations and diagrams in Concept VI.c first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Link conservation measures with testable ecological outcomes

Degree of integration ★★★★☆

Key ideas

① A protected area can reduce local fishing and give fish more opportunities to survive and reproduce. Adult movement or larval dispersal may benefit surrounding areas. Test this inference using data inside and outside the area and at different distances; the name 'protected area' alone is insufficient. Wetland management, restrictions on clam collection and indicator-species monitoring must connect the measure with the habitat, species or pollutant exposure actually affected.

② **Check how far recovery has progressed before comparing its speed**

③ Suppose the photosynthetic rates of two plant groups are each set to 100 before disturbance. After disturbance, they fall to 40 and 20. Both groups then return to their original, identical environmental conditions. The question is not merely whether photosynthesis occurs, but how far each rate remains below its original value of 100.

④ On day 1, group A rises from 40 to 80 and group B from 20 to 45. On day 3, A reaches 100 while B reaches only 70. A recovers faster and reaches its original baseline by day 3; B has not fully recovered within the observation period. A substantial rise to 70 is still not a return to 100.

⑤ 'Not fully recovered by day 3' does not mean 'unable ever to recover': no later data are supplied. Repeated disturbance before recovery may keep photosynthesis low for a prolonged period, reducing organic-matter production and accumulation. Include this intermediate process when inferring effects on survival.

⑥ **Compare each group with its own original baseline after both return to the same conditions**

⑦

Time	Relative photosynthetic rate: A	Relative photosynthetic rate: B
Before disturbance	100	100
After disturbance, at the start of recovery	40	20
Recovery day 1	80	45
Recovery day 3	100	70

How to recognise the problem State what the measure changes, then evaluate it using population, community or pollution data. If evidence concerns only one species, keep the conclusion within that scope.

Example 1 [HKDSE Biology 2017 Paper 2 Q2]

(a) A marine protected area is established in open sea where fishing is prohibited to ensure the fishery resources are sustainable. Outside the protected area, bottom trawling is allowed. A preliminary study was conducted to estimate the animal biomass (kg km^{-2}) at six randomly chosen sites as shown in Diagram I. The animal biomass of each site is shown in Diagram II.

Diagram I

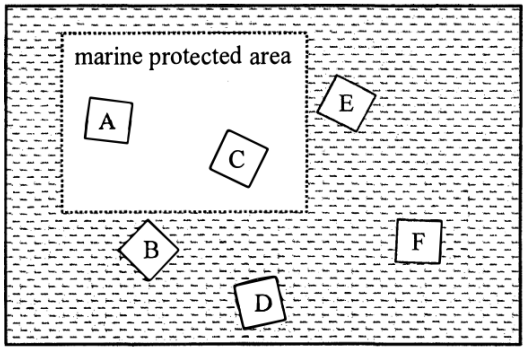
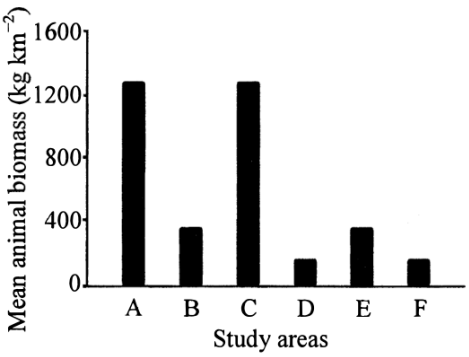


Diagram II



(i) Bottom trawling is a fishing method in which fish nets are laid on the sea bottom and towed by fishing boats. Suggest ***two*** potential effects caused by bottom trawling on the physical environment on the sea bottom. (2 marks)

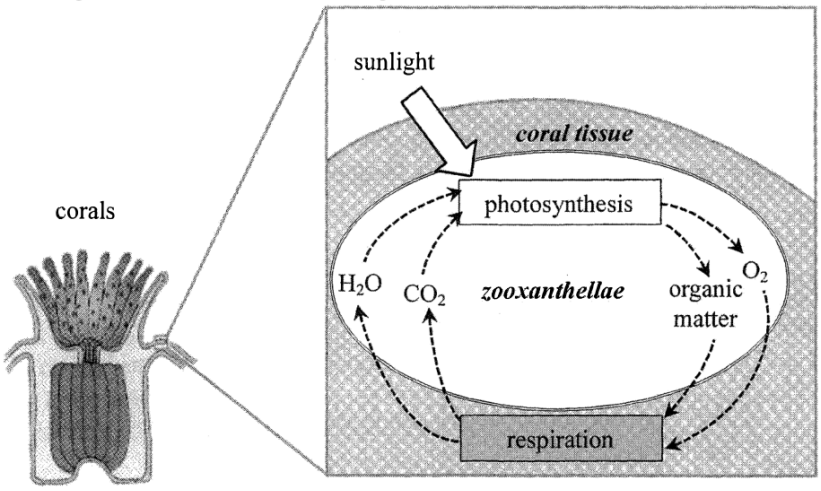
(ii) (1) Comparing the biomass of the sites within and outside the protected area, what pattern do you observe? (2 marks)

(2) Suggest a possible explanation for this pattern. (3 marks)

(iii) What is the limitation of this preliminary study? What measurement should be taken to increase the validity of the study? (4 marks)

(iv) State ***one*** marine protected area in Hong Kong. (1 mark)

2(b) Corals appear colourful because they have mutualistic unicellular algae called zooxanthellae in their bodies. The diagram below shows the exchange of materials between them:



(i) When the seawater temperature increases, corals will expel zooxanthella. This is known as coral bleaching. After bleaching, the corals may eventually die due to a deterioration in their health. Based on the information from the diagram, suggest why the health of coral deteriorates. (1 mark)

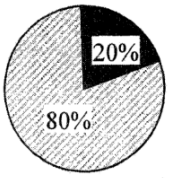
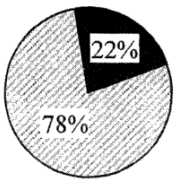
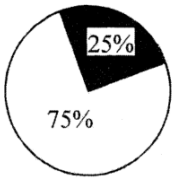
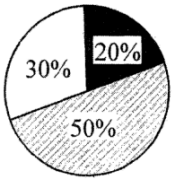
(ii) Increased atmospheric carbon dioxide level is believed to be the cause of increased seawater temperature. Explain why. (3 marks)

(iii) A transplantation experiment was conducted to study the effect of increased seawater temperature on the health of the corals, as follows:

Site A Temperature range: 27°C - 30°C	→ Corals from site A were transplanted to site B →	**Site B** Temperature range: 27°C - 35°C

After 14 months, native corals from Site A and transplanted coral from Site B were collected and put into tanks of seawater with different temperature settings. The charts below show the health of the corals after 6 months:

Key: ▨ Healthy ☐ Bleached ■ Dead

Tanks with seawater at 27°C		*Tanks with seawater at 32°C*	
Native corals from site A	Transplanted corals from site B	Native corals from site A	Transplanted corals from site B
			

(1) Compare the effect of increased seawater temperature on the health of the native corals and transplanted corals. Support your answer with information from the above charts. (3 marks)

(2) Under the threat of global warming, what is the implication of this study for coral bleaching? (1 mark)

Solution (a)(i); original allocation: 2 mark(s)

Bottom trawling disturbs seabed sediments, increasing turbidity or releasing pollutants. It also destroys seabed habitats, shelters and breeding sites.

The question concerns physical environmental effects. Sediment disturbance and damage to benthic habitat are more relevant than merely "catching many fish". Distinguish effects on water conditions from effects on habitat structure.

✓ *Method: Identify what the measure changes, then evaluate the outcome with data.*

(a)(ii)(1); original allocation: 2 mark(s)

A and C inside the protected area have the highest biomass, nearby B and E intermediate values, and more distant D and F the lowest. Animal biomass is higher closer to the protected area.

A and C are approximately 1300 kg km^{-2}, B and E approximately 350, and D and F approximately 150. Match map distances with bar heights to identify the spatial pattern. Sites outside the reserve are not all equally low.

(a)(ii)(2); original allocation: 3 mark(s)

The no-fishing area allows animals to survive, reproduce and build up biomass. Some move into neighbouring areas, making nearby biomass higher than that farther away.

Protection reduces capture and habitat disturbance and provides feeding and breeding conditions. As populations grow, some individuals move outward, creating a spillover benefit for nearby areas. This explains both high biomass inside and higher biomass near than far outside.

(a)(iii); original allocation: 4 mark(s)

Total biomass does not show which species benefit or suffer and cannot represent diversity. Add measurements such as species number to assess biodiversity effects of protection.

For example, an increase in one large fish species can raise total biomass even if other species decline. Recording species richness and, where appropriate, each species' abundance addresses this limitation of measuring only the total. Explain which limitation the proposed measurement corrects.

(a)(iv); original allocation: 1 mark(s)

Hoi Ha Wan Marine Park.

Name one established Hong Kong marine protected area. An ordinary bathing beach is not necessarily a protected area.

(b)(i); original allocation: 1 mark(s)

Loss of symbiotic algae reduces the organic nutrients supplied to coral by photosynthesis.

The diagram shows zooxanthellae supplying photosynthetic organic matter to coral. Expulsion of algae during bleaching interrupts this supply, reducing available energy and impairing coral health.

(b)(ii); original allocation: 3 mark(s)

Additional CO_2 absorbs outgoing long-wave infrared radiation from the Earth's surface, retaining more heat in the surface–atmosphere system. Air temperatures rise and seawater warms.

The greenhouse effect involves infrared radiation emitted after the surface absorbs sunlight, not simply heat "reflected" by a mirror-like Earth. Connect CO_2 absorption to reduced energy loss and then seawater temperature.

(b)(iii)(1); original allocation: 3 mark(s)

32°C affects native corals more severely. None remain healthy: 75% are bleached and 25% dead. Transplanted corals are still 50% healthy, with 30% bleached and 20% dead.

At 27°C, most corals are healthy in both groups: 80% native and 78% transplanted. At 32°C, the healthy proportion falls more in the native group. Lower bleaching and mortality in corals transplanted to warmer site B support greater heat tolerance. Support the comparison with at least two data points, not one selected percentage.

(b)(iii)(2); original allocation: 1 mark(s)

Previous exposure to warmer conditions may increase tolerance of later warming, suggesting some capacity for adaptation or adjustment in corals.

The experiment supports a relationship between exposure history and heat-tolerance responses. It does not establish that genetic mutation necessarily occurred or that all corals can completely withstand global warming.

Examiner's comment (source page(s) 5)

Comparisons inside and outside marine protected areas should consider distance from the boundary. Total biomass does not substitute for species diversity. Corals are animals; loss of their symbiotic algae affects food and oxygen supply.

Marking reference: HKDSE Biology 2017 Paper 2 Q2, official marking scheme, source page(s) 9.

Practice VI.a Human impacts on the environment

Related type 1 Evaluate management through renewal, competition and diversity

Degree of integration ★★★☆☆

Practice

1. [HKDSE Biology 2021 Paper 2 Q2]

← *Hint: Set the comparison basis, then examine resource renewal and competition.*

(a) Conventional farming engages the use of artificial chemicals such as herbicides and insecticides while organic farming does not. A survey comparing a conventional farm and an organic farm was conducted. The survey involved random sampling in areas within the farm and the areas around the farm at different times of a year. The data are shown in the table below:

		Conventional farm		Organic farm	
		Around the farm	Within the farm	Around the farm	Within the farm
Species richness (number of species)	Plants other than crops	7	3	28	18
	Pollinators	1	0	10	6
Abundance (number of individuals)	Pest A	310	160	51	49
	Predators of A	9	3	24	11

(Note: The numbers shown are the mean values rounded up to the nearest integer.)

(i) With reference to the data collected within the farms, discuss the effectiveness of the use of herbicides. How can the use of herbicides bring about an increase in the yield of crop production? (3 marks)

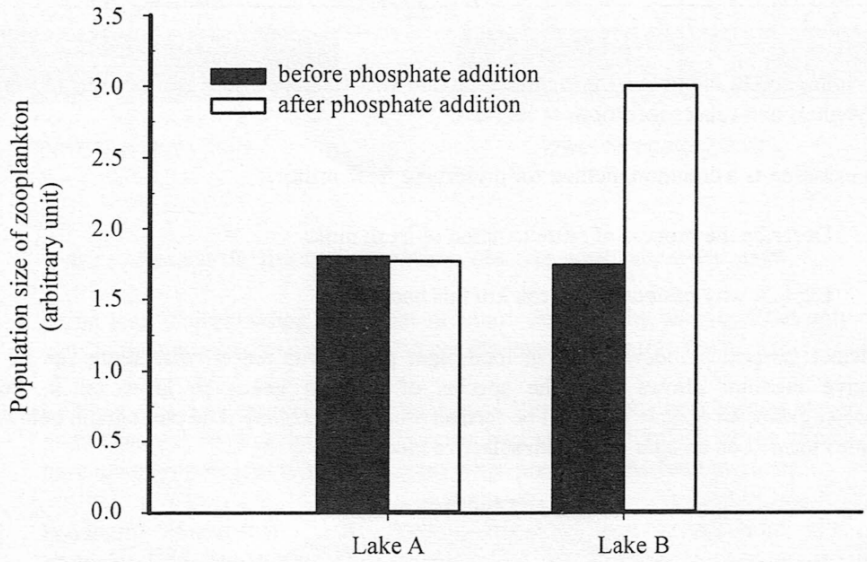
(ii) According to the data collected within the farms, discuss the effectiveness of chemical control and biological control on the population of pests. Explain your answer with supporting evidence from the data. (4 marks)

(iii) With reference to species richness, explain why organic farming is beneficial to the sustainable development of communities in the areas around the farm. (3 marks)

2(b) Algal bloom commonly occurs when an aquatic ecosystem is polluted by domestic sewage. Lakes A and B have similar environmental conditions while the frequency of occurrence of algal bloom in Lake B is lower than that in Lake A. The major food chains of the two lakes are shown below:

Lake A: phytoplankton → zooplankton → Fish Species 1
Lake B: phytoplankton → zooplankton → Fish Species 1 → Fish Species 2

It is hypothesised that the number of trophic levels of the food chain affects the frequency of occurrence of algal bloom in the two lakes. To test this hypothesis, phosphate was added to the lakes and the population sizes of the zooplankton were monitored. The bar chart below shows the population sizes of zooplankton in Lakes A and B before and after the addition of phosphate:



(i) What would be the effect of phosphate addition on the population size of phytoplankton? Explain your answer. (2 marks)

(ii) With reference to the bar chart, suggest why the frequency of occurrence of algal bloom in Lake B is lower than that in Lake A. (3 marks)

(iii) Based on the food chain of Lake B, explain why there is a higher zooplankton biomass in Lake B after phosphate addition. (3 marks)

(iv) Explain why algal bloom leads to the reduction of dissolved oxygen in water at night. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 3 mark(s)**

Herbicides reduce weeds and their competition with crops for resources, potentially increasing yield.

Use the within-farm data: the conventional farm has only 3 non-crop plant species, compared with 18 on the organic farm. This is consistent with weed removal by herbicides. Fewer weeds compete for light, water and minerals, leaving more resources for crops.

a(ii); original allocation: 4 mark(s)

In this survey, biological control on the organic farm is more effective against pest A.

Within the organic farm, pest A numbers 49, compared with 160 on the conventional farm. Its predators number 11 and 3 respectively. Conventional insecticides may also kill natural predators, reducing predation pressure. More predators remain on the organic farm and can suppress the pest population. Restrict this conclusion to the survey; it does not establish that all chemical control is ineffective.

a(iii); original allocation: 3 mark(s)

Greater plant and pollinator species richness around the organic farm helps sustain community reproduction and food supply.

There are 28 non-crop plant species and 10 pollinator species around the organic farm, exceeding the conventional farm's 7 and 1. A wider range of pollinators supports reproduction of different flowering plants. Greater plant diversity supplies varied food and habitat resources, supporting continued animal communities.

b(i); original allocation: 2 mark(s)

Phosphate promotes phytoplankton proliferation.

Phosphorus is required to synthesise nucleic acids and other substances. If initially insufficient, phosphate addition removes this growth limitation and allows the phytoplankton population to increase.

b(ii); original allocation: 3 mark(s)

Zooplankton in lake B increase markedly after phosphate addition. Their greater grazing can control phytoplankton and reduce the likelihood of an algal bloom.

Zooplankton remain approximately unchanged in lake A but rise from approximately 1.7 to 3.0 arbitrary units in lake B. The additional zooplankton feed on phytoplankton, allowing lake B to transfer extra primary production more effectively to the next trophic level.

b(iii); original allocation: 3 mark(s)

In lake B, fish species 2 preys on fish species 1, reducing species 1's predation pressure on zooplankton.

Phosphate addition increases phytoplankton and hence food supply. Simultaneously, reduced predation allows zooplankton to use this food and increase. Include both top-down predation control and food supply; an additional trophic level does not automatically guarantee an increase.

b(iv); original allocation: 2 mark(s)

At night, algae stop photosynthesising but continue consuming dissolved oxygen through respiration.

Daytime photosynthesis replenishes oxygen. At night, the light-dependent reactions stop, reducing replenishment while large numbers of algal cells still respire, so dissolved oxygen falls.

Examiner's comment (source page(s) 9)

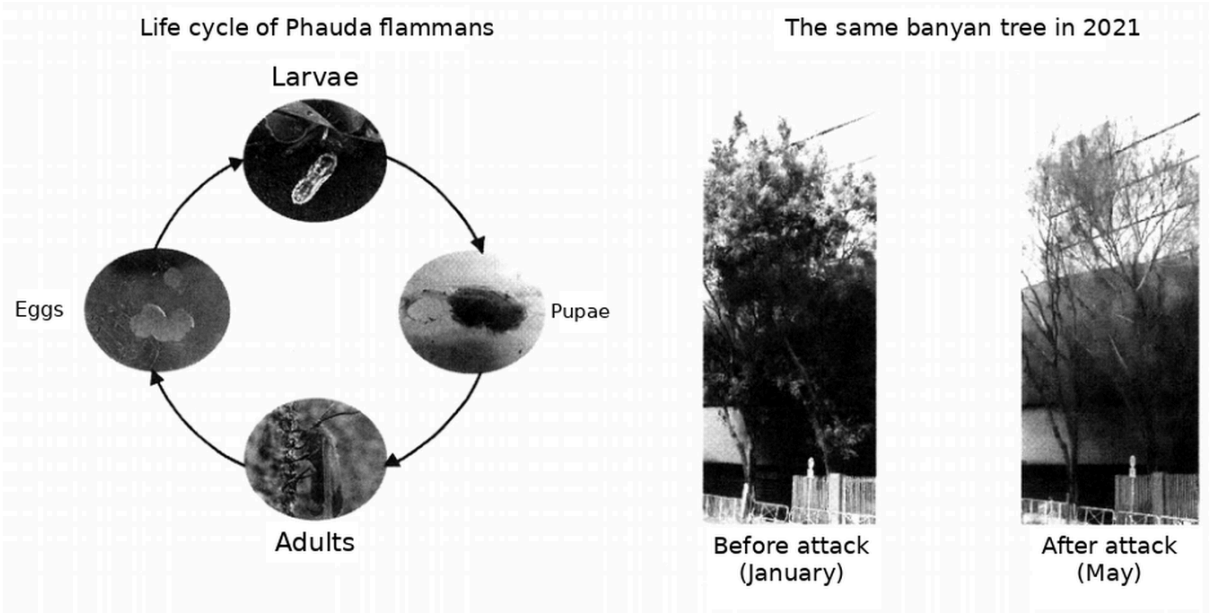
Use the relevant biological data to compare farming systems and connect species richness with sustainability. Trace phosphate enrichment, phytoplankton and zooplankton changes fully in aquatic questions. Zooplankton are not photosynthetic organisms.

Marking reference: HKDSE Biology 2021 Paper 2 Q2, official marking scheme, source page(s) 8, 9.

2. [HKDSE Biology 2025 Paper 2 Q2 (English translation)]

Hint: Set the comparison basis, then examine resource renewal and competition.

(a) *Phauda flammans* is a local moth species whose larvae feed on banyan leaves. The following diagrams show its life cycle and photographs of the same banyan tree before and after attack by *Phauda flammans* in 2021:

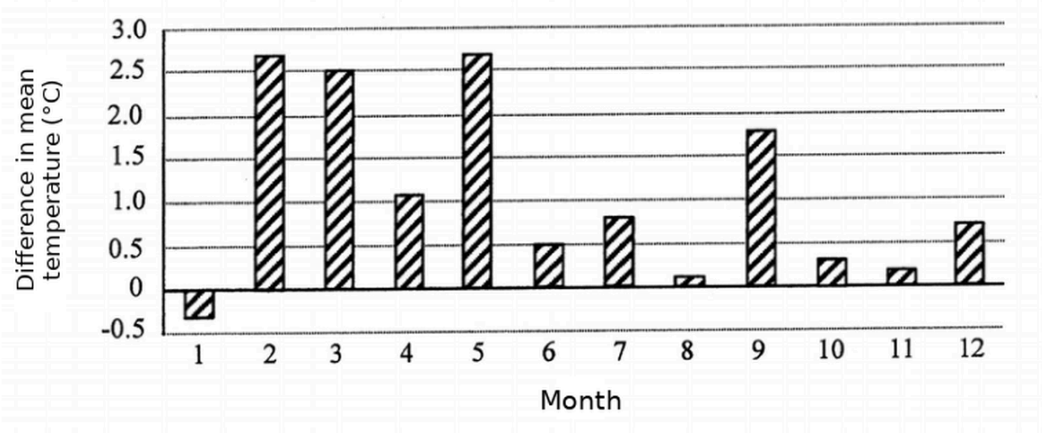


The table below gives the estimated duration of development at different stages of *Phauda flammans* at different temperatures:

Mean temperature (°C)	Eggs (days)	Larvae (days)	Pupae (days)	Adults (days)
20	16.0	105.6	29.0	11.0
24	12.6	72.0	19.0	7.7
28	9.3	38.4	8.9	4.4

(i) With reference to the tabulated data, describe the general pattern observed for the effect of temperature on the duration of development of *Phauda flammans*, and explain the pattern. (3 marks)

(ii) The bar chart below shows the difference between the monthly mean temperature in Hong Kong in 2021 and the monthly mean temperature for 1991–2020:

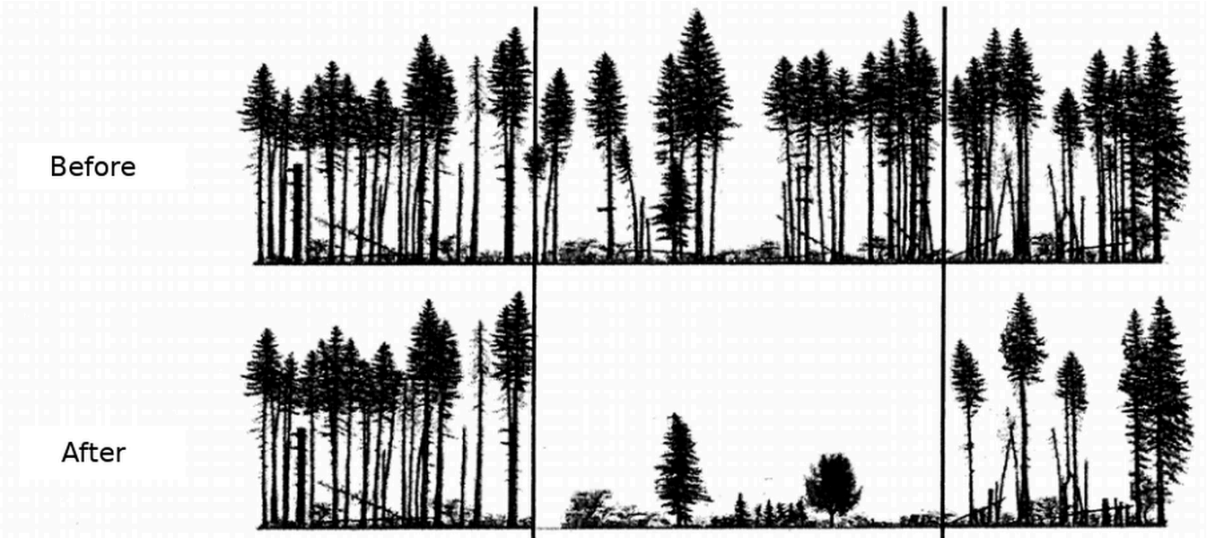


(1) What information does the bar chart provide about the mean temperatures in 2021 compared with the monthly mean temperatures for 1991–2020? (1 mark)

(2) *Phauda flammans* usually produces three to four generations each year. Using the preceding diagrams and table and the bar chart above, explain why the outbreak of *Phauda flammans* in 2021 caused the changes in the banyan tree shown in the photographs. (4 marks)

(iii) Why is widespread use of chemical insecticides not recommended for controlling *Phauda flammans*? Give two reasons. (2 marks)

(b) Early afforestation in Hong Kong mainly involved planting fast-growing exotic tree species to control soil erosion and protect water catchments. However, forests formed by a single exotic tree species have low biodiversity. In recent years, ecologists have adopted thinning, a new strategy involving removal of exotic trees, to address this problem. The diagrams below illustrate different methods of managing the trees:



Method I (left)	Method II (centre)	Method III (right)
No thinning	Intensive thinning: remove most exotic trees, retain shrubs and plant native tree seedlings.	Moderate thinning: remove some exotic trees.

(i) After several years of growth, the exotic trees under Method I had a smaller mean trunk diameter and crown cover than those under Method III. Suggest an explanation for this observation. (4 marks)

(ii) Six plots (20 m × 20 m) were randomly selected in a local plantation for testing. The table below shows the treatment applied to each plot and its species richness, absolute abundance and biodiversity index initially and after one year of growth:

Plot	Method	Initial species richness	Initial absolute abundance	Initial biodiversity index*	Species richness after 1 year	Absolute abundance after 1 year	Biodiversity index* after 1 year
1	I	4	174	1.086	8	133	1.326
2	I	5	297	1.120	6	171	1.258
3	II	4	247	0.824	9	195	1.417
4	II	7	201	1.197	10	128	1.671
5	III	5	224	0.958	9	147	1.372
6	III	4	255	1.095	5	142	1.251

* A higher value indicates greater biodiversity in the habitat.

(1) Based on the data in the table, which method would you recommend? Show how you use the relevant data to reach your answer. (3 marks)

(2) Although no native tree seedlings were planted in the plots receiving moderate thinning under Method III, their species richness still increased. Suggest one explanation for this result. (2 marks)

(3) Suggest one benefit to wildlife in the habitat when plant biodiversity increases. (1 mark)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation (a)(i); original allocation: 3 mark(s)

Within the table's 20–28°C range, increasing mean temperature shortens the duration of every stage: egg, larva, pupa and adult. Higher temperature can increase metabolic and growth rates, allowing the whole life cycle to be completed faster.

Compare each stage across temperatures first. For example, the larval period falls from 105.6 to 72.0 and then 38.4 days. Then state the common trend across stages. Include metabolic rate as the intermediate mechanism. Do not extrapolate this limited temperature range into a claim that development always accelerates indefinitely as temperature rises.

(a)(ii)(1); original allocation: 1 mark(s)

Except for January, mean temperatures in the other 11 months of 2021 exceed the corresponding monthly means for 1991–2020.

Each bar is a difference from the baseline for the same month. Above zero means warmer and below zero means colder; the bars do not compare adjacent months of 2021.

(a)(ii)(2); original allocation: 4 mark(s)

Warmer conditions in most months can shorten each generation's life cycle, allowing more generations per year. The larval population therefore increases faster, and more larvae eat fig-tree leaves, producing the more severe leaf loss shown in the photograph.

Combine the two datasets: the table links temperature to developmental duration, and the bar chart shows warmer conditions in most months of 2021. The chain is shorter life cycle → more generations per year → more larvae → greater total feeding. "Warm weather means more insects" omits the crucial reasoning.

(a)(iii); original allocation: 2 mark(s)

For example, chemical insecticides may kill other beneficial insects. They may also kill the moth's natural enemies, weakening natural control of its population.

These reasons concern non-target beneficial organisms and natural enemies respectively. "Toxic" and "polluting" alone are not two explained reasons. Other appropriate effects include harmful substances accumulating along food chains or selection of resistant individuals.

(b)(i); original allocation: 4 mark(s)

Method I retains more trees of the same species, causing stronger intraspecific competition for the same light, soil nutrients and space. Each tree obtains fewer resources and grows less, producing a smaller mean trunk diameter and canopy cover than method III.

The original question compares trunk diameter and canopy cover, not mean tree height. Method III removes some trees, lowering density and increasing resources available to those remaining. Establish the competition difference from tree numbers before linking it to growth.

(b)(ii)(1); original allocation: 3 mark(s)

Recommend method II. The mean increases in biodiversity index are I = 0.189, II = 0.534 and III = 0.285. Method II has the largest mean increase.

For each plot, subtract the initial value from the value after one year, then average the two plots using the same method.

I: [(1.326−1.086)+(1.258−1.120)]÷2 = (0.240+0.138)÷2 = 0.189.

II: [(1.417−0.824)+(1.671−1.197)]÷2 = (0.593+0.474)÷2 = 0.5335, approximately 0.534.

III: [(1.372−0.958)+(1.251−1.095)]÷2 = (0.414+0.156)÷2 = 0.285.

Comparing mean increases accounts for different starting values. Do not label these differences as final index values.

(b)(ii)(2); original allocation: 2 mark(s)

After thinning in method III, more light penetrates the forest understorey. Previously shaded plants requiring greater light intensity can establish and flourish, increasing species richness even without planting additional native seedlings.

Explain the increase in plant species through changed understorey light conditions. "Providing bird habitat" does not explain how plant diversity increases.

(b)(ii)(3); original allocation: 1 mark(s)

Greater plant diversity provides more kinds of food for wildlife occupying different ecological niches.

Give one specific benefit. A wider range of habitats or breeding sites is also acceptable. Avoid simply restating that the change "benefits animals".

Examiner's comment (source page(s) 76)

Explain temperature effects on life cycles through metabolic rate and generations per year. Subtract each forest plot's starting value before calculating mean increases. Increased plant richness under method III is linked to understorey light.

Marking reference: HKDSE Biology 2025 Paper 2 Q2, official marking scheme, source page(s) 62, 63.

3. [HKDSE Biology 2022 Paper 2 Q2]

Hint: Set the comparison basis, then examine resource renewal and competition.

(a) The major purpose of deforestation in the tropical rainforest is to turn it into a grassland for cattle farming.

(i) The table below shows the comparison of plant communities within a primary rainforest and a grassland formed after deforestation:

Type of plant community	Average plant height (m)	Number of plant species	* Percent coverage (%) of individual plant species	
Primary rainforest	28	18	Species A	15
			Species B	19
			Species C	12
			Species D	20
			Species E	14
Grassland	0.3	3	Species H	95

* Only species with at least 10% of coverage are included.

(1) What would be the effect of converting a tropical rainforest to a grassland on the average plant height and number of plant species? (1 mark)

(2) For estimating the relative abundance of plant species in the above case, why was percent coverage a better measurement than the number of individual species? (1 mark)

(3) With reference to the percent coverage of individual plant species, what was the effect of deforestation on the composition of the plant community? (3 marks)

(ii) Soil porosity (space between soil particles) was determined for soil samples collected from the rainforest and grassland. The soil samples were then incubated in the laboratory for 7 days. After that, the net changes in the amounts of ammonium content and inorganic nitrogen (i.e. nitrate and nitrite) were calculated. The results are shown in the table below:

Soil sample	Soil porosity (%)	Amount of ammonium (mg kg^{-1} soil)	Amount of inorganic nitrogen (mg kg^{-1} soil)
Primary rainforest	80	13	14
Grassland	60	7.3	9.8

(1) Based on the information in the table, deduce the effect of deforestation on the oxygen level of the soil. (2 marks)

(2) With reference to the nitrogen cycle and the answer in (1), answer the following questions:

(I) Explain the difference in the amount of ammonium between the samples taken from the primary rainforest and the grassland. (2 marks)

(II) Explain the difference in the amount of inorganic nitrogen between the samples taken from the primary rainforest and the grassland. (2 marks)

2(b) During World War II, much of the forests in Hong Kong had been cleared for firewood. After the war, the Government began to build new reservoirs and restore the existing ones. At that time, imported tree species were planted in areas around these reservoirs.

(i) Explain why planting trees in the area nearby is important to the functioning of reservoirs. (2 marks)

(ii) Tai Po Kau Nature Reserve comprises a secondary forest with native tree species and an afforestation area with imported tree species. A survey about the bird community was conducted in these two areas. The results are shown in the table below:

	Secondary forest with native tree species	Afforestation area with imported tree species
Total number of bird species	44	46
Number of forest-dependent bird species	16	9
Number of migrating bird species	28	37
Number of species nested in the forest	12	2
Number of individual birds per hectare	44	12

(1) What is meant by a secondary forest? (1 mark)

(2) In what ways are forests important to bird communities? State ***two*** of them. (1 mark)

(3) Suggest ***one*** way by which birds are beneficial to tree communities. (1 mark)

(4) Based on the above results, deduce which type of tree, native tree species or imported tree species, has a greater contribution to the local bird community. (4 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i)(1); original allocation: 1 mark(s)**

Mean plant height and the number of plant species decrease.

The primary rainforest has a mean height of 28 m and 18 plant species; the grassland has only 0.3 m and 3 species. Together, these show shorter vegetation and reduced species richness.

a(i)(2); original allocation: 1 mark(s)

Plant sizes differ greatly, so percentage cover better reflects their occupation of space or contribution to biomass in the community.

Counting one large tree and one small grass plant as one individual each treats their actual community contributions as equal. Cover provides a more reasonable comparison of occupied space and relative importance in primary production.

a(i)(3); original allocation: 3 mark(s)

The grassland is overwhelmingly dominated by species H, whereas the principal rainforest species have more even relative cover. Species composition also differs.

H covers 95% of the grassland, while rainforest species A–E each cover approximately 12–20%. Thus, clearing reduces species richness and converts a relatively even multispecies community into one in which H occupies nearly all the space.

a(ii)(1); original allocation: 2 mark(s)

Soil oxygen is lower after clearing.

Grassland soil has 60% porosity, below the rainforest's 80%. Fewer pores accommodate and exchange air, suggesting poorer aeration and lower oxygen supply. Infer from the given physical data rather than simply claiming that fewer trees produce less photosynthetic oxygen.

a(ii)(2)(I); original allocation: 2 mark(s)

Grassland soil contains less ammonia because lower oxygen supply reduces decomposition of organic nitrogen compounds by aerobic decomposers.

Ammonification converts organic nitrogen into ammonia or ammonium compounds. Poorer aeration slows aerobic decomposition, producing less ammonia within seven days. This explains 7.3 mg in grassland compared with 13 mg in rainforest.

a(ii)(2)(II); original allocation: 2 mark(s)

Grassland has less inorganic nitrogen such as nitrate and nitrite because oxygen shortage slows nitrification and may accelerate denitrification.

Nitrifying bacteria oxidise ammonium compounds to nitrite and nitrate in an oxygen-requiring process. Insufficient oxygen reduces their production. More oxygen-poor conditions also favour denitrification, converting nitrate to gaseous nitrogen that is lost. Thus, grassland has 9.8 mg compared with rainforest's 14 mg.

b(i); original allocation: 2 mark(s)

Roots stabilise soil and the canopy reduces raindrop impact, limiting sediment entry into the reservoir and preventing siltation and loss of capacity.

Connect plant functions to reservoir operation. Intercepting rain and stabilising slopes reduce erosion. Less sediment is carried into the reservoir, preserving more storage space.

b(ii)(1); original allocation: 1 mark(s)

A secondary forest develops naturally on the same site after the original forest has been disturbed.

Soil, seeds or nearby biological sources generally remain, allowing secondary succession. This differs from primary forest development beginning on bare rock.

b(ii)(2); original allocation: 1 mark(s)

Food and habitats for nesting or shelter.

Fruits, seeds and insects on trees provide food; branches, foliage and tree holes provide breeding sites and refuge from predators. The question asks for two items, so distinguish resources from places.

b(ii)(3); original allocation: 1 mark(s)

Birds can disperse tree seeds.

After eating fruits, birds may deposit viable seeds elsewhere in their droppings, moving them away from the parent tree and promoting tree-community renewal. Removing leaf-eating pests is another acceptable example.

b(ii)(4); original allocation: 4 mark(s)

Native tree species are more favourable for local bird communities.

Although the exotic-tree forest has slightly more bird species overall (46 versus 44), it also has more migratory species (37 versus 28). Secondary forest with native trees has more forest-dependent species (16 versus 9) and nesting species (12 versus 2), indicating support for more birds using forest as permanent habitat and breeding sites. Total birds per hectare does not distinguish local from migratory birds and is not direct evidence of more local birds.

Examiner's comment (source page(s) 9, 10)

Species H's 95% grassland cover demonstrates dominance and evenness; explain these rather than merely repeating the figure. Increased soil ammonia involves decomposition and ammonification, not nitrification. Use forest-dependent and nesting species to infer benefits to local birds. Undifferentiated total birds per hectare cannot directly establish more local birds.

Marking reference: HKDSE Biology 2022 Paper 2 Q2, official marking scheme, source page(s) 10, 11.

Practice VI.b Pollution control and global environmental issues

Related type 1

Explain ecological outcomes from pollution and environmental variables

Degree of integration ★★★★☆

Practice

1. [HKDSE Biology 2013 Paper 2 Q2]

Hint: Read the environmental variable, then use its mechanism to explain ecological effects.

(a) An experiment was conducted to study the effect of acid rain on the growth of bean seedlings planted in pots containing two different soils, either with or without heavy metal X. The same amount of water with a pH of either 6 or 3 was used to water the seedlings every day and their average increase in fresh weight was measured after 30 days. The results are shown in the table below:

	Average increase in fresh weight of bean seedling (g)	
	pH 6	pH 3
Soil without heavy metal X	13.0	7.7
Soil with heavy metal X	7.5	4.3

Note: Rainwater normally has a pH value of 6.

(i) What conclusion can you draw from the results of the experiment? (3 marks)

(ii) During the experiment, excess water that came out of the potted plants was collected and the amount of heavy metal ion X in it was determined. The table below shows the results:

	pH 6	pH 3
Amount of heavy metal ion X in excess water (arbitrary unit)	5	10

Based on this information, explain the effect of adding water of pH 3 to the seedlings in soil containing heavy metal X. (3 marks)

(iii) State the air pollutants that cause acid rain and state the human health problem associated with these air pollutants. (3 marks)

2(b) Pollutant Y was found in sewage discharge from industry, leading to bioaccumulation in organisms.

(i) Give ***three*** properties of chemicals that make them liable to bioaccumulation. (3 marks)

(ii) Specimens were collected from a site where pollutant Y was known to be discharged. The levels of pollutant Y in sea water, sediment and some organisms collected from the site are listed in the following table:

	Concentration of pollutant Y (arbitrary unit)
Seawater	2
Sediment	36
Clams	163
Birds	1557
Fish	519

Based on the information in the above table, which organism is most likely the top consumer of the food chain in the site? Explain your answer. (3 marks)

(iii) With reference to the functional role of organisms in an ecosystem, give ***one*** group of organisms not included in the above table. State the ecological significance of this group of organisms. (3 marks)

(iv) If humans consumed fish contaminated with pollutant Y, which human organ would have a high concentration of this pollutant? Explain your answer. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation a(i); original allocation: 3 mark(s)

Low pH inhibits seedling growth, as does heavy metal X in the soil. Growth is lowest when both occur together. Without X, mean fresh-mass increases at pH 6 and pH 3 are 13.0 and 7.7 g; with X, they are 7.5 and 4.3 g respectively.

Use appropriate paired controls to distinguish the effects of pH and heavy metal, then identify the lowest result when both are present. Comparing only the table's maximum and minimum does not separate the factors.

a(ii); original allocation: 3 mark(s)

Acidic water releases more soil heavy metal X as ions, increasing X in the outflow from 5 to 10 units. The higher available metal-ion concentration is more toxic to seedlings and further inhibits growth.

The second table supplies the intermediate mechanism: low pH changes metal solubility and accessible concentration; it does not create additional metal from nothing.

a(iii); original allocation: 3 mark(s)

Sulphur dioxide and nitrogen oxides. These pollutants can irritate the eyes and respiratory tract, causing respiratory discomfort or disease.

Each gas category earns 1 mark, and an appropriate health problem earns 1 mark. Carbon dioxide is not the principal answer for strongly acidic rain caused by combustion pollution in this question.

b(i); original allocation: 3 mark(s)

Substances that readily accumulate commonly have high lipid solubility, resist metabolism or biodegradation, and are difficult to excrete.

If a substance persists after entering the body and intake exceeds removal, it accumulates. "Highly toxic" alone does not explain increasing concentration over time.

b(ii); original allocation: 3 mark(s)

Birds are most likely the top consumers. They have the highest Y concentration, 1557 units. Higher-level consumers eat many contaminated prey, and poorly eliminated Y becomes biomagnified along the food chain, generally giving top consumers the highest concentration.

This is the most likely inference based on biomagnification. Bird size or activity range is not the main evidence.

b(iii); original allocation: 3 mark(s)

Producers: convert solar energy into chemical energy in organic matter, supplying other organisms with food and energy.

Alternatively, decomposers: break organic matter into inorganic substances, returning minerals to the environment for reuse.

Choose one group and give both levels of its role. Seawater and sediment are environmental components, not a group of organisms as requested.

b(iv); original allocation: 2 mark(s)

The liver. It processes many foreign and toxic substances in the blood and may therefore take up and accumulate pollutant Y in the stated situation.

Explain the organ's role in handling foreign substances within this paper's marking context. Do not infer that every pollutant always accumulates exclusively in the liver.

Examiner's comment (source page(s) 4)

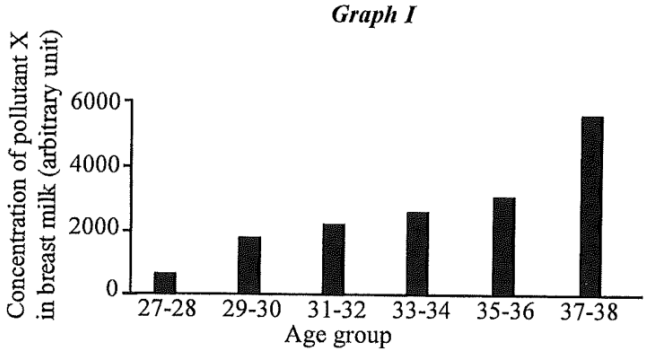
Identify pH 6 as normal rainwater and pH 3 as acid rain before explaining acid-induced metal-ion release. Accumulation depends on properties including lipid solubility and difficulty of elimination.

Marking reference: HKDSE Biology 2013 Paper 2 Q2, official marking scheme, source page(s) 8.

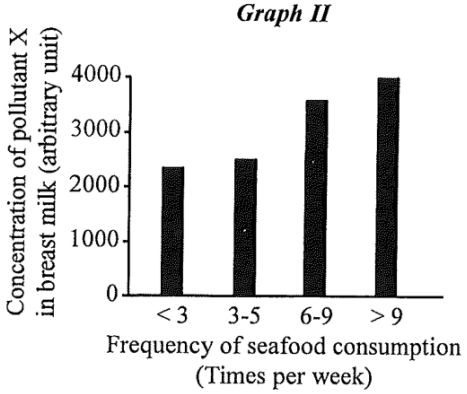
2. [HKDSE Biology 2023 Paper 2 Q2]
Hint: Read the environmental variable, then use its mechanism to explain ecological effects.

(a) In 2000, a survey was conducted in Hong Kong to assess the bioaccumulation of pollutant X in the human body. In the survey, human breast milk samples were collected for analysis. A questionnaire was prepared to obtain information of the participants such as age, dietary intake and health condition. The graphs below show the concentration of pollutant X in the breast milk collected in relation to the age groups (Graph I) and the frequency of sea food consumption per week (Graph II).

Graph I



Graph II



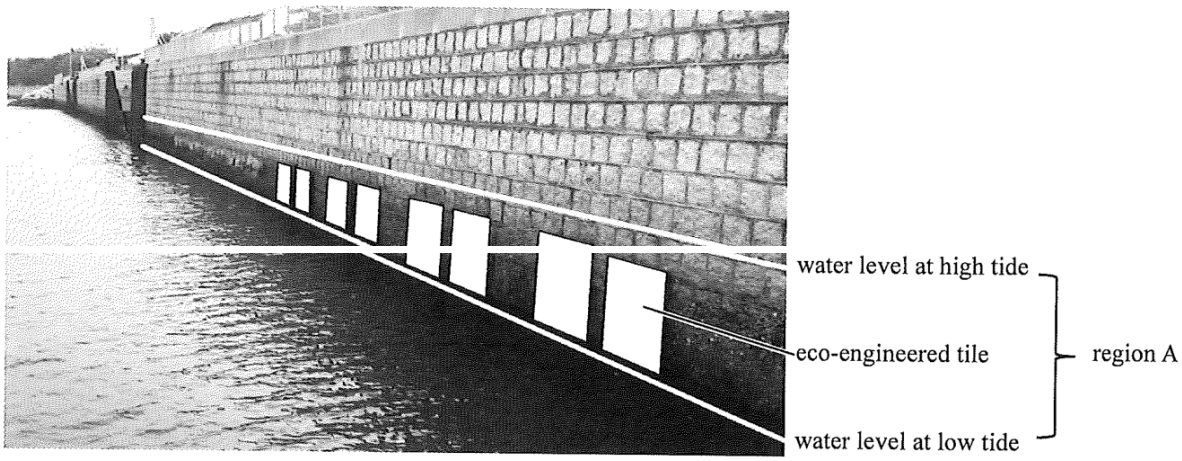
(i) With reference to the above graphs, give ***two*** pieces of evidence which support that pollutant X can be accumulated in the human body. Explain your answer. (4 marks)

(ii) (1) List ***three*** common properties of the pollutants which will lead to bioaccumulation. (3 marks)

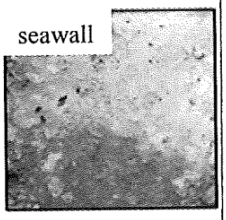
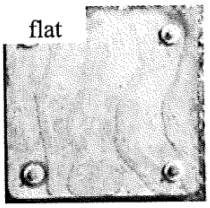
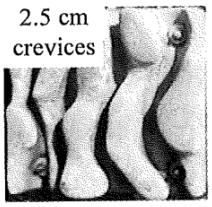
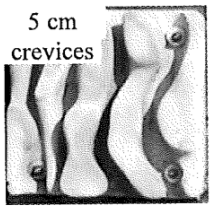
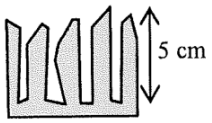
(2) Suggest why milk could be used as one of the indicators for bioaccumulation. (2 marks)

(iii) With reference to the above case, suggest ***two*** human activities which would lead to the release of bioaccumulative pollutants to the environment. (2 marks)

2(b) Eco-engineered tiles were installed on a local seawall in an attempt to increase the biodiversity of the shoreline. The photograph below shows a seawall where eco-engineered tiles are installed:



An experiment was conducted with the installation of different eco-engineered tiles on a local seawall. The table below shows four treatments and the number of invertebrate species living on each surface after 12 months:

Treatment	Surface View	Side View	Number of invertebrate species found on the surface
(1) Original seawall surface		---	4
(2) Tiles with flat surface installed on the seawall			8
(3) Tiles with crevices of 2.5 cm depth installed on the seawall			13
(4) Tiles with crevices of 5 cm depth installed on the seawall			17

(i) By comparing relevant treatments, deduce ***two*** effects of installing the eco-engineered tiles. Tabulate your answer in the answer book according to the following format. (4 marks)

Comparison of the results of two treatments	Deduction

(ii) Suggest how the crevices of the eco-engineered tiles could help organisms in Region A to cope with threats posed by the following environmental conditions:

(1) exposed seawall surface during low tide (1 mark)

(2) wave action (1 mark)

(iii) (1) Suggest another group of data which should be collected to assess the effect of installing eco-engineered tiles on the biodiversity in Region A. (1 mark)

(2) To confirm if the installation of eco-engineered tiles has increased the biodiversity of Region A, all the data collected should fulfil certain requirements. List these requirements. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation a(i); original allocation: 4 mark(s)

Older age is associated with higher breast-milk X, supporting accumulation with exposure duration. More frequent weekly seafood consumption is associated with higher X, supporting accumulation through repeated dietary intake. Figure I rises from a low value in the 27–28 age group to approximately 5600 in the 37–38 group, consistent with retention after prolonged exposure. Figure II rises from approximately 2400 with seafood fewer than 3 times weekly

to approximately 4000 with more than 9 times weekly, supporting greater retention with greater dietary intake. These support associations; one cross-sectional survey does not exclude all other influences.

a(ii)(1); original allocation: 3 mark(s)

Lipid-soluble, resistant to breakdown or metabolism, and difficult to excrete.

Lipid-soluble substances can enter and remain in fatty tissues. If breakdown is difficult and excretion slow, repeated intake may exceed clearance, increasing total body burden over time. The three properties concern distribution, persistence and clearance respectively.

a(ii)(2); original allocation: 2 mark(s)

Breast milk contains fat into which lipid-soluble pollutants can partition. It is produced from maternal substances, so its pollutant content can reflect pollutants already present in the mother.

Milk is synthesised by mammary glands using maternal materials. Detecting persistent, lipid-soluble X in it provides evidence of entry into the mother and partitioning with lipids, making it a possible indicator.

a(iii); original allocation: 2 mark(s)

Rain washes persistent agricultural pesticides into rivers and seas. Industrial discharges introduce relevant pollutants into aquatic environments.

Seafood can accumulate X in contaminated food webs. For both human activities, describe release and transport into the environment rather than merely listing "agriculture" or "industry".

b(i); original allocation: 4 mark(s)

Treatment 2 versus 1: 8 versus 4 species, suggesting that bricks themselves increase species richness. Treatment 4 versus 3: 17 versus 13 species, suggesting that deeper crevices increase richness further.

The first comparison contrasts the original seawall with flat bricks, testing the attachment environment provided by the brick material or surface. The second compares creviced bricks differing only in depth, from 2.5 to 5 cm, supporting a depth effect. Alternatively, treatment 3 versus 2, with 13 versus 8 species, supports more species on creviced than flat surfaces.

b(ii)(1); original allocation: 1 mark(s)

Crevices retain moisture and provide shade, reducing drying or overheating during low-tide exposure.

Flat surfaces dry readily at low tide. Recessed crevices lose water more slowly, maintaining a moister and cooler microenvironment for organisms sheltered inside.

b(ii)(2); original allocation: 1 mark(s)

Crevices provide shelter and attachment sites, reducing removal of organisms by waves.

Waves act directly on organisms projecting from a flat surface. Grooves reduce impact and increase contact surfaces for attachment, improving the chance of remaining on the seawall.

b(iii)(1); original allocation: 1 mark(s)

The number of individuals or relative abundance of each species.

Species number measures richness only. Knowing whether one species overwhelmingly dominates is necessary to assess evenness and biodiversity more fully.

b(iii)(2); original allocation: 2 mark(s)

Species richness increases after brick installation, and relative abundances become more even.

Adding a few rare species to a community still overwhelmingly dominated by one species differs from supporting several species at appreciable abundances. Check improvements in both richness and evenness to confirm increased diversity.

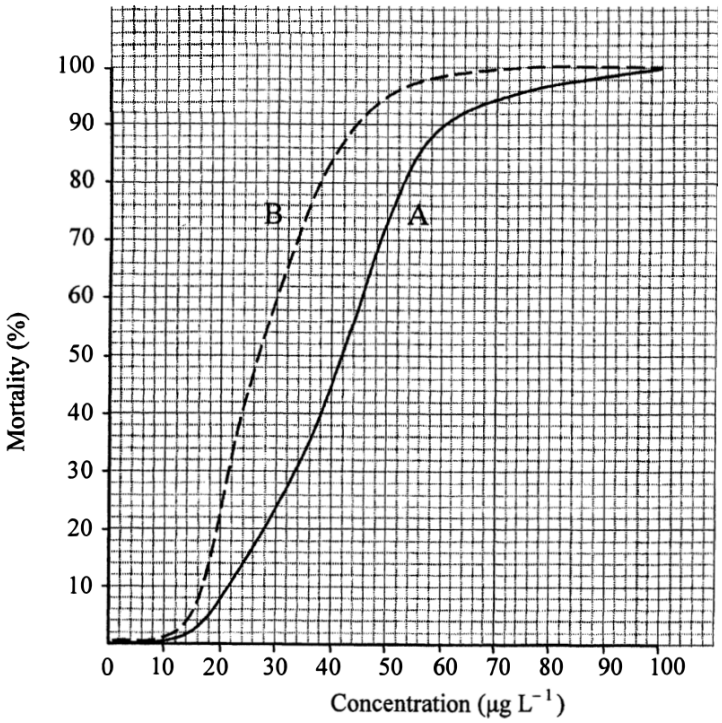
Examiner's comment (source page(s) 10, 11)

Link breast-milk indicators to milk fat and pollutant lipid solubility. Increased species number on seawall bricks establishes increased richness only; individual counts for each species are needed to assess evenness and overall diversity.

Marking reference: HKDSE Biology 2023 Paper 2 Q2, official marking scheme, source page(s) 11, 12.

3. [HKDSE Biology 2014 Paper 2 Q2]
Hint: Read the environmental variable, then use its mechanism to explain ecological effects.

(a) The graph below shows the mortality of a species of freshwater shrimp after it has been exposed to various concentrations of two **fat-soluble pesticides**, A and B, for 24 hours:



(i) (1) Determine the concentrations of pesticides A and B at which 50% of the shrimps were killed. (1 mark)

(2) Deduce which pesticide, A or B, was more toxic. (2 marks)

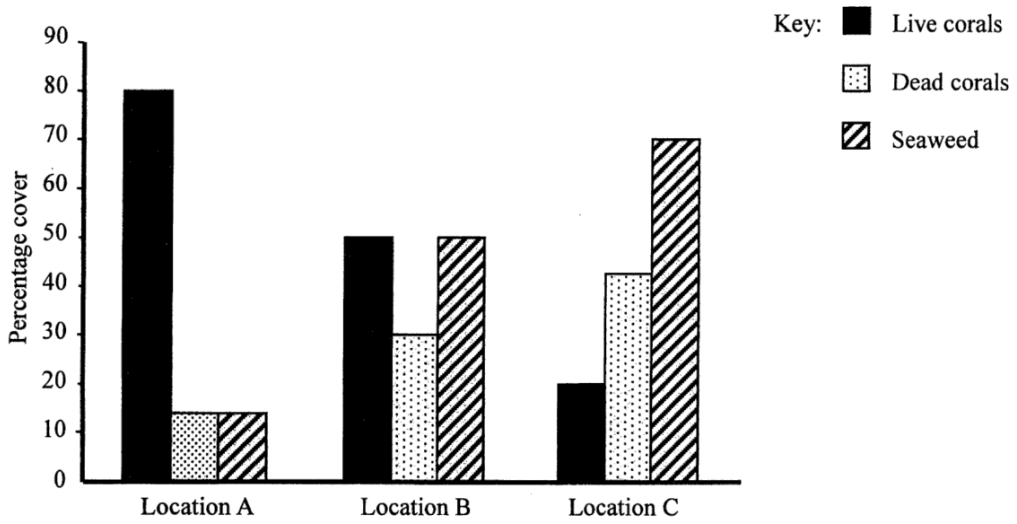
(ii) To further study the toxicity of pesticides A and B, the shrimps were exposed to either pesticide A or B at a concentration of 5 μg L^{-1} for 4 days. At the end of the study, the concentrations of pesticides in the body tissue of the shrimps were determined.

(1) 50 μg kg^{-1} of pesticide A and 80 μg kg^{-1} of pesticide B were found in the body tissue of the shrimps. Suggest ***three*** possible reasons why there was a higher concentration of pesticide B than pesticide A in the body tissue. (3 marks)

(2) Why was a concentration of 5 μg L^{-1} adopted for the pesticides used in the experiment? (1 mark)

(iii) A field survey was conducted to investigate the concentration of pesticide A in the body tissue of herbivorous fish and carnivorous fish. What difference in the concentrations of pesticide A would you expect to find between these two types of fish? Explain your answer. (3 marks)

2(b) A field survey was conducted to determine the health status of the coral communities at three locations A, B and C on the sea bottom along a coastline. At each location, the percentage cover of live corals, dead corals and seaweed was determined. The results are shown in the graph below:



(i) At which location did the coral communities appear to be the healthiest? Support your answer with data. (2 marks)

(ii) (1) Suggest ***one*** human activity which may have led to the highest percentage cover of seaweed in location C. Explain your answer. (3 marks)

(2) Suggest ***one*** possible consequence of this human activity that may have resulted in the low percentage cover of live corals at location C. (1 mark)

(iii) (1) Hoi Ha Wan was chosen to be a marine park for the richness of its coral community. Explain the ecological importance of coral communities. (3 marks)

(2) Suggest ***one*** human activity that should be prohibited in a marine park. (1 mark)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i)(1); original allocation: 1 mark(s)**

A: approximately 42 μg L^{-1}. B: approximately 27 μg L^{-1}.

From 50% mortality, draw a horizontal line to each curve, then read concentration vertically. Both correct values earn 1 mark.

a(i)(2); original allocation: 2 mark(s)

B is more toxic because a lower concentration causes the same 50% mortality.

Hold the effect constant before comparing the dose/concentration required to assess toxicity.

a(ii)(1); original allocation: 3 mark(s)

B may be absorbed more readily by shrimp, excreted less readily, and broken down or metabolised less readily.

These independently affect entry, exit and internal elimination, allowing a higher internal B concentration under the same external conditions. They are not three repetitions of "B accumulates more".

a(ii)(2); original allocation: 1 mark(s)

5 μg L^{-1} is below the concentration at which appreciable mortality begins in the graph, helping keep shrimp alive while accumulation in living organisms is measured.

Extensive mortality and subsequent decomposition would interfere with comparisons of internal concentrations.

a(iii); original allocation: 3 mark(s)

Carnivorous fish are expected to have a higher A concentration. A is lipid-soluble and can accumulate in organisms.

Carnivorous fish occupy a higher trophic level and consume many prey already containing A, causing biomagnification along the food chain.

Include trophic level, dietary intake and increasing concentration through successive levels. Larger body size alone is insufficient because the question concerns concentration.

b(i); original allocation: 2 mark(s)

A: live-coral cover is highest, approximately 80%, and dead-coral cover lowest, approximately 13%.

Both live and dead cover directly support the health assessment more effectively than simply stating that seaweed is scarce.

b(ii)(1); original allocation: 3 mark(s)

Fertiliser-containing agricultural run-off enters seawater, bringing abundant nitrate, phosphate and other nutrients. These promote rapid seaweed growth and increase its percentage cover.

Include all three links: human activity, substances entering the water, and biological response.

b(ii)(2); original allocation: 1 mark(s)

Abundant seaweed competes with coral for light and space, shading it and hindering growth, thereby reducing live-coral cover.

Alternatively, explain how a specific pollutant makes seawater unsuitable for coral. Avoid "more seaweed means less coral" without an intermediate mechanism.

b(iii)(1); original allocation: 3 mark(s)

Coral communities provide shelter and refuge from predators, together with food or breeding habitats. These support many other marine species and increase community biodiversity.

Select two specific resource/habitat functions and connect them to support for more species.

b(iii)(2); original allocation: 1 mark(s)

Ban fishing practices that directly damage coral, such as trawling.

Relate the measure to coral protection: trawling across the seabed damages fragile coral structures.

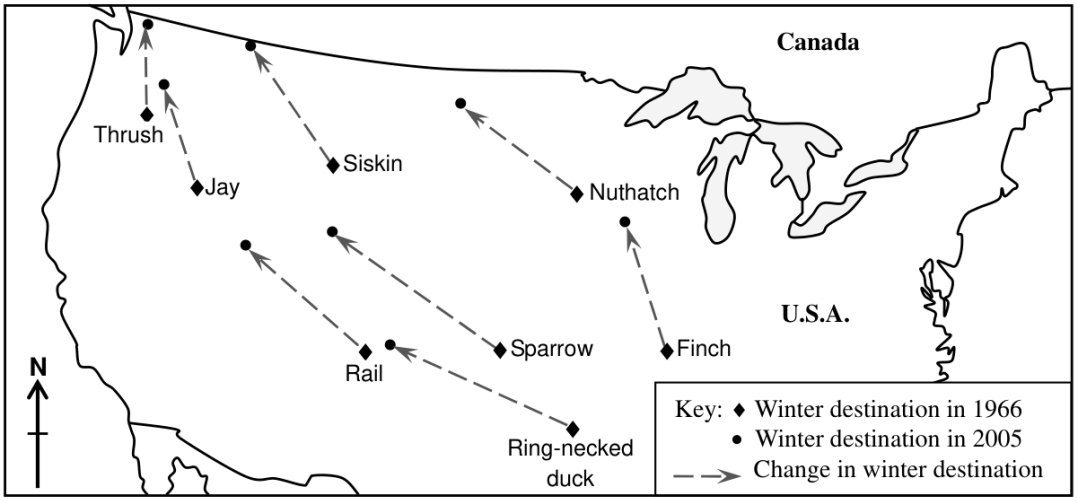
Examiner's comment (source page(s) 5)

Include units and comparisons for toxicity readings. Degradation, excretion and egestion are not synonymous. Assess coral health using both live and dead cover. Identify inorganic nutrients in agricultural run-off as promoting algal growth.

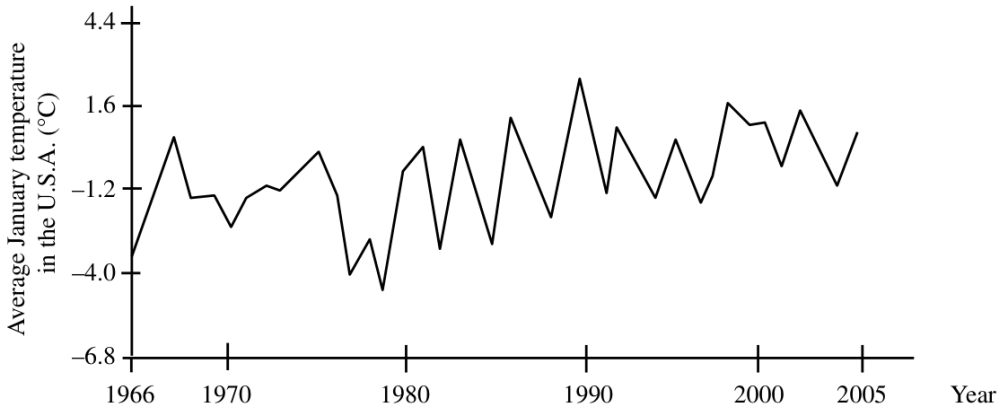
Marking reference: HKDSE Biology 2014 Paper 2 Q2, official marking scheme, source page(s) 8.

4. [HKDSE Biology Practice Paper Paper 2 Q2]
Hint: Read the environmental variable, then use its mechanism to explain ecological effects.

(a) Many bird species living in Canada migrate southwards to the U.S.A. to spend the winter. The diagram below shows the destinations of some of these bird species in the U.S.A. in the winters of 1966 and 2005:



. The average January temperatures in the U.S.A. from 1966 to 2005 are shown in the following graph:



(i) Suggest one way which can be used to track the migration route of birds. (1 mark)

(ii) (1) State the general trend shown in the average January temperature in the U.S.A. from 1966 to 2005. (1 mark)

(2) Relate the change in the winter destination of bird species in the U.S.A. with the change in average January temperature in the U.S.A. from 1996 to 2005. Suggest, from an ecological point of view, a reason for the change in the winter destination. (2 marks)

(3) Suggest **two** possible effects caused by the change in the winter destination of these migratory bird species on native bird species. (2 marks)

(4) It is believed that the trend in the average January temperature in the U.S.A. is due to human activities. Explain how human activities may have caused this trend. (4 marks)

2. (b) Forests provide humans with valuable resources. Proper forest management is needed when exploring new uses of forests to ensure sustainable forestry.

(i) The combustion of fossil fuel releases acidic gases which may cause acid rain. State and explain **two** environmental impacts of acid rain. (4 marks)

(ii) Some scientists are exploring the use of forest woody biomass to replace the fossil fuel used in power plants. Trees are logged from two forests, A and B, for this research and other uses. The table below shows the distribution of the inorganic nutrients nitrogen and magnesium in these two forests:

	Forest A		Forest B	
	Nitrogen (%)	Magnesium (%)	Nitrogen (%)	Magnesium (%)
Biomass above forest floor	35	70	5	25
Biomass on forest floor	5	5	5	5
Biomass below forest floor	60	25	90	70
Total	100	100	100	100

Based on the difference in the distribution of inorganic nutrients of the two forests, explain which forest will reestablish itself more quickly after logging. (3 marks)

(iii) Over-exploitation of forest resources leads to a decrease in biodiversity.

(1) Provide **one** reason why biodiversity is important. (1 mark)

(2) Reforestation is a way to restore a forest. In earlier years, reforestation depended mainly on exotic tree species imported from other countries and most of the plantations were formed by a single exotic tree species. State **one criterion** when choosing an appropriate exotic tree species for reforestation. Give **one disadvantage** of forming a plantation using a single tree species. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 1 mark(s)**

Fit birds with suitable GPS trackers to record their migration routes.

Alternatively, use identification rings and resighting/recapture records from different locations. The method must connect locations for the same bird.

a(ii)(1); original allocation: 1 mark(s)

Mean January temperature rises overall from 1966 to 2005, despite year-to-year fluctuations.

An increase need not occur every year for an overall upward trend to exist.

a(ii)(2); original allocation: 2 mark(s)

Wintering destinations shift northwards overall. Warmer northern winters allow birds to find suitable habitat and sufficient food further north, reducing the need to travel as far south as before.

Use temperature changes to explain the map changes, then supply the ecological food/habitat mechanism.

a(ii)(3); original allocation: 2 mark(s)

Newly arriving migrants may compete with native birds for food or habitat. They may also become prey for local predatory birds, increasing those birds' food supply.

Effects can differ in direction depending on the food web. Do not assume that every arriving bird preys on native birds.

a(ii)(4); original allocation: 4 mark(s)

Greater fossil-fuel combustion increases CO_2 emissions and atmospheric greenhouse-gas concentrations. These gases absorb outgoing long-wave infrared radiation from the Earth's surface and re-emit it in all directions, reducing net heat loss to space at the same surface temperature and causing warming.

Connect human activity, a named gas, increased concentration and the infrared-radiation mechanism. Do not confuse the greenhouse effect with ozone depletion allowing more ultraviolet radiation through.

b(i); original allocation: 4 mark(s)

Acid rain lowers water pH, harming or killing acid-sensitive aquatic organisms and reducing populations and diversity.

It also makes soil mineral nutrients more readily dissolved and leached, lowering fertility and hindering plant growth.

Each effect needs a cause and biological consequence, giving two pairs of 2 marks each. The context is forest ecology, so damage to statues alone does not answer the ecological question.

b(ii); original allocation: 3 mark(s)

B recovers faster. Logging mainly removes above-ground biomass. B retains 90% of nitrogen and 70% of magnesium below ground, whereas A retains only 60% and 25% respectively. B therefore preserves a larger nutrient store to support new plant growth sooner.

Compare the nutrient proportions remaining in the system after trees are removed, rather than assuming that greater initial above-ground biomass means faster recovery.

b(iii)(1); original allocation: 1 mark(s)

Biodiversity supplies varied foods, medicines and other resources and helps maintain ecosystem functioning.

The question needs only one reason. Develop one adequately rather than listing every possible use.

b(iii)(2); original allocation: 2 mark(s)

Selection criterion: a tree species able to grow in the damaged site's soil and climate. Disadvantage of using one species: a specialist pathogen or pest could rapidly affect a large area, making the whole plantation vulnerable.

Fast growth is another possible criterion, and low biodiversity another monoculture disadvantage. Each part earns 1 mark; keep the selection criterion separate from the disadvantage.

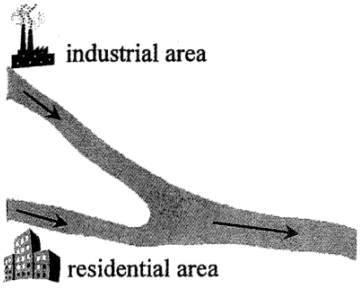
Examiner's comment (source page(s) 7, 8)

A moderate answer identifies northward movement and overall warming, but tree-selection criteria must concern vegetation establishment. Give ecological consequences for acid rain. Explain forest B's recovery through removal of above-ground biomass and retention of below-ground nutrients.

Marking reference: HKDSE Biology Practice Paper Paper 2 Q2, official marking scheme, source page(s) 12.

5. [HKDSE Biology 2016 Paper 2 Q2]
Hint: Read the environmental variable, then use its mechanism to explain ecological effects.

(a) Water samples A and B are collected from an industrial area and residential area along a river respectively. Their locations are shown in the map and the analysis of water samples is shown in the table below:



	Water sample A (industrial area)	Water sample B (residential area)
Biochemical oxygen demand, (BOD, 5 days 20°C) (mg L^{-1})	20	100
Heavy metal (ppm)	30	0.01
Antibiotic residues (μg L^{-1})	0.1	50

(i) Give the particular type of pollutant in water sample B that can contribute to the difference in BOD between water samples A and B. Explain your answer. (4 marks)

(ii) Some people like to go fishing in the river next to the industrial area and consume the fish. Explain the health risks of consuming the fish. (4 marks)

(iii) (1) Suggest why antibiotic residues are present in water sample B. (1 mark)

(2) Suggest ***one*** possible effect of antibiotic residues on the ecosystem. (1 mark)

2(b) Arsenic is poisonous to humans. Contamination of soil with arsenic can occur as a result of using arsenical pesticides and herbicides, mining activity and waste disposal. An experiment was conducted to study the effect of phosphate addition on the growth of wheat and the accumulation of arsenic in different parts of the wheat. 100 pots with uncontaminated soil and 100 pots with arsenic-contaminated soil were prepared for growing wheat. Phosphate was added to 50 pots from each group. The dry masses of grain and plant body (shoot and root), and the distribution of arsenic in different parts of the wheat after seven months are shown in the table below:

	Dry mass of wheat (g pot^{-1})		Distribution of arsenic in the wheat grown in arsenic-contaminated soil (%)
	Uncontaminated soil	Arsenic-contaminated soil	
No phosphate added			
• grain	8	2	10
• plant body	14	5	90
Phosphate added			
• grain	20	12	2
• plant body	15	14	98

(i) Compare the effects of arsenic on the growth of grain and plant body when no phosphate was added. (2 marks)

(ii) With regard to the growth of wheat in the two types of soils, what can you conclude about the effect of phosphate addition on the growth of grain and plant body? (4 marks)
(Note: You should refer to the growth of wheat in uncontaminated soil as the control.)

(iii) Comment on the effect of phosphate addition on the distribution of arsenic in different parts of the wheat. (2 marks)

(iv) With reference to the above results, what is the significance of phosphate addition to the agricultural production of wheat in arsenic-contaminated land? (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation (a)(i); original allocation: 4 mark(s)

B contains more biodegradable organic matter from domestic sewage. Microorganisms consume more oxygen through respiration during decomposition, so BOD is higher.

A has a BOD of 20 mg L^{-1} and B 100 mg L^{-1}. BOD measures oxygen required for microbial decomposition of available organic matter under specified conditions, not the dissolved oxygen already present at that moment. More organic matter in residential sewage → more decomposition → greater oxygen consumption → higher BOD.

(a)(ii); original allocation: 4 mark(s)

The industrial area's high heavy-metal content can accumulate in fish and pass through food chains. Prolonged consumption of contaminated fish may cause excessive heavy-metal intake and poisoning.

A contains 30 ppm heavy metals, far above B's 0.01 ppm. Certain heavy metals are not readily biologically degraded or eliminated. Long-term uptake increases body burden, and feeding relationships may intensify accumulation. The risk from eating fish therefore concerns pollutants accumulated over time, not simply whether the river looks clear.

(a)(iii)(1); original allocation: 1 mark(s)

Antibiotics taken by residents, or their residues, can enter domestic sewage through urine and faeces.

Link the source to residential drainage. Drugs not completely absorbed or metabolised are excreted and enter water through the sewage system.

(a)(iii)(2); original allocation: 1 mark(s)

Antibiotic residues can select for a higher proportion of resistant microorganisms.

Sensitive individuals are more readily inhibited or killed, while those already possessing resistance variants are more likely to survive and reproduce. Resistance therefore becomes more common. Antibiotics do not purposefully induce the required mutation in every bacterium.

(b)(i); original allocation: 2 mark(s)

Arsenic reduces both grain and vegetative growth, with proportionally greater suppression of grain growth.

Without added phosphorus, grain dry mass falls from 8 to 2 g, a 75% reduction. Vegetative dry mass falls from 14 to 5 g, approximately 64%. Both are inhibited, but compare relative changes rather than only the absolute losses of 9 g and 6 g.

(b)(ii); original allocation: 4 mark(s)

Added phosphorus increases grain dry mass in both soils, although contaminated soil still gives a lower value. It has little effect on vegetative dry mass in uncontaminated soil but raises that in contaminated soil from 5 to 14 g, close to the uncontaminated value.

Grain mass rises from 8→20 g in uncontaminated soil and 2→12 g in contaminated soil. Both increase, but 20 g still exceeds 12 g after phosphorus addition. Vegetative mass changes little from 14→15 g in uncontaminated soil, whereas 5→14 g in contaminated soil shows marked recovery. Separate grain from vegetative mass, then compare soils within each.

(b)(iii); original allocation: 2 mark(s)

Phosphorus addition increases the proportion of arsenic in vegetative parts from 90% to 98% and reduces that in grains from 10% to 2%.

These are percentage distributions between parts, not total arsenic amounts or concentrations per gram. They establish a smaller proportion in edible grains and a larger proportion in roots and shoots, but do not determine the milligrams absorbed by the whole plant.

(b)(iv); original allocation: 2 mark(s)

It can increase grain yield and reduce the proportion of arsenic entering edible grains, potentially lowering dietary exposure risk.

Connect agricultural benefit with pollutant distribution in food. Grain mass rising from 2 to 12 g in contaminated soil supports increased yield. Grain arsenic allocation falling from 10% to 2% supports a favourable change in risk. The table alone does not show that any safe concentration has been reached.

Examiner's comment (source page(s) 4)

BOD is the oxygen required for microbial decomposition of organic matter in water, not existing dissolved oxygen. Arsenic-distribution percentages alone do not give absolute arsenic content in food. Assessing food safety also requires actual content in the edible portion.

Marking reference: HKDSE Biology 2016 Paper 2 Q2, official marking scheme, source page(s) 9.

Practice VI.c Conservation of species and habitats

Related type 1

Link conservation measures with testable ecological outcomes

Degree of integration ★★★★☆

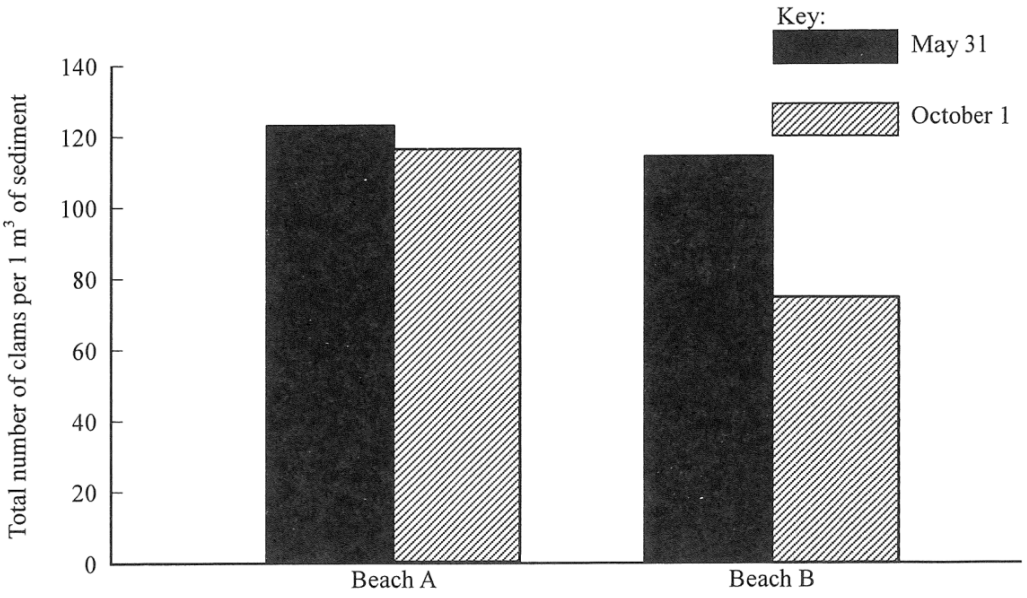
Practice

1. [HKDSE Biology 2020 Paper 2 Q2]

Hint: Identify what the measure changes, then evaluate the outcome with data.

(a) A study was conducted on beaches A and B to investigate the effect of clam harvesting on the animal community on sandy shores. On beach A, clam harvesting was forbidden all year round. On beach B, clam harvesting was allowed from June to September. Field sampling was conducted on both beaches on May 31 and October 1. Animals in the sediment were identified and counted.

(i) The diagram below shows the number of clams collected from the two beaches on May 31 and October 1:



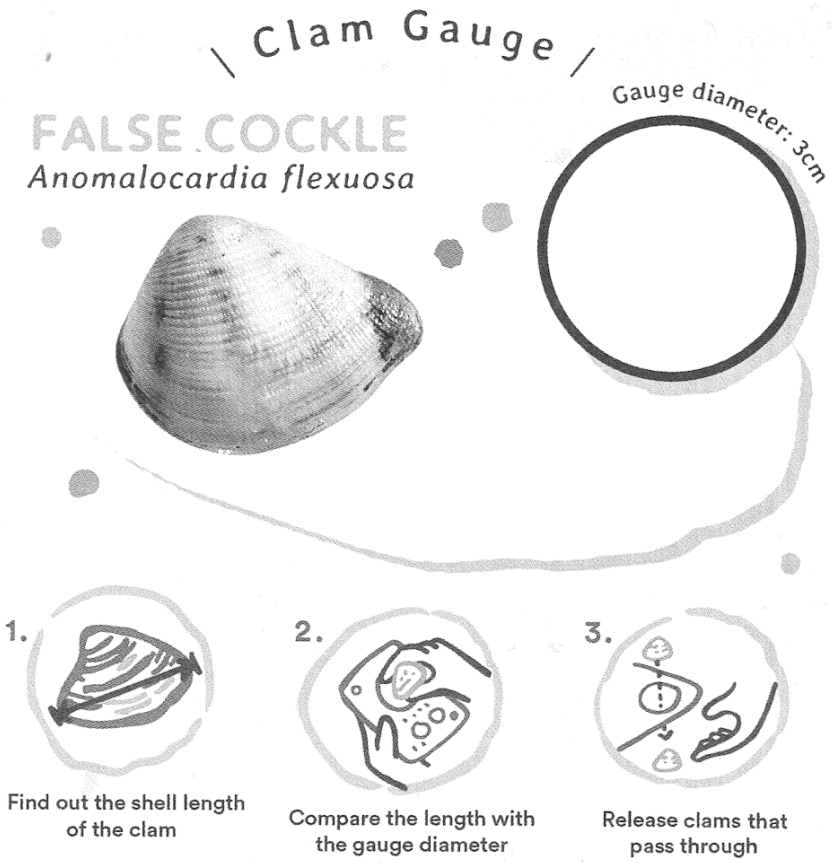
Describe the change in the density of clams on the two beaches. (2 marks)

(ii) The table below shows the animal species composition of beaches A and B on May 31 and October 1 (the data on clams are excluded):

		May 31		October 1	
	Animal species	No. of individuals per 1 m^3 of sediment	Percentage of total individuals	No. of individuals per 1 m^3 of sediment	Percentage of total individuals
Beach A	Species 1	20	31.3	19	30.2
	Species 2	15	23.4	16	25.4
	Species 3	8	12.5	8	12.7
	Species 4	21	32.8	20	30.7
Beach B	Species 1	19	30.6	7	20.6
	Species 2	16	25.8	5	14.7
	Species 3	7	11.3	21	61.8
	Species 4	20	32.3	1	2.9

Based on the results in (a) (i) and (a) (ii), deduce the effect of clam harvesting on the species diversity of the animal community on sandy shores. (4 marks)

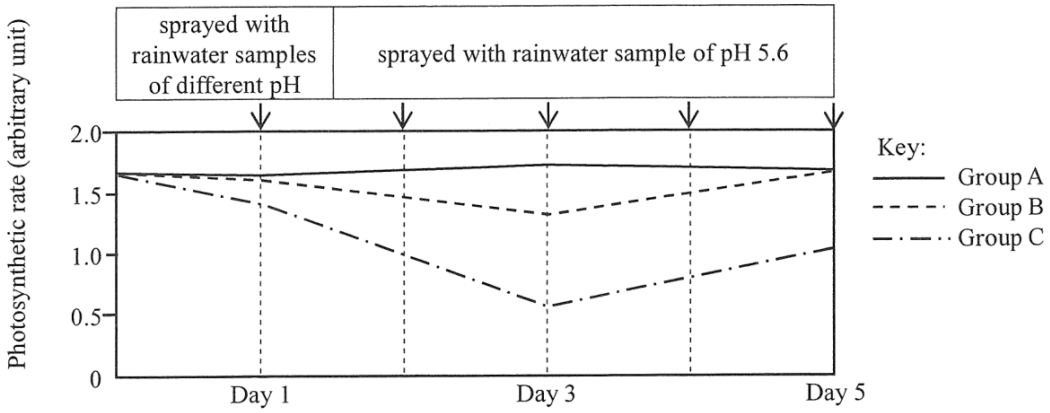
(iii) The World Wildlife Fund in Hong Kong has developed a Clam Gauge as a guideline for harvesting clams. The gauge for a particular calm is shown below:



Explain how we can help conserve the clam community by harvesting only clams which cannot pass through the gauge. (3 marks)

2(b) Normal rainwater has a pH of around 5.6. Acid rain was first noted in the 17th century. At that time, rainwater with pH readings below 2.5 were recorded in industrial areas.

(i) In 1986, an experiment was conducted to study the effect of acidity of rainwater on the photosynthetic rate of a lichen species. The lichens were divided into three groups: Group A served as the control, which was sprayed with rainwater sample of pH 5.6 every day; Groups B and C were sprayed with rainwater samples of pH 3.5 and 2.5 respectively on Day 1 and then sprayed with rainwater sample of pH 5.6 on Day 2 until Day 5.



(1) Compare the effects of spraying rainwater samples of different pH on the photosynthetic rate of the lichen. (4 marks)

(2) The chart below shows the weather in city Y in a month during the rainy season. Based on the results of the experiment, explain why the lichen is rarely found in the industrial area of city Y. (3 marks)

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

(ii) State *two* major air pollutants and describe how they cause the formation of acid rain. (2 marks)

(iii) State *two* effects of acid rain on soil minerals. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation a(i); original allocation: 2 mark(s)

Clam density at beach A falls slightly from approximately 120 to 113 individuals/m³. At beach B it falls markedly from approximately 110 to 70 individuals/m³.

Compare 31 May with 1 October within each beach, then identify the larger decrease at B. A prohibits clam collecting year-round, whereas B permits it between the surveys. Saying only that A exceeds B does not describe the changes. Use reasonable approximate graph readings; perspective distortion from a slanted photograph does not justify precise decimals.

a(ii); original allocation: 4 mark(s)

The data support reduced species diversity in the sandy-shore animal community following clam collecting, chiefly through reduced evenness.

At A, where no collecting occurs, the four species' relative abundances remain broadly similar, providing a comparison without collecting. At B, species 1, 2 and 4 decrease, while species 3 increases from 7 to 21 individuals. Its proportion rises from 11.3% to 61.8%, making it clearly dominant. Species 4 falls from 32.3% to 2.9%.

All four species remain in B's second survey; species 4 still has one individual. Do not claim disappearance or reduced species number. The evidence for lower diversity is less even relative abundance. Connect B's collecting activity with the A control for a supported inference. A fall in total individuals alone does not necessarily mean lower diversity.

a(iii); original allocation: 3 mark(s)

Collecting only larger clams leaves small juveniles to grow and reproduce, producing offspring that replace collected individuals and maintain the population.

Smaller individuals able to pass through the 3 cm diameter hole are retained in the habitat. The point is their opportunity to reach reproductive age, not that they contain too little meat. The conservation gauge links collection restrictions to reproductive replenishment, promoting sustainable use. Do not reverse the rule and say only juveniles are collected.

b(i)(1); original allocation: 4 mark(s)

Rain below pH 5.6 reduces photosynthetic rate, with pH 2.5 causing a greater fall than pH 3.5. After switching back to pH 5.6 rain, the pH 3.5 group returns close to normal by day 5, whereas the pH 2.5 group remains incompletely recovered.

A receives pH 5.6 daily and remains approximately stable. B and C receive pH 3.5 and 2.5 respectively only on day 1, then pH 5.6 on days 2–5. B reaches a minimum near 1.3 and C near 0.5, showing stronger inhibition with greater acidity. By day 5, B is near A's level, but C remains near 1.0, below the normal approximately 1.6. Cover both damage and recovery, identifying each group's pH rather than comparing only A, B and C labels.

b(i)(2); original allocation: 3 mark(s)

Highly acidic industrial-area rain can markedly reduce lichen photosynthesis. Frequent rainy-season exposure may affect lichens again before recovery, preventing adequate long-term organic-nutrient production and threatening survival.

After one pH 2.5 treatment, the experiment shows incomplete recovery by day 5. The calendar also shows closely spaced rainy days with little time for prolonged recovery. Combine both to explain scarce lichens in industrial areas rather than merely repeating that acid rain lowers photosynthesis. Lichens depend on their photosynthetic partners for organic matter, so sustained inhibition affects growth and survival.

b(ii); original allocation: 2 mark(s)

Sulphur dioxide and nitrogen oxides. After atmospheric release, oxidation and related reactions form acidic substances that dissolve in rainwater, causing acid rain.

Give both pollutants and acid formation, for example sulphuric or nitric acid. Normal rain can already be weakly acidic because of carbon dioxide. CO_2 should not replace SO_2 and NO_x as the main causes of the strongly acidic industrial rain asked about here.

b(iii); original allocation: 2 mark(s)

Acid rain increases the solubility of certain toxic soil metals and makes mineral nutrient ions more readily leached.

First, metal ions such as aluminium that were less soluble enter soil solution more readily under acidic conditions, increasing toxicity risk. Second, useful mineral nutrients are washed away, reducing fertility. The question specifies soil minerals; root damage or lower soil pH alone does not describe the mineral changes.

Marking reference: HKDSE Biology 2020 Paper 2 Q2, official marking scheme, source page(s) 10.

2. [HKDSE Biology 2024 Paper 2 Q2]
Hint: Identify what the measure changes, then evaluate the outcome with data.

(a) Every year, migratory birds travel long distances between breeding grounds in the north and wintering grounds in the south. During their long journeys, wetlands along the coastal areas are important stopovers. Hong Kong is one of these stopovers.

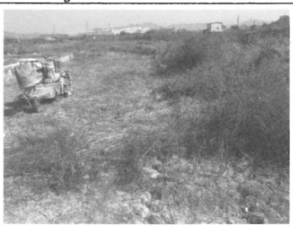
(i) Yellow-breasted bunting, *Emberiza aureola*, is one of these migratory birds which visit Hong Kong during the winter. The following table shows the change in the conservation status of yellow-breasted bunting according to the International Union for Conservation of Nature:

Year	***Conservation status***
2000	Least concern
2004	Near threatened
2008	Vulnerable
2013	Endangered
2017	Critically endangered

Explain ***one*** human activity that might have threatened the yellow-breasted bunting. (1 mark)

(ii) In 2005, the government and local green groups worked together to implement habitat management measures for the abandoned fresh water wetland in Long Valley (northern part of the New Territories). The photographs below show the habitats before and after the measures:

Before the measures | ***After the measures***



Abandoned farmland



Dry farmland



Wet farmland



Dried wetland



Marsh



Water flea pond

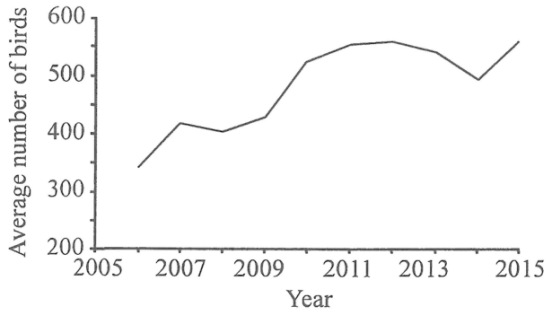


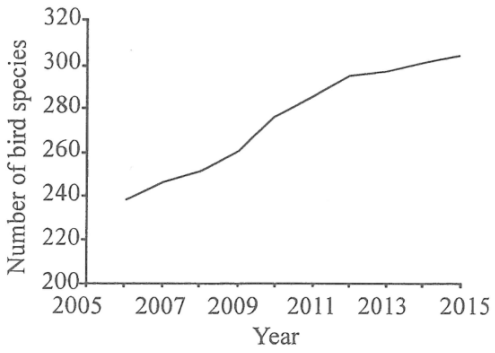
Lotus pond



Fish pond

The graphs below show the average number of birds recorded and the number of bird species recorded annually from 2006 to 2015 in Long Valley:





(1) Based on the data shown, what can you conclude about the effect of the habitat management on the conservation for migratory birds? (4 marks)

(2) Explain why the habitat management is important in bringing about the changes shown in the graphs. (4 marks)

2(b) Microplastics (MPs) are small plastic pieces (less than five millimeters). They can be plastic debris resulting from the breakdown of large plastic wastes or microbeads in cosmetic and cleansing products. They can be harmful to aquatic environments. Scientists are looking for biological ways to monitor and solve MPs pollution.

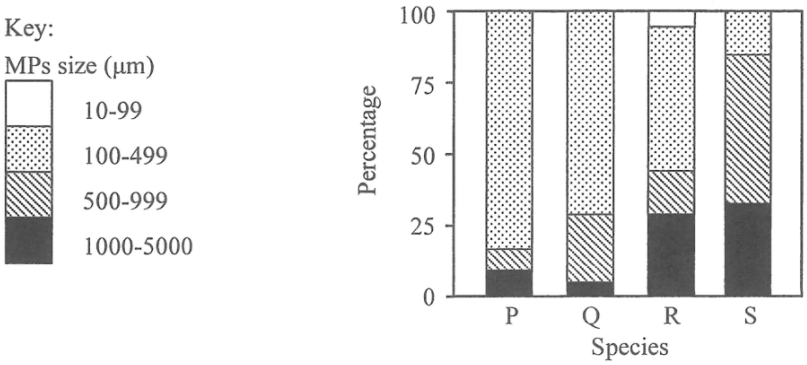
(i) Suggest why sessile (immovable) organisms are more suitable than movable organisms for use in monitoring MPs pollution in aquatic environments. (2 marks)

(ii) The table below shows the occurrence of four species of sessile organisms which are commonly found in Hong Kong waters:

Site	*Habitat*	*Species*
1	Rocky shore	A
2	Rocky shore	A, B
3	Rocky shore	A, B
4	Rocky shore	A, C
5	Mudflat	C
6	Mudflat	C
7	Mudflat	D
8	Mudflat	D
9	Pier	C
10	Pier	C
11	Pier	D
12	Pier	D

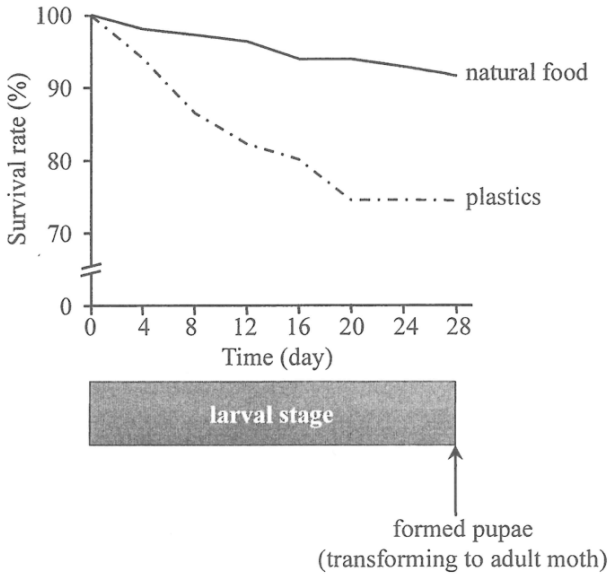
Based on the data in the table, which species should be chosen for monitoring MPs pollution? Explain your answer. (3 marks)

(iii) The diagram below shows the relative portions of different sized MPs retained in the guts of four species of sessile organisms:



Based on the data shown in the diagram, which species (P, Q, R or S) should be used for monitoring MPs pollution? Explain your answer. (3 marks)

(iv) The larvae of a certain species of wax moth (*Achroia grisella*) was found to be able to feed on and degrade plastics. In an investigation, two groups of *A. grisella* larvae were fed with plastics and natural food separately and their survival rates were recorded until the larvae formed pupae. The results are shown below:



(1) With reference to the data shown, do the data support that the larvae can obtain nutrients from plastics? Explain your answer with evidence. (1 mark)

(2) To study the feasibility of a large-scale application of *A. grisella* in treating plastic pollution, suggest another measurement for the investigation. Explain your answer. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 1 mark(s)**

For example, converting coastal wetlands for development destroys stopover sites where migrating birds replenish food reserves.

Yellow-breasted buntings need rest and energy replenishment during long migrations. Loss of wetland food and habitat reduces survival and migration-completion chances. Illegal hunting for food is another possible specific activity, provided its threat is explained.

a(ii)(1); original allocation: 4 mark(s)

Both recorded mean bird abundance and bird-species number show increasing trends, supporting the view that habitat management attracts more visiting/staying individuals and more species.

Mean bird abundance rises overall, despite fluctuations, from approximately 340 in 2006 to 550 in 2015. Species number increases continuously from approximately 240 to 300. The former measures individual abundance and the latter species richness. Use both separately rather than replacing them with one vague "increase in numbers".

a(ii)(2); original allocation: 4 mark(s)

Restoration and establishment of varied habitats increase food resources and available ecological niches, allowing more individuals and species to use the site.

The photographs show abandoned or dried areas becoming dry farmland, paddy fields, marshes and several pond types. More food supports larger populations and greater abundance. Different water depths, vegetation, food and habitat conditions meet different birds' needs, increasing available niches and hence species number.

b(i); original allocation: 2 mark(s)

Microplastics ingested by sessile organisms mainly originate in their local water, making internal pollutant levels easier to associate with local pollution sources.

Mobile organisms may ingest microplastics elsewhere before moving to the sampling site, leaving the represented location uncertain. Organisms fixed in place are better suited to comparing sites.

b(ii); original allocation: 3 mark(s)

Species C.

C occurs in all three habitat types: rocky shores, mudflats and piers. Other species have more restricted distributions. Using one species across habitats reduces confounding from differences in feeding or accumulation between species, making pollution comparisons fairer. Occurrence at all twelve individual sites is unnecessary; the key is coverage across the three habitat types.

b(iii); original allocation: 3 mark(s)

Species R.

R's gut contains all four microplastic size categories in the legend, including the smallest, 10–99 μm. P, Q and S lack some categories. R covers a fuller particle-size range and can better reflect pollution by different-sized microplastics rather than monitoring only one preferentially ingested size.

b(iv)(1); original allocation: 1 mark(s)

Support: approximately 75% of larvae fed only plastic survive to day 28 and can form pupae.

Sustained survival and development support obtaining some nutrition from plastic. However, survival is lower than with natural food, so this does not show identical nutritional value.

b(iv)(2); original allocation: 2 mark(s)

Examine the chemical properties and particle sizes of plastic in larval waste to determine whether it is truly degraded rather than merely fragmented.

If larvae only chew large plastics into microplastics and pass them out, pollution persists and may become harder to collect. Actual degradation must be demonstrated before proposing large-scale pollution treatment. Intake, reproductive capacity or growth can also be measured, but connect the measurement to treatment efficiency or continued usability.

Examiner's comment (source page(s) 75, 76)

More bird individuals and species do not automatically establish greater overall diversity without evenness data. C permits same-species comparisons across three habitat types; R covers every particle-size category. Inferences from larval survival should cite sustained development to day 28 or pupation, not simply which survival curve is higher.

Marking reference: HKDSE Biology 2024 Paper 2 Q2, official marking scheme, source page(s) 61, 62.

Chapter 9

Microorganisms and humans: question types and practice

VII.a Microbiology

Read the explanations and diagrams in Concept VII.a first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Estimate microorganisms using growth curves, optical density and colonies

Degree of integration ★★★☆☆

Key ideas

① An increase from 100 to 400 cells is a fourfold increase, or two doublings. If this takes two hours, the mean doubling time is one hour. Optical density measures turbidity and may reflect living cells, dead cells and other particles. Colony counts measure units capable of forming colonies; a cluster of cells can form one colony.

② Before comparing the two kinds of result, state what each actually measures.

How to recognise the problem Read the time axis and check whether the vertical scale is logarithmic. Distinguish total cell amount, optical density and colony-forming units. Limit conclusions to the evidence actually supplied.

Example 1 [HKDSE Biology 2018 Paper 2 Q3]

3(a) Trillions of microorganisms live in the human intestine. Most of them are non-pathogenic and play an important role in many functions in the human body, including food digestion, defence against pathogens and vitamin production.

(i) What is the ecological relationship between humans and these microorganisms in the intestine? Explain your answer. (2 marks)

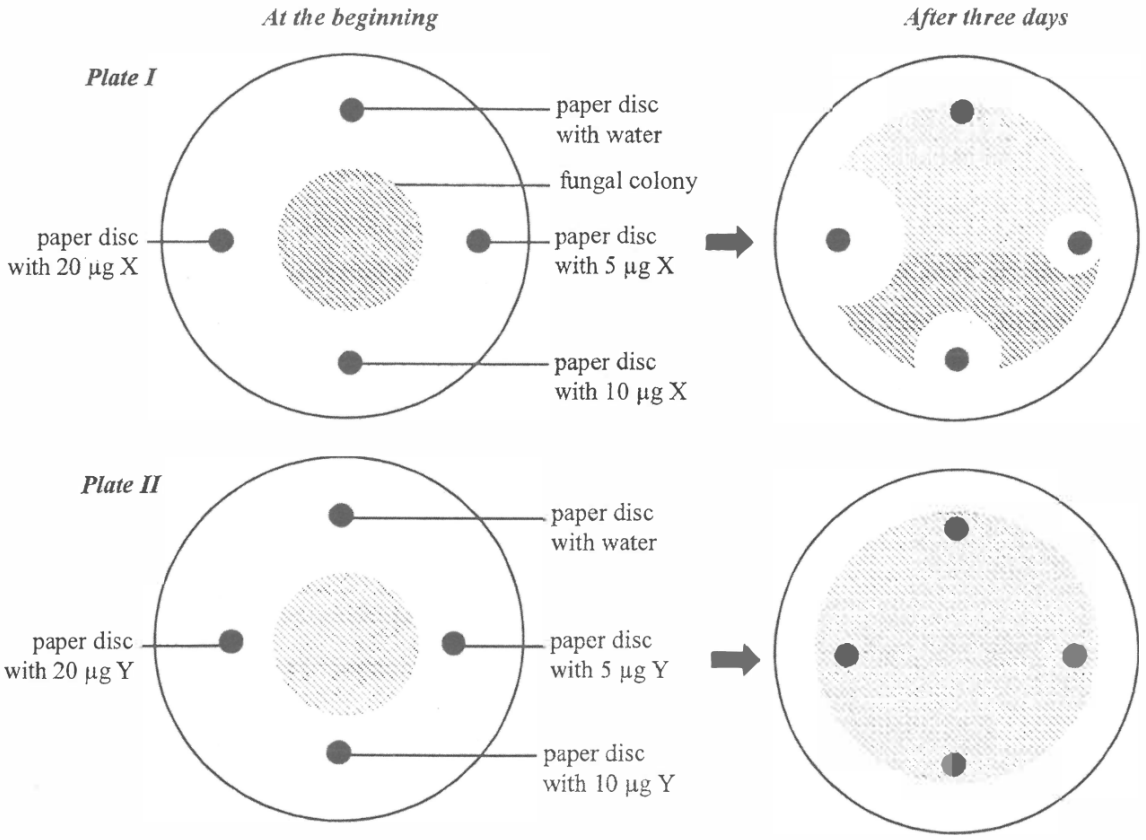
(ii) According to the World Health Organization, probiotics are microorganisms that confer health benefits on humans. However, a large amount of probiotics has to be ingested so that a sufficient number can survive gastric digestion and reach the intestine.

(1) You are given a flask of cultured probiotic bacteria and a 37°C incubator. In order to design an investigation to estimate the number of probiotic bacteria that can survive gastric digestion, suggest ***two*** other pieces of information that are required to simulate, in a laboratory setting, the physiological conditions in the stomach. (2 marks)

(2) Describe how you can estimate the number of living probiotic bacteria at the end of the experiment. (4 marks)

(3) It is believed that patients who have completed a course of antibiotic treatment should consume food that contains probiotics. Suggest why this may protect patients from infection. (3 marks)

3(b) An experiment was conducted to study the effects of compounds X and Y on the growth of a fungal colony. Paper discs with different dosages of compound X or Y were placed on nutrient agar plates previously inoculated with a fungal colony. The appearance of the fungal colony at the beginning of the experiment and after three days are shown in the diagrams below:



(i) Describe the effects of compounds X and Y on the growth of this fungus. (3 marks)

(ii) In another experiment, the fungus was incubated for 1 hour with compound X or Y. After that, a green fluorescent dye which cannot pass through cell membranes was added. Green fluorescence stained the nuclei of the fungal cells exposed to compound X while no fluorescence was detected in fungal cells exposed to compound Y.

(1) With reference to the results, suggest the possible action of compound X on the fungus. (2 marks)

(2) Based on your answer in (1), suggest why clear zones were present in plate I after three days as shown in the diagram above. (2 marks)

(iii) Aseptic technique should be used during the inoculation of the fungus on the agar plate. Explain the importance of this. (1 mark)

(iv) This fungus occurs commonly in air and grows on our household goods. Suggest ***one*** possible daily use of compound X in our household products. (1 mark)

Solution a(i); original allocation: 2 mark(s)

Mutualism: microorganisms obtain food and a habitat from the host, while the host benefits through assistance with digestion, protection against pathogens or vitamin supply.

Identify a concrete benefit for each partner. The gut provides suitable conditions and nutrients. The stated microorganisms help break down food, restrict pathogens or synthesise vitamins, benefiting the host. "Symbiosis" describes living together; benefits to both explain why the relationship is mutualistic.

Method: Check the scale; distinguish optical density, cell number and colonies.

a(ii)(1); original allocation: 2 mark(s)

The stomach pH and the normal residence time of food or microorganisms in the stomach are needed.

A microbial culture and 37°C incubator are already provided, so identify missing information about stomach conditions. pH determines the acidity to simulate, while residence time determines the duration of acid exposure. Both affect survival. Do not list the already supplied 37°C as missing information.

a(ii)(2); original allocation: 4 mark(s)

Prepare suitable serial dilutions of the treated culture. Spread a known volume on culture medium, incubate and count colonies. Use the dilution and plated volume to calculate viable concentration.

Dilution separates colonies, preventing excessive merging that makes counting impossible. Select a plate suitable for counting and estimate CFU on the assumption that one viable colony-forming unit produces one colony.

Original viable concentration (CFU/mL) = colony count ÷ dilution fraction ÷ plated volume (mL).

For the total in the culture, multiply by total culture volume. This method identifies viability through colony formation. Turbidity measurement or direct counting of every cell may include dead cells. A colony-forming unit is not necessarily one cell, since a cluster can produce a single colony.

a(ii)(3); original allocation: 3 mark(s)

Antibiotics may also kill beneficial gut bacteria. Probiotic supplementation can help restore beneficial populations that compete with pathogens for food and attachment sites, reducing pathogen multiplication.

First explain the intestinal change caused by treatment. Structures or metabolic pathways targeted by antibiotics are not necessarily exclusive to pathogenic bacteria, so beneficial bacteria may also be affected. Fewer existing competitors allow pathogens greater access to space and nutrients. Restoring beneficial bacteria aims to re-establish this competitive barrier. “Boosting immunity” alone is insufficient, and probiotics do not neutralise every antibiotic.

b(i); original allocation: 3 mark(s)

X inhibits fungal growth, and greater amounts of X on the paper discs produce larger inhibition zones. Under these test conditions, Y shows no inhibitory effect.

X discs containing 5, 10 and 20 μg show progressively larger zones without fungal growth, supporting a stronger effect at higher doses. The water control shows that the disc or added water alone is not responsible. No corresponding clear zone appears at any tested Y dose. Conclude only that no inhibition is observed at the doses and conditions tested, not that Y is ineffective at every possible concentration.

b(ii)(1); original allocation: 2 mark(s)

X damages fungal cell membranes or increases their permeability, allowing dye normally excluded by intact membranes to enter cells and produce green nuclear fluorescence.

Connect the stated detection principle with the observation. Intact membranes exclude the dye, but nuclei fluoresce after X treatment, indicating passage across the original barrier. Y provides a comparison without the same effect. “X stains the cells green” does not identify membrane integrity or permeability as the property tested.

b(ii)(2); original allocation: 2 mark(s)

X diffuses from the paper disc into surrounding medium and damages fungal membranes. Nearby fungi cannot grow or die, producing an inhibition zone.

The disc supplies X, which diffuses outward and damages membranes wherever its concentration is sufficient. Loss of normal selective permeability can allow cellular contents

to leak, preventing sustained fungal growth and leaving an area without fungal colonies. Explain diffusion → membrane damage → growth inhibition, not merely the appearance of a clear circle.

b(iii); original allocation: 1 mark(s)

Prevent contamination of the culture and medium by other microorganisms so observed growth responses can be attributed to the fungus being tested.

Introduced bacteria or fungi could form extra colonies or alter local conditions, interfering with inhibition-zone interpretation. In this comparative experiment, aseptic technique directly maintains culture purity and reliable results. “For safety” alone does not answer the experimental purpose.

b(iv); original allocation: 1 mark(s)

An ingredient in mould-resistant paint.

For example, a substance with suitable antifungal activity can be incorporated into a coating to limit mould on damp surfaces. This directly follows from the fungal-inhibition evidence. A generic “germ-killing product” does not specify mould as the target.

Marking reference: HKDSE Biology 2018 Paper 2 Q3, official marking scheme, source page(s) 8.

Example 2 [HKDSE Biology 2023 Paper 2 Q3]

(a) Microbial contaminations happen during food processing if the food is not properly handled. The photographs below show two meat products: minced beef and steak.

Minced beef



Steak



(i) Suggest why minced beef is more susceptible to microbial contamination than steak. (2 marks)

(ii) To assess the safety and quality of minced beef, four minced beef samples were collected for microbial analysis. The samples were incubated at two different temperatures (5°C and 20°C). At regular time intervals, samples were plated for colony count of bacterium X and bacterium Y respectively. The time taken for the colony count to increase 10-fold were recorded. The results are shown in the table below:

Sample	Time taken for the colony counts to increase 10-fold (hour)			
	Bacterium X		Bacterium Y	
	5°C	20°C	5°C	20°C
1	34.95	8.93	79.78	7.28
2	41.51	7.74	65.83	8.47
3	32.86	11.60	44.60	9.65
4	20.69	14.03	40.39	17.66

(1) Calculate the mean time taken for the colony count to increase 10-fold for bacterium X and bacterium Y at 5°C and 20°C. Tabulate your results in the answer book according to the following format. (2 marks)

	Bacterium X		Bacterium Y	
	5°C	20°C	5°C	20°C
Mean time (hour)				

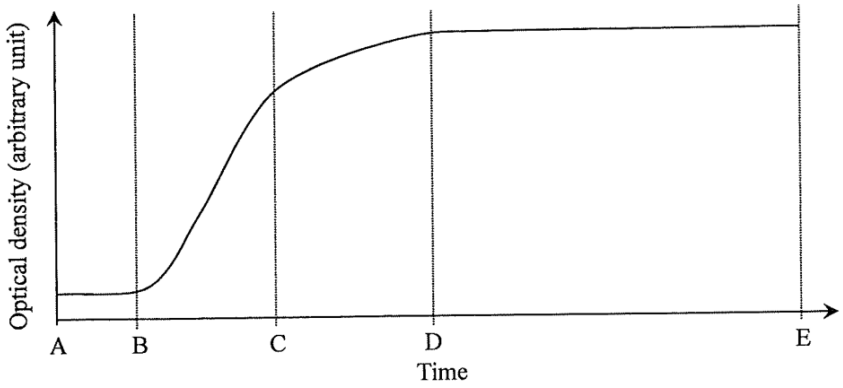
(2) Compare the growth rates of bacteria X and Y at different temperatures. (2 marks)

(3) According to the answer in (2), which bacterium is more likely to cause food spoilage of the samples stored in the refrigerator? Explain your answer. (3 marks)

(4) Bacterium Y is commonly found in the intestinal tract of animals. Explain why bacterium Y is often used as an indicator of microbial contamination of meat during food processing. (2 marks)

3(b) The growth of a bacterial culture was monitored by measuring the turbidity of the culture in terms of optical density. The results are shown in the Graph I below:

Graph I



(i) What are the characteristics of the stationary phase of bacterial growth? (2 marks)

(ii) With reference to the principle of optical density measurement, explain why CD is the stationary phase instead of DE with supporting evidence from the graph. (3 marks)

(iii) With reference to the principle of optical density measurement, explain why the bacterial culture should be mixed well before measuring the optical density. (2 marks)

(iv) *Pseudomonas aeruginosa* is a bacterial species with resistance to a number of antibiotics. It causes serious infections in hospital patients with weak immune systems.

To assess the effectiveness of different antibiotics in treating patients infected with *P. aeruginosa*, *P. aeruginosa* was isolated from the patients to prepare a culture. A fixed amount of the culture was added to mini-wells containing equal volume of antibiotics at different concentrations for incubation as shown in Diagram II. The results after incubation are shown in Diagram III:

Diagram II (3D view)

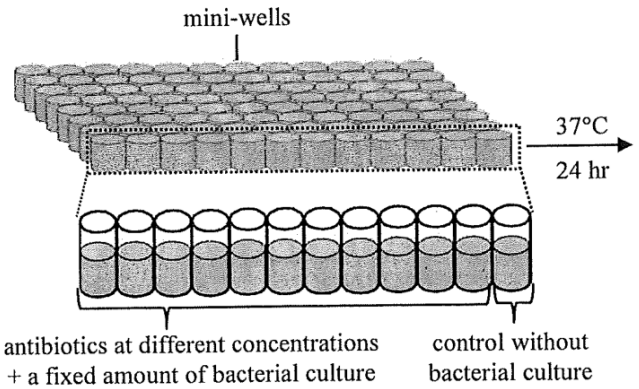
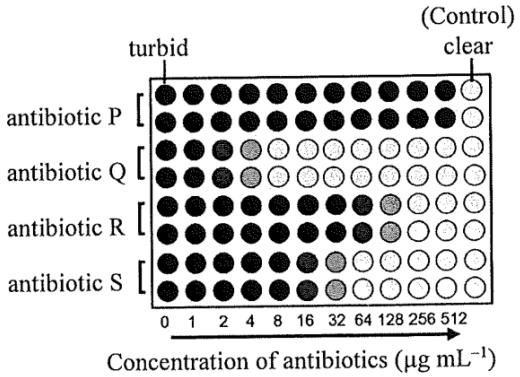


Diagram III (top view)



(1) Arrange the antibiotics in ascending order of their effectiveness in treating patients infected with *P. aeruginosa*. (1 mark)

(2) What is the minimum concentration of antibiotic S required to treat the patients effectively? (1 mark)

Solution a(i); original allocation: 2 mark(s)

Mincing increases exposed surface area and adds processing steps involving contact with equipment, hands and the environment.

Dividing the same meat volume into many small pieces exposes more surface to microorganisms. Mixing also distributes surface contamination into the interior. Minced beef is therefore more readily contaminated at multiple locations than an intact steak.

Method: Check the scale; distinguish optical density, cell number and colonies.

a(ii)(1); original allocation: 2 mark(s)

Mean times: X takes 32.50 h at 5°C and 10.58 h at 20°C; Y takes 57.65 h and 10.77 h respectively.

Add the four values in each column and divide by 4. For example, X at 5°C:
(34.95+41.51+32.86+20.69)÷4 = 32.5025 h, or 32.50 h to two decimal places.

The other three unrounded means are 10.575, 57.65 and 10.765 h respectively.

a(ii)(2); original allocation: 2 mark(s)

X grows faster than Y at 5°C. At 20°C their growth rates are approximately equal.

A shorter time for the same tenfold increase means faster growth. At 5°C, X's 32.50 h is markedly shorter than Y's 57.65 h. At 20°C, 10.58 and 10.77 h are very close. Do not mistake a longer time for faster growth.

a(ii)(3); original allocation: 3 mark(s)

X is more likely to spoil refrigerated food sooner.

Refrigerator temperature is close to the experimental 5°C. X needs only 32.50 h for a tenfold increase there, compared with Y's 57.65 h. It reaches a large population sooner, so metabolic effects and food decomposition accumulate earlier, causing faster spoilage from equal starting conditions.

a(ii)(4); original allocation: 2 mark(s)

Y commonly lives in animal intestines, so its presence in food may indicate contamination by intestinal contents or faeces during processing.

Intestinal contents can contact meat during slaughter or be transferred through contaminated equipment or hands. A bacterium normally associated with the intestine can indicate processing hygiene and cross-contamination. This does not mean that every Y bacterium necessarily causes disease directly.

b(i); original allocation: 2 mark(s)

During the stationary phase, the rate of viable-cell formation is approximately equal to the death rate, keeping viable-cell number approximately constant.

As resources decline and metabolites accumulate, new cells may still form but are offset by deaths. "Stationary" means no net increase in viable count, not that every cell stops metabolising or dividing.

b(ii); original allocation: 3 mark(s)

Optical density is affected by living cells and still-intact dead cells. New cells continue forming and light-scattering particles accumulate during CD, so optical density can rise even though the viable population has reached the stationary phase.

In this question, dead cells still scatter light, so the optical-density curve is not a viable-count curve. Even when births and deaths balance, total cellular particles may increase, explaining the slow rise during CD. Do not automatically equate the DE optical plateau with the viable population's stationary phase.

b(iii); original allocation: 2 mark(s)

Mixing suspends cells evenly, preventing settling or clumping and making the optical reading representative of the culture.

Optical density results from suspended particles scattering or blocking light. If many cells settle, fewer lie in the light path and the reading is too low. Uniform distribution allows a fixed sampling position to represent overall cell quantity more reliably.

b(iv)(1); original allocation: 1 mark(s)

From lowest to highest: P, R, S, Q.

In this test, an antibiotic that maintains clear wells at a lower concentration has greater inhibitory activity. Q gives clear wells first, followed by S, then R, while P requires a higher concentration. Thus the order from weakest to strongest is P<R<S<Q.

b(iv)(2); original allocation: 1 mark(s)

64 μg/mL.

Both replicate rows for S remain cloudy at 32 μg/mL and become clear at 64 μg/mL. The lowest concentration shown to inhibit visible growth is therefore 64 μg/mL. Include the concentration unit.

Examiner's comment (source page(s) 11, 12)

Less time for a tenfold increase means faster growth. Optical density includes scattering by live and dead cells, so it cannot directly identify growth phases from viable counts. Find clear wells to identify the lowest effective antibiotic concentration, and include units.

Marking reference: HKDSE Biology 2023 Paper 2 Q3, official marking scheme, source page(s) 12, 13.

VII.b Uses of microorganisms

Read the explanations and diagrams in Concept VII.b first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Track microbial substrates, products and population changes

Degree of integration ★★★★☆

Key ideas

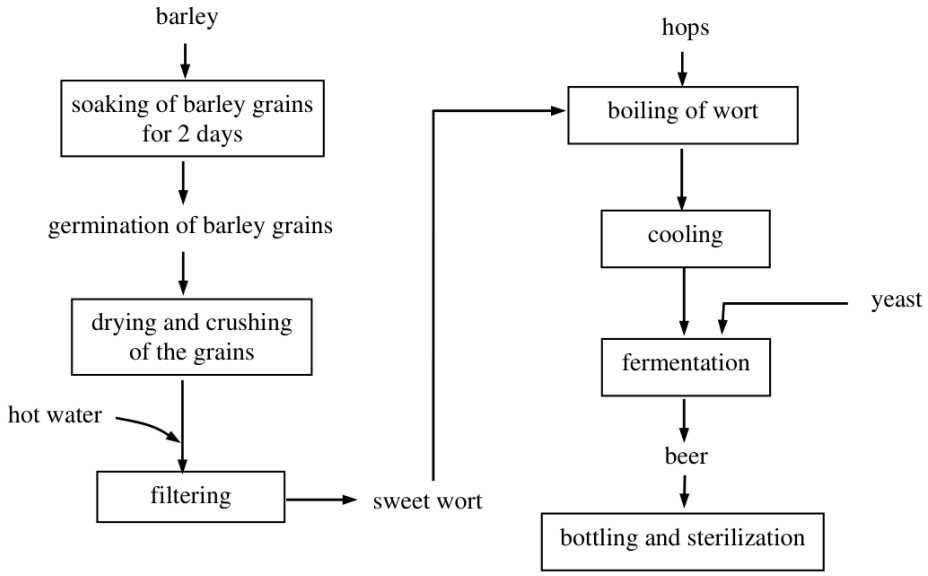
① Stored starch in barley is not already glucose available to yeast. Hydrolytic enzymes during germination first break stored materials into smaller usable molecules, which microorganisms then metabolise.

② In alcoholic fermentation, sugar can form ethanol and carbon dioxide under anaerobic conditions. Dairy fermentation involves different principal products and microorganisms. A population's later failure to increase may result from nutrient limitation, accumulated products or other environmental changes. The explanation must fit the stated conditions.

How to recognise the problem Connect raw material → usable substrate → microbial activity → product. Match each interval of a growth curve with pH, sugar or other measurements made at the same time.

Example 1 [HKDSE Biology Sample Paper Paper 2 Q3]

3.(a) The diagram below outlines the sequence of processes involved in the brewing of beer:



(i) Explain the importance of soaking the barley grains in water for two days in the brewing of beer. (4 marks)

(ii) Give ***two*** reasons why it is necessary to boil the wort. (2 marks)

(iii) Describe how yeast can help in beer production. (2 marks)

(iv) Sketch a graph to show the change in the population of yeast from the time when it is added to the wort to the time of bottling and sterilization of the beer. (3 marks)

3.(b) Mary bought two boxes of fresh milk from the supermarket and put them in the refrigerator. After two weeks, she found that one box of milk had turned sour and curdled. The photograph below shows the appearance of the spoiled milk:



(i) Fresh milk is usually pasteurized before it is sent to the supermarket for sale. How does pasteurization help to preserve fresh milk? (1 mark)

(ii) Briefly explain how the milk became sour and curdled after being left for two weeks. (3 marks)

(iii) Mary found the other box of milk appeared normal and she drank the whole box. A few hours later, she suffered from diarrhoea as a result of food-borne infection.

(1) Explain how drinking this box of milk may bring about the above food-borne infection. (2 marks)

(2) Name a pathogen that causes food-borne infections. (1 mark)

(iv) Butter and cheese are products made from milk, but they can be kept unspoiled for a longer period of time than milk. Why? (2 marks)

Solution a(i); original allocation: 4 mark(s)

Method: Explain each interval through substrate, microorganism and product.

Soaking allows barley to absorb water, soften its seed coat and begin germinating. Germination produces or activates hydrolytic enzymes such as amylase, converting stored starch into fermentable sugars for subsequent yeast use.

Starch in dry grains is not directly available to yeast in large quantities in this process. Water uptake initiates germination. The embryo and associated tissues provide enzymes that hydrolyse starch to maltose, with further conversion to glucose and other sugars. These soluble sugars enter the sweet wort and become fermentation substrates. Explain the two-day soaking through water → germination → enzymes → sugars, not merely grain cleaning.

a(ii); original allocation: 2 mark(s)

Reduce microorganisms that could contaminate fermentation, and remove dissolved oxygen to provide more oxygen-poor conditions for subsequent alcoholic fermentation.

Boiling kills most introduced microorganisms, reducing competition with yeast and spoilage risk. Heating also expels dissolved gases. The wort must then cool before yeast is added, otherwise the heat would damage the desired yeast too. Boiling should not automatically be called complete sterilisation that destroys every heat-resistant spore; focus on contamination control.

a(iii); original allocation: 2 mark(s)

Yeast carries out alcoholic fermentation, breaking down glucose to form ethanol, the alcohol in beer.

Under oxygen-poor conditions, yeast still gains a little ATP through glycolysis. Subsequent reactions convert pyruvate to ethanol and regenerate NAD, allowing the process to continue.

Glucose → ethanol + carbon dioxide + a small amount of energy.

Yeast converts available sugar into alcohol; this step does not turn barley directly into yeast.

a(iv); original allocation: 3 mark(s)

Viable yeast numbers increase slowly, then rapidly, then approach a plateau. During bottling and heat treatment they fall rapidly to very low levels or zero.

Plot time horizontally and viable cells per unit volume vertically. Newly added yeast first adapts, then multiplies rapidly with plentiful nutrients. Depletion of sugar and other resources and ethanol accumulation slow growth. Since the endpoint includes bottling and heat treatment, the graph must end with a sharp decrease caused by yeast death, not an indefinitely high plateau. This is a viable-population graph; total cellular debris does not explain the post-treatment fall.

b(i); original allocation: 1 mark(s)

Pasteurisation uses controlled heating to kill many bacteria in fresh milk, reducing pathogens and spoilage microorganisms.

It delays spoilage but usually does not eliminate all microorganisms and heat-resistant spores. Appropriate refrigeration is still needed, and storage is not indefinite. Refrigeration slows multiplication rather than killing every remaining microorganism.

b(ii); original allocation: 3 mark(s)

Surviving or subsequently introduced lactic acid bacteria multiply during storage and ferment lactose to lactic acid, making milk sour. Greater acidity coagulates casein, forming curds.

Explain both observations: lactic acid accumulation accounts for sourness; lower pH destabilises dispersed milk proteins, producing curds. Curds are not merely accumulated bacteria, and refrigeration does not stop all microbial activity.

b(iii)(1); original allocation: 2 mark(s)

Apparently normal milk may still contain pathogenic bacteria. After ingestion, living pathogens enter and multiply in the digestive tract, causing food-borne infection and diarrhoea.

Appearance, sourness and curdling reflect some spoilage processes but do not accompany every pathogen. The question specifies food-borne infection, so include entry and multiplication of living pathogens rather than discussing only preformed toxins and omitting infection.

b(iii)(2); original allocation: 1 mark(s)

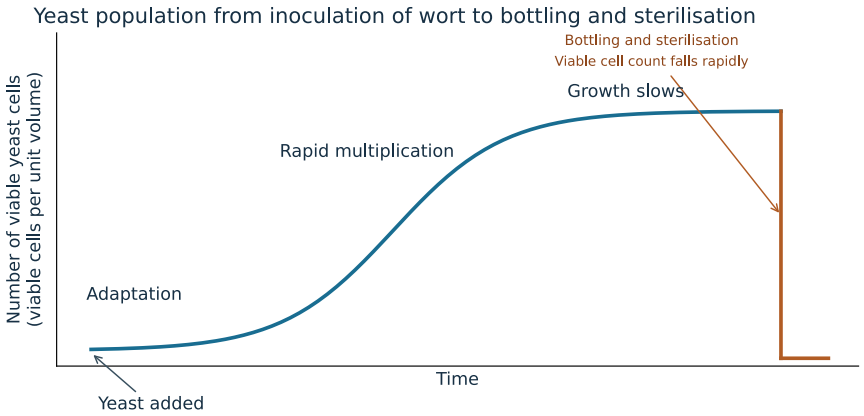
Salmonella.

It is an example of a bacterium entering the digestive tract through contaminated food and causing infection. “Bacteria” alone does not name a pathogen as requested.

b(iv); original allocation: 2 mark(s)

Butter and cheese generally contain less available water than fresh milk, slowing microbial growth and metabolism and improving storage life.

Enzyme reactions, transport and multiplication require suitable water availability. Converting milk into lower-moisture products reduces water available to microorganisms. Salt in some products lowers availability further. This explains longer storage, not an inability of all butter or cheese to spoil.

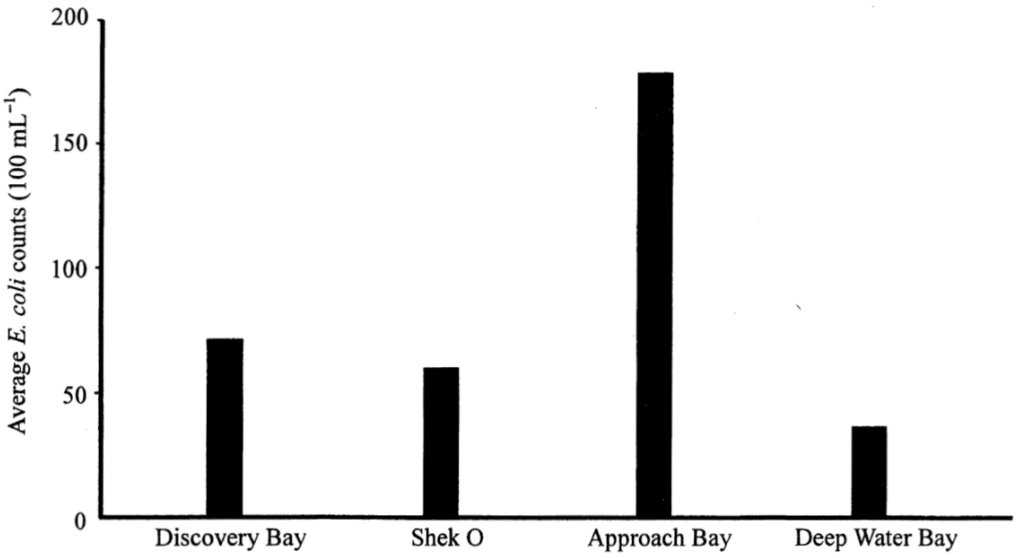


Viable yeast population from inoculation to bottling and heat treatment.

Marking reference: HKDSE Biology Sample Paper Paper 2 Q3, official marking scheme, source page(s) 10.

Example 2 [HKDSE Biology 2014 Paper 2 Q3]

(a) In Hong Kong, *Escherichia coli* counts have been used to judge whether the water quality of beaches is suitable for swimming. The bar chart below shows the levels of *E. coli* in the water samples taken from four beaches in 2012:



(i) Why can *E. coli* counts be used to reflect the water quality? (3 marks)

(ii) Based on the above data, which beach would you prefer for swimming? Why? (3 marks)

(iii) State ***two*** limitations of using *E. coli* counts in estimating water quality. (2 marks)

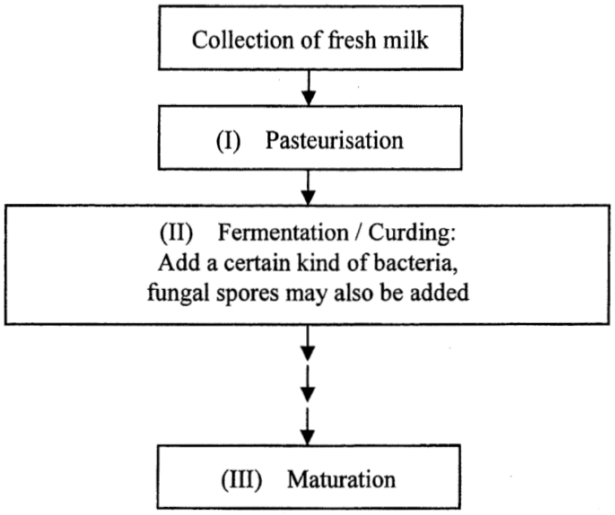
(iv) To measure the number of *E. coli* in a water sample, a culturing step is needed and aseptic techniques must be used.

(1) What is the importance of using aseptic techniques in this process? (1 mark)

(2) Describe ***one*** commonly used aseptic technique for the preparation of the culture media and outline its principles. (3 marks)

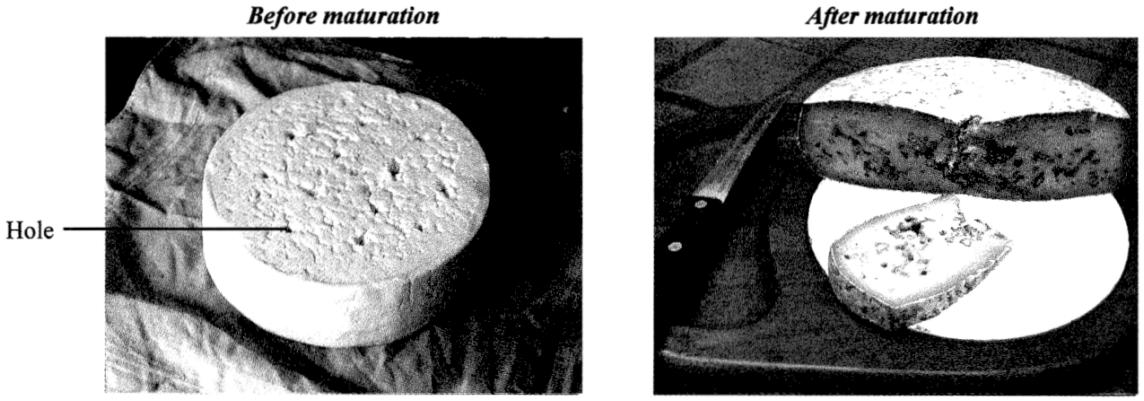
3(b) (i) In the past, cheese was made from milk by the action of environmental microorganisms. Its flavour was mainly due to the metabolites of microorganisms. Different batches of cheese produced during different seasons of a year might end up having different flavours. Explain this phenomenon. (3 marks)

(ii) Nowadays, mass production of cheese is carried out in a controlled manner to ensure the quality and consistency of the cheese. The following flowchart shows some major steps involving microorganisms during the manufacture of cheese:



(1) In terms of quality and consistency, explain the importance of processes (I) and (II). (2 marks)

(2) In the production of blue cheese, a small number of the spores of a species of aerobic fungus is added into the cheese curds during process (II). In process (III), tiny holes are punched in the cheese using a needle. When a large number of spores appear in the cheese, the blue colour of the spores becomes visible. The photographs below show the appearance of the blue cheese before and after maturation:



Suggest how the step of punching holes in the cheese leads to the appearance of the blue cheese after maturation. (3 marks)

Solution a(i); original allocation: 3 mark(s)

Escherichia coli normally lives in the intestines of humans and other warm-blooded animals and is excreted in faeces. Higher E. coli counts in water can indicate faecal contamination and the possible presence of other faecally transmitted pathogens.

It is an indicator of contamination; not every counted E. coli bacterium is necessarily pathogenic.

a(ii); original allocation: 3 mark(s)

Deep Water Bay. It has the lowest E. coli reading, approximately 35 per 100 mL, indicating the lowest faecal-contamination indicator level among the four samples.

Method: Explain each interval through substrate, microorganism and product.

This compares the question's 2012 data. Historical samples are not a present-day beach-safety notice.

a(iii); original allocation: 2 mark(s)

The indicator does not detect all other pollution, such as chemical contamination. E. coli and different pathogens also survive and disappear at different rates, so their densities need not remain in a fixed ratio.

These are distinct limitations: omission of other pollution types and an imperfect correspondence between the indicator and actual hazards.

a(iv)(1); original allocation: 1 mark(s)

Prevent contamination by microorganisms not originating in the water sample, so the count represents that sample.

For measurement, aseptic technique ensures the correct source of colonies. It also helps protect the operator.

a(iv)(2); original allocation: 3 mark(s)

Sterilise the medium in an autoclave. Pressure allows steam to reach a higher temperature, whose heat kills bacteria, fungi and their spores, reducing pre-existing microbial contamination.

This concerns sterilising culture medium, a different treatment level from the subsequent milk-pasteurisation example.

b(i); original allocation: 3 mark(s)

Seasonal differences in temperature and other conditions affect the microorganisms entering milk and their metabolic activity. Different organisms or metabolic conditions produce different mixtures of metabolites, changing cheese flavour.

Connect season → microorganisms → metabolites rather than claiming that weather directly changes flavour.

b(ii)(1); original allocation: 2 mark(s)

Step I, pasteurisation, reduces existing microorganisms and pathogens, limiting uncontrolled fermentation. Step II introduces selected strains, making major metabolites and flavour more predictable and improving consistency.

The older official wording about eliminating all original microorganisms is too absolute: pasteurisation is not complete sterilisation and does not guarantee destruction of every spore. The correct mechanism is reduced background contamination followed by fermentation controlled using a selected starter culture.

b(ii)(2); original allocation: 3 mark(s)

Piercing allows oxygen into the cheese. Penicillium can then carry out aerobic respiration for energy, supporting hyphal growth and spore formation. Abundant spores produce the visible blue markings.

The question states that the blue colour comes from numerous spores. Explain how oxygen supplied by piercing promotes their formation; the holes themselves are not stained blue.

Examiner's comment (source page(s) 5)

E. coli indicates faecal contamination; its presence does not guarantee disease. Link piercing to oxygen, aerobic respiration, hyphae and spores. Distinguish pasteurisation from complete sterilisation.

Marking reference: HKDSE Biology 2014 Paper 2 Q3, official marking scheme, source page(s) 9.

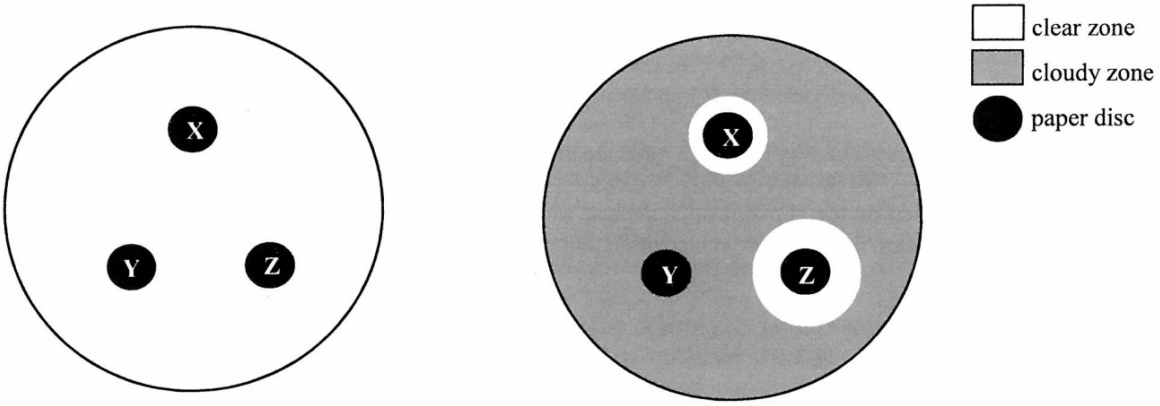
Example 3 [HKDSE Biology 2022 Paper 2 Q3]

(a) During the Coronavirus Disease 2019 (COVID-19) pandemic, hand sanitiser was one of the most common anti-pandemic products. The following lists the procedure of a test which assesses the antibacterial effectiveness of hand sanitiser samples X, Y and Z:

1. A bacterial culture was spread evenly over the surface of a sterile agar plate using a sterile spreader.
2. A sterile filter paper disk soaked with 10 μL of hand sanitiser sample was placed on the agar plate.
3. The agar plate was incubated at 37°C for 24 hours.
4. The diameters of the clear zones were measured.

The diagrams below show the appearance of the agar plate before and after incubation:

Before incubation (top view) ***After incubation (top view)***



(i) Explain why there were cloudy and clear regions on the agar plate after incubation. (2 marks)

(ii) Assuming that the various hand sanitiser samples have the same diffusion rate on the agar plate, arrange the hand sanitiser samples in ascending order of their antibacterial ability. Explain your answer. (2 marks)

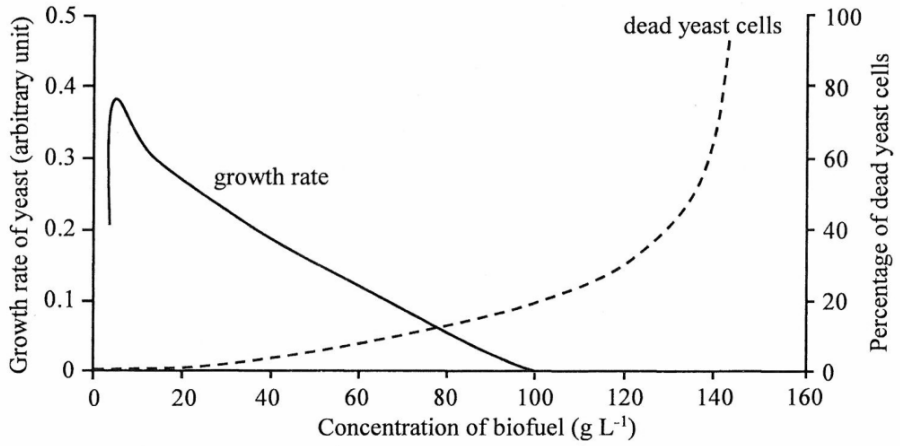
(iii) Do the results truly reflect the effectiveness of the hand sanitiser samples against COVID-19? Why? (2 marks)

(iv) State ***two*** aseptic techniques that should be employed during the spreading of bacterial culture over the agar plate. Explain your answer. (4 marks)

3(b) Biofuel can be produced in yeast fermentation.

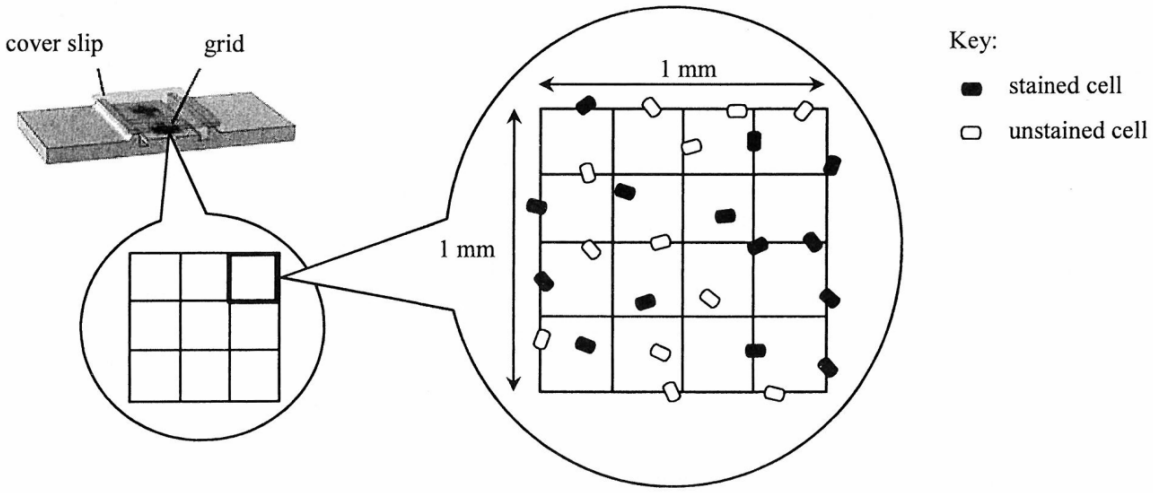
(i) Write the word equation for the yeast fermentation. (2 marks)

(ii) The graph below shows the impact of the production of biofuel on the yeast culture:



(1) With reference to the graph, what is the impact of biofuel production on the yeast? (2 marks)

(2) The percentage of dead cells can be estimated by using a cell counter (a hemocytometer). The diagram below shows the appearance of a diluted yeast culture sample stained with a blue dye which stains dead cells. Count the number and calculate the percentage of dead yeast cells as shown in the diagram below: (2 marks)



(3) The large square measures 1 mm x 1 mm and has a depth of 0.1 mm. The yeast sample has been diluted with a dilution factor of 10^4. Calculate the total cell count per cm^3 of the original yeast culture. (2 marks)

(iii) Traditionally, biofuel was produced from the edible part of corn or sugarcane. A genetically modified (GM) yeast with the ability to convert cellulose to biofuel has been developed. State ***two*** advantages of using this GM yeast over a traditional yeast strain for biofuel production. (2 marks)

Solution a(i); original allocation: 2 mark(s)

Bacteria grow in the cloudy area. The clear area indicates that hand rub has killed or inhibited nearby bacteria.

Multiplying bacteria form a visible lawn or colonies, making the agar surface cloudy. Hand rub diffuses from the paper disc, inhibiting growth wherever sufficient active ingredient is present and leaving a clear inhibition zone.

Method: Explain each interval through substrate, microorganism and product.

a(ii); original allocation: 2 mark(s)

From weakest to strongest: Y < X < Z.

Assuming equal diffusion rates, a larger clear zone indicates inhibition farther from the disc. Y has no appreciable zone, X a smaller zone, and Z the largest, giving this order.

a(iii); original allocation: 2 mark(s)

No. The experiment tests antibacterial activity only, whereas COVID-19 is caused by a virus.

Bacteria and viruses differ in structure and reproduction. Antibacterial effectiveness cannot directly establish effectiveness against the relevant virus; testing that virus is needed.

a(iv); original allocation: 4 mark(s)

Open the Petri-dish lid only slightly to reduce entry of airborne microorganisms. Avoid talking and wear a mask during handling to reduce droplet contamination.

Both measures prevent external microorganisms entering the culture. A smaller opening and shorter exposure reduce airborne deposition; avoiding speech and covering the mouth and nose reduce microorganism-containing droplets. Explain each contamination source rather than only listing "keep clean".

b(i); original allocation: 2 mark(s)

Glucose → ethanol + carbon dioxide.

Yeast fermentation partially breaks glucose down to ethanol and releases carbon dioxide. Ethanol is the biofuel here. Include the starting material and both products in the word equation.

b(ii)(1); original allocation: 2 mark(s)

As biofuel concentration increases, yeast growth rate generally decreases and the percentage of dead cells increases.

Apart from a short initial low-concentration interval, the solid curve declines from its peak to zero near 100 g/L. The broken curve rises with concentration, showing that accumulated ethanol inhibits growth and increases cell death.

b(ii)(2); original allocation: 2 mark(s)

Including cells on the top and left boundaries but excluding those on the bottom and right gives 11 dead cells out of 21, or approximately 52.4% mortality.

Dark cells are stained dead cells; white cells are living. Use one consistent boundary rule to avoid double-counting adjacent squares. This gives 11 dark and 10 white cells:

Percentage dead = $11 \div (11+10) \times 100\% = 52.38\ldots\%$.

Do not include every boundary cell or exclude them all.

b(ii)(3); original allocation: 2 mark(s)

The original culture contains approximately 2.1×10^9 yeast cells/cm³.

Counting-square volume = 1 mm×1 mm×0.1 mm = 0.1 mm³.

Diluted-sample concentration = $21 \div 0.1 = 210$ cells/mm³.

Since 1 cm³ = 1000 mm³, this is 2.1×10^5 cells/cm³.

Multiply by the dilution factor of 10^4 to obtain 2.1×10^9 cells/cm³ in the original culture.

b(iii); original allocation: 2 mark(s)

Using inedible crop parts or non-food cellulose feedstocks reduces competition with food production. A wider feedstock supply can also increase potential fuel output.

Conventional yeast cannot directly use cellulose and often requires edible sugar or starch feedstocks. The stated GM yeast adds cellulose to the usable range, enabling use of underused straw, bagasse and similar materials. Edible parts remain available for people or livestock, while fuel sources expand.

Examiner's comment (source page(s) 10, 11)

Rank inhibition from weakest to strongest and connect the order to zone size. Use a consistent adjacent-boundary rule with a haemocytometer. Concentration calculation then requires chamber volume, unit conversion and dilution correction.

Marking reference: HKDSE Biology 2022 Paper 2 Q3, official marking scheme, source page(s) 11, 12.

VII.c Microbial genetics

Read the explanations and diagrams in Concept VII.c first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Interpret viruses and microbial communities using host specificity and controls

Degree of integration ★★★☆☆

Key ideas

① A virus multiplying in one bacterial species does not imply that it can complete the same process in every bacterium or human cell. Host specificity depends on recognition of the host surface and the ability to use host systems after entry.

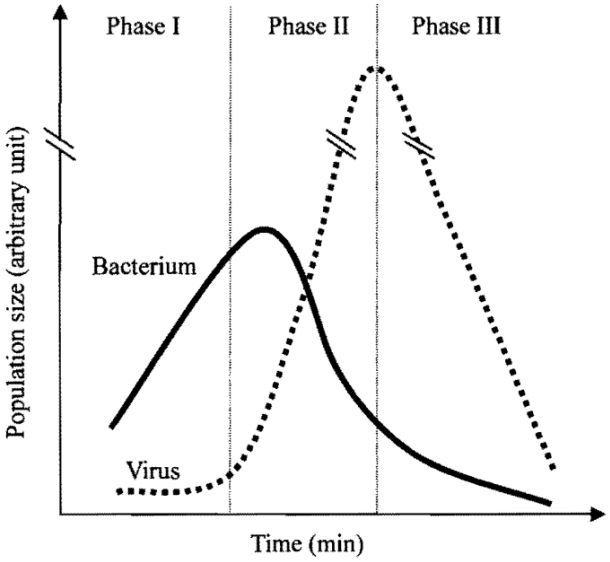
② When comparing microbial communities or disease outcomes before and after treatment, check whether controls retain the same background conditions. Only then can differences support the proposed factor. Simultaneous changes in microbial composition and symptoms still require a distinction between correlation and causation.

How to recognise the problem Identify the virus, host and treatments, then ask which alternative explanation each group excludes. Do not generalise one host's results to all organisms.

Example 1 [HKDSE Biology 2012 Paper 2 Q3]

(a) Viruses that infect bacteria may be used to eliminate pathogenic bacteria in food production lines. Figure 3A below indicates variations in the population size of a virus and its bacterial host:

Figure 3A



(i) What is the importance of the bacterial host to the virus? (1 mark)

(ii) With reference to the life cycle of viruses, explain the difference in the virus population in Phase I and Phase II. (4 marks)

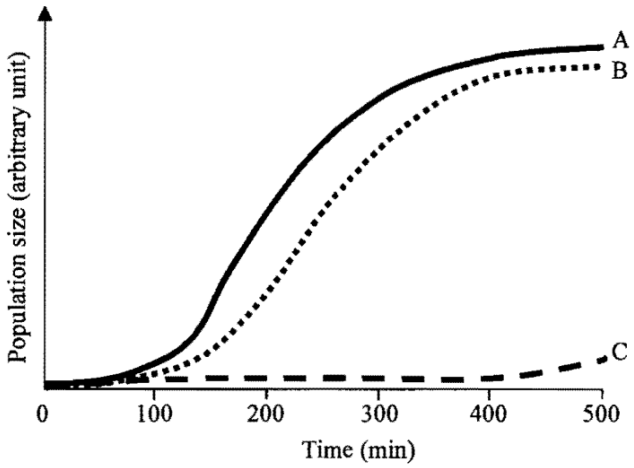
(iii) Explain the drop in the population size of the virus in Phase III. (1 mark)

(iv) The results of scientific research have suggested that adopting such technology in food production would be safe for humans. Why would it be safe? (2 marks)

(v) Compared to conventional disinfection techniques, such as UV irradiation and steam sterilization, viral disinfection has limitations. Suggest ***two*** limitations. (2 marks)

3.(b) The wild type and mutant of a bacterial species were examined for growth under different culture conditions. The mutants were unable to produce cell walls. Figure 3B below indicates the change in the cell number of the wild type grown in an isotonic liquid medium (A), the mutant in an isotonic liquid medium (B), and the mutant in a hypotonic liquid medium (C):

Figure 3B



(i) Suggest ***two*** methods of determining the population size of bacteria in a liquid culture. (2 marks)

(ii) With reference to the function of cell walls, explain the different growth rates observed for the two mutant cultures, B and C. (4 marks)

(iii) What would happen to the population size of the wild type (A) if the culture at the 400th minute was mixed with an equal volume of the fresh culture medium? Explain your answer. (4 marks)

Solution a(i); original allocation: 1 mark(s)

Bacteria are the hosts in which the viruses replicate.

Viruses cannot reproduce independently and must use appropriate host-cell machinery.

Method: Identify the host and controls, then limit the conclusion accordingly.

a(ii); original allocation: 4 mark(s)

In period I, after attachment and infection, viruses require time to replicate inside bacteria, so the free-virus count remains low. In period II, infected bacteria lyse and release many new viruses, causing the sharp population increase.

Intracellular replication precedes lysis and release, so the curve does not immediately peak at initial infection. Explain both the process in each interval and its effect on the curve.

a(iii); original allocation: 1 mark(s)

As bacterial hosts decline, viruses have fewer suitable cells for replication. Viruses that do not infect hosts gradually become inactive or degrade, so their number falls.

Do not say viruses “eat all the bacteria” and starve. The limitation is lack of hosts for replication.

a(iv); original allocation: 2 mark(s)

Viruses are host-specific. These bacteriophages infect bacteria and cannot infect human cells.

The safety rationale concerns differences in host recognition, not small viral size or a claim that all viruses are beneficial.

a(v); original allocation: 2 mark(s)

A bacteriophage targets only bacteria it can infect, giving a narrower range than ultraviolet or steam disinfection. Bacteria may also develop resistance preventing infection by that phage.

Explain limitations relative to conventional disinfection. Effectiveness against some bacteria does not mean removal of every pathogenic microorganism at once.

b(i); original allocation: 2 mark(s)

Direct microscopic cell counting; plating a suitably diluted culture and counting colonies after incubation; or estimating density from turbidity.

Choose any two. Colony counts represent viable colony-forming units, while turbidity requires calibration under comparable conditions. The methods do not measure exactly the same quantity.

b(ii); original allocation: 4 mark(s)

C's hypotonic medium has a higher water potential than the mutant cells, so water enters by osmosis. Without cell walls, the mutants cannot withstand expansion and turgor pressure; some lyse, limiting population growth. B's isotonic medium has a water potential close to that of the cells, preventing substantial net water entry and osmotic lysis, so the population can increase normally.

Compare B and C for the same wall-deficient mutant. Their difference is medium water potential, not sudden nutrient deficiency in C. Explain water movement, wall function and why B avoids lysis.

b(iii); original allocation: 4 mark(s)

Adding fresh medium increases nutrients and dilutes accumulated harmful metabolites. Bacteria resume reproduction and population size increases. Eventually nutrients are depleted again or wastes accumulate, returning the population to the stationary phase.

The original question labels this b(iii); the official marking page misprints b(iv). The numbering here follows the question. The later supported population size increases. Adding an equal medium volume initially dilutes concentration, so distinguish that immediate density change from subsequent growth in total cell number.

Examiner's comment (source page(s) 2)

The low-virus interval is not a bacterial-style acclimatisation lag phase. Wall-deficient bacteria cannot resist swelling from water uptake. State a measurement method, not only an instrument name.

Marking reference: HKDSE Biology 2012 Paper 2 Q3, official marking scheme, source page(s) 8.

VII.d Harmful effects and control of microorganisms

Read the explanations and diagrams in Concept VII.d first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Draw bounded conclusions from inhibition, survival and food data

Degree of integration ★★★★☆

Key ideas

① With the same materials and comparable conditions, a larger clear inhibition zone supports greater inhibition of nearby microbial growth. Zone size also depends on factors such as diffusion, so entirely different experiments cannot be compared without checking conditions. Reheating food can reduce some viable microorganisms without guaranteeing destruction of every existing toxin.

② Increasing drug resistance can result from existing variants surviving and reproducing more successfully under selection. Bacteria do not deliberately produce a specified mutation because they need it.

How to recognise the problem Distinguish measurements of growth inhibition, microbial survival and toxins. Keep conclusions within the scope supported by the controls and measurements.

Example 1 [HKDSE Biology 2016 Paper 2 Q3]

(a) Sushi is a high risk food item with regard to food poisoning. One of the causative agents for food poisoning due to consumption of sushi is *Bacillus cereus*. The table below shows the growth conditions for this pathogen:

	Range	Optimum
pH	5 - 8	6 - 7
Temperature	10°C - 49°C	30°C

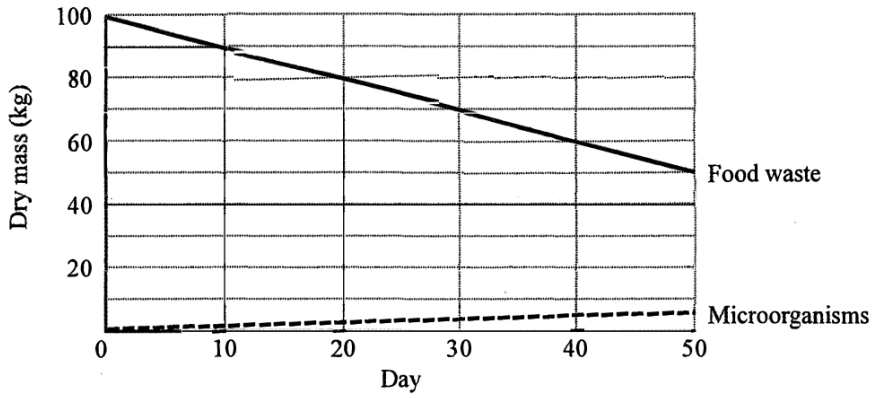
(i) Give ***two*** reasons why sushi itself is a high risk food item. (2 marks)

(ii) During the preparation of sushi, vinegar is added for its palatability. In fact, this also helps improve the safety of the food if enough vinegar is added. Explain why. (2 marks)

(iii) Briefly describe how the growth of *B. cereus* under various pH conditions can be measured experimentally. (4 marks)

(iv) Some sushi restaurants serve plates with sushi on a rotating conveyor belt that moves past every table. Explain why this way of serving sushi increases the risk of food poisoning. (2 marks)

3(b) In natural ecosystems, bacteria and fungi decompose dead bodies and release minerals into the environment for other organisms to use. Composting utilizes such biological activity to treat food waste. The diagram below shows the change in the dry mass of food waste over time and the change in dry mass of the microorganisms that grow on the food waste during the same period of time:



(i) Explain the overall changes in the dry mass of the food waste and the changes in the dry mass of the microorganisms growing on the food waste. (4 marks)

(ii) (1) Determine the total dry mass of food waste and microorganisms on day 0 and day 50. (1 mark)

(2) Explain the change in the total dry mass of food waste and microorganisms as determined in (1). (4 marks)

(iii) State ***one*** way that treated food waste can be utilised in daily life. (1 mark)

Solution (a)(i); original allocation: 2 mark(s)

Sushi often contains uncooked ingredients whose microorganisms have not been killed by heating. Direct hand contact during preparation also increases contamination opportunities. Give independent reasons. Nutrient richness favouring microbial multiplication is another possibility. "Sushi tastes good" is not a microbiological explanation; specify how pathogens remain or enter the food.

Method: Distinguish growth inhibition, survival and toxins.

(a)(ii); original allocation: 2 mark(s)

Enough vinegar lowers pH below the bacterium's growth range, for example below 5, inhibiting growth.

The table gives a growth range of pH 5–8 and an optimum of pH 6–7. Vinegar creates acidic conditions; if final pH actually falls outside the range, multiplication becomes harder. A change in flavour does not address the growth data.

(a)(iii); original allocation: 4 mark(s)

Compare the same medium at different pH values, inoculating equal initial bacterial quantities. After equal incubation times at the same temperature, compare growth through turbidity or cell counts.

pH is the sole independent variable. Keep medium composition and volume, initial bacterial amount, temperature and incubation time constant. Optical density or counts provide the dependent variable; replication improves reliability. Following the given conditions, temperature and time can be fixed at 30°C and 24 hours. Answer through design principles without expanding into additional culture details.

(a)(iv); original allocation: 2 mark(s)

Prolonged room-temperature display allows extensive bacterial multiplication or toxin production, increasing food-poisoning risk.

The conveyor-belt issue is longer exposure to temperatures favourable for growth. Microbial numbers and possible toxins accumulate over time, making infection or intoxication more likely when eaten.

(b)(i); original allocation: 4 mark(s)

Food-waste dry mass decreases continuously while microbial dry mass increases. Microorganisms decompose the waste, absorb breakdown products, synthesise their own materials and reproduce.

From day 0 to day 50, waste falls from approximately 100 to 50 kg, while microorganisms rise from approximately 0 to 5 kg. Decomposition converts large molecules into absorbable smaller ones, some used in new cells, explaining one declining and one rising curve. Not all lost waste becomes microbial biomass because the changes are unequal.

(b)(ii)(1); original allocation: 1 mark(s)

Approximately 100 kg on day 0 and 55 kg on day 50.

Add waste and microbial masses at each time: initially 100 + approximately 0; finally 50 + approximately 5. Do not copy only the waste curve.

(b)(ii)(2); original allocation: 4 mark(s)

Total dry mass decreases because microbial respiration converts some organic carbon into carbon dioxide, which escapes into the air.

Some food waste becomes microbial biomass; some is consumed in respiration. CO_2 leaves the compost, reducing the dry material remaining to be weighed. Because dry mass is measured, water evaporation cannot explain this 45 kg difference. Hydrogen sulphide and sulphur dioxide contain no carbon and are not direct destinations of organic carbon.

(b)(iii); original allocation: 1 mark(s)

Use as compost or a soil conditioner.

Decomposed material supplies soil with organic matter and some mineral nutrients. One use suffices without repeating the whole decomposition process.

Examiner's comment (source page(s) 5)

Calculate total dry mass by adding all components, then explain loss of organic carbon as respiratory carbon dioxide. Dry mass excludes water, so its decline cannot be attributed to evaporation.

Marking reference: HKDSE Biology 2016 Paper 2 Q3, official marking scheme, source page(s) 10.

Practice VII.a Microbiology

Related type 1

Estimate microorganisms using growth curves, optical density and colonies

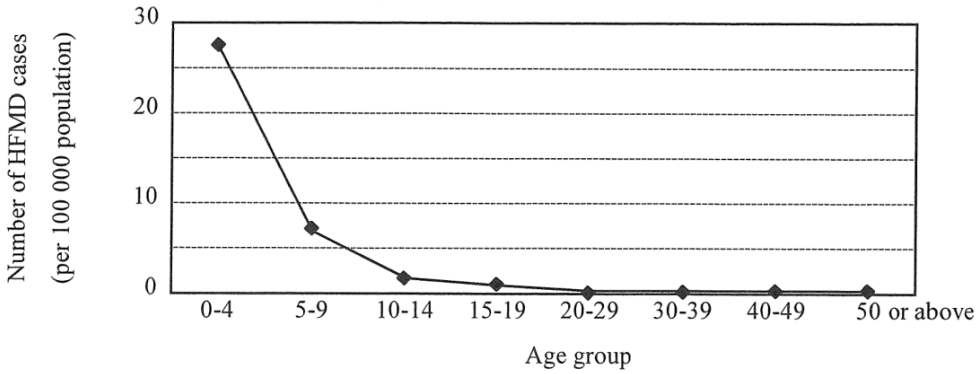
Degree of integration ★★★☆☆

Practice

1. [HKDSE Biology 2020 Paper 2 Q3]

Hint: Check the scale; distinguish optical density, cell number and colonies.

(a) Hand-foot-and-mouth disease (HFMD) is a viral infection which is transmitted through contact with the stool or secretions of an infected person. There was an outbreak of HFMD in Hong Kong in 2008. The graph below shows the number of HFMD cases during the outbreak:

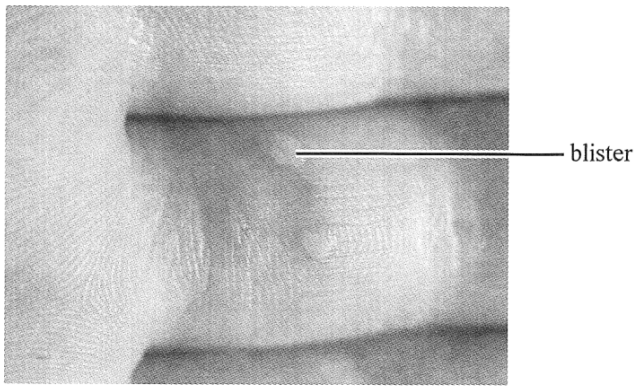


(i) In age groups below 20, how does the number of HFMD cases vary with the age of patients? (2 marks)

(ii) Suggest ***two*** explanations for the change described in (a) (i). (2 marks)

(iii) Can antibiotics effectively treat HFMD? Explain your answer. (2 marks)

(iv) One of the symptoms of HFMD is the formation of blisters in the mouth cavity, and on the palms and soles of the feet. The fluid inside the blisters contains a large number of viral particles. The photograph below shows some blisters on a finger:



With reference to the life cycle of viruses, describe how a large number of viral particles forms inside the blister. (4 marks)

3(b) The Centre for Food Safety has established guidelines for assessing the safety and quality of some ready-to-eat food items. One of the assessments involves estimating the bacterial colonies formed per 1 gram of food sample. Table 1 shows the categories of food quality according to the guidelines and Table 2 presents the results of the colony count in sushi samples collected from local restaurants.

Table 1

Colony count per 1 g of food sample	Category of food quality	Recommendations
<1 000 000	Satisfactory	No actions to take
1 000 000 – <10 000 000	Borderline	Still considered acceptable for human consumption
≥ 10 000 000	Unsatisfactory	Consider investigating causes for high count

Table 2

Sushi samples	Colony count per 1 g of food sample
A	610 000
B	11 240 000
C	9 800 000
D	16 000 000
E	140 000

(i) List the sushi samples which are unsatisfactory in terms of food quality according to the guidelines from the Centre for Food Safety. (1 mark)

(ii) Even though the food quality of some sushi samples is unsatisfactory, consumption of these sushi samples will not necessarily result in food poisoning or infection. What is the limitation of using bacterial colony count as an indication of food safety? (1 mark)

(iii) Suggest ***one*** possible cause of a high bacterial colony count in the sushi samples. (1 mark)

(iv) Below is a flowchart showing the major steps for conducting an aerobic bacterial colony count for a sushi sample:

Blend 50 g sample to prepare a 500 mL stock solution

↓

Perform a serial dilution of the stock solution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4})

↓

Spread each of the diluted solutions over a nutrient agar plate

↓

Incubate at 30°C for 48 hours

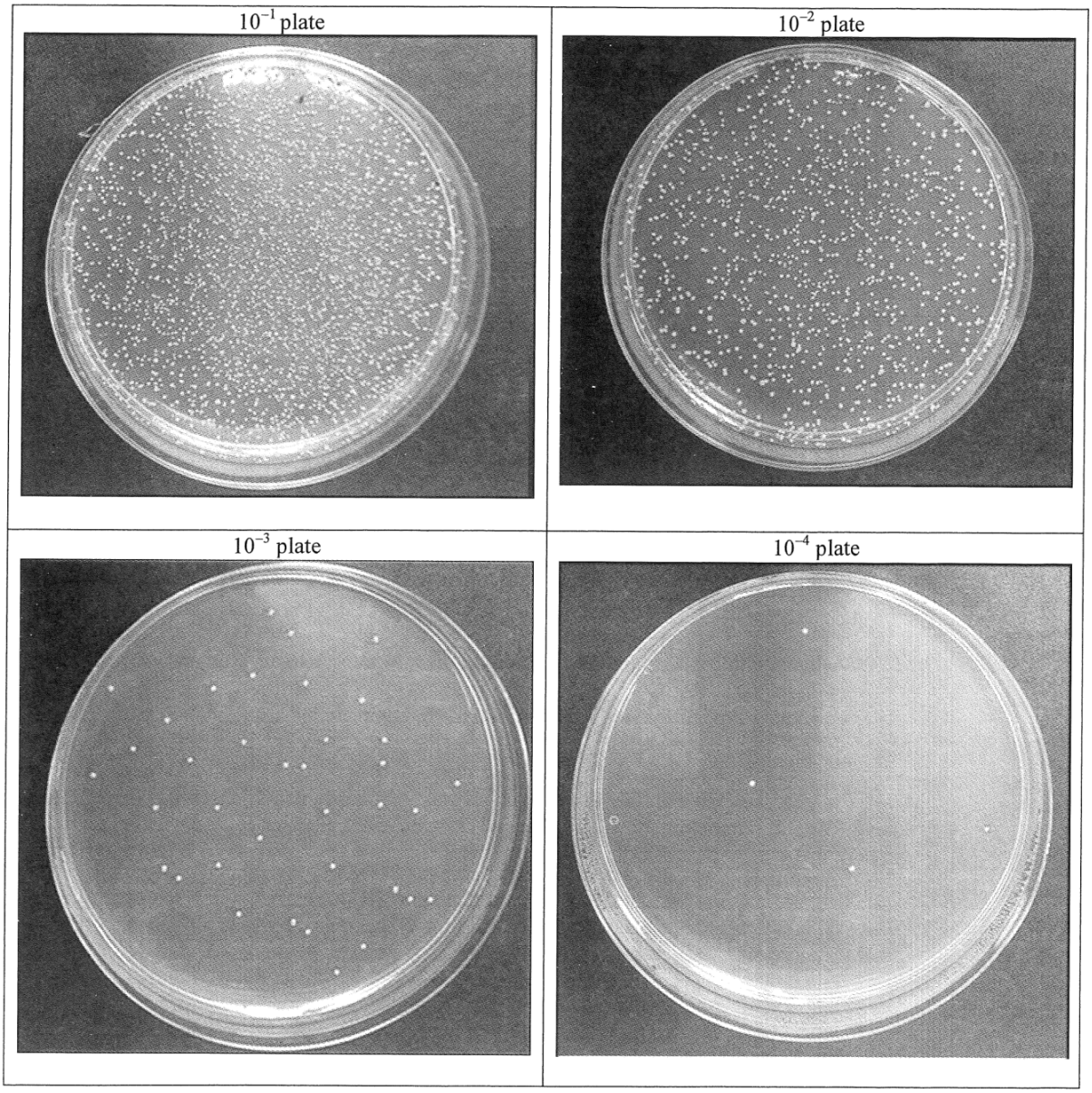
↓

Count the number of bacterial colonies formed

↓

Calculate the aerobic bacterial colony count

The photographs below show the bacterial colonies in four agar plates after incubation:



(1) Which agar plate should be selected to count the number of bacterial colonies formed? Explain why other agar plates are not suitable for this counting process. (3 marks)

(2) Count the number of bacterial colonies on the selected agar plate. Calculate the number of bacterial colonies formed per gram of the sushi sample. (2 marks)

(3) In the above process, aseptic techniques should be used to avoid contamination. Describe ***one*** aseptic technique for spreading the solution over the agar plate. Explain the principle involved. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 2 mark(s)**

Below age 20, hand, foot and mouth disease case numbers decrease with increasing age group. The decrease is greater from ages 0–4 to 10–14, then smaller from 10–14 to 15–19.

Compare the extent of decline rather than merely listing values. The vertical axis shows cases per 100,000 people, standardising for age-group size; it does not mean the older groups simply contain fewer people. As the question specifies under-20s, focus on these four groups.

a(ii); original allocation: 2 mark(s)

Older children generally have better personal hygiene, reducing infection through contaminated contacts. Their more mature immune systems also provide stronger resistance.

Give two different causes. Frequent handwashing and less hand-to-mouth behaviour are both hygiene explanations and should not be presented as independent mechanisms simply by changing wording. Previous infection with the relevant virus and resulting immune memory is another acceptable reason. These are plausible explanations, not factors measured separately by the graph.

a(iii); original allocation: 2 mark(s)

Antibiotics cannot effectively treat the causative viruses directly.

Antibiotics mainly target bacterial structures or processes, killing bacteria or inhibiting their growth. The hand, foot and mouth disease pathogens are viruses and lack those same targets. The explanation is the difference between viruses and bacteria, not that this viral strain has become antibiotic-resistant.

a(iv); original allocation: 4 mark(s)

The virus attaches to a suitable host cell and introduces its nucleic acid. It uses host synthetic machinery to replicate nucleic acid and produce viral proteins. New components assemble into numerous viruses, which are released by host-cell lysis or another release process, producing a high viral concentration in blister fluid.

Connect the viral life cycle to the fluid. Suitable host cells, including relevant epidermal cells, provide conditions for replication. Viruses do not independently divide by binary fission in blister fluid like bacteria. Include release from infected cells to explain abundant viruses in the fluid.

b(i); original allocation: 1 mark(s)

B and D.

The unsatisfactory threshold is at least 10,000,000 colony-forming units per gram. B has 11,240,000 and D 16,000,000, both above it. C has 9,800,000, close but below; do not round it into the unsatisfactory category.

b(ii); original allocation: 1 mark(s)

Total colony count does not identify whether pathogenic bacteria are present, so a high count does not necessarily indicate infection or food-poisoning risk.

The method counts viable microorganisms able to form colonies under the specified culture conditions, including possible non-pathogens. The main limitation is lack of pathogenicity identification. Do not claim that dead bacteria also form counted colonies; they cannot reproduce during incubation.

b(iii); original allocation: 1 mark(s)

The sushi was inadequately refrigerated, for example stored above 4°C, allowing microorganisms to multiply more readily.

Warmer conditions accelerate metabolism and reproduction in many food contaminants, allowing an initially small population to grow during storage. Contaminated hands, environmental contamination or high initial contamination of ingredients are also reasonable causes, provided each is linked to increased microbial numbers.

b(iv)(1); original allocation: 3 mark(s)

Choose the 10^{-3} dilution plate.

The 10^{-1} and 10^{-2} plates have excessive, crowded and partly overlapping colonies, making individual colonies difficult to distinguish and encouraging undercounting. The 10^{-4} plate has very few colonies, so a one-colony error produces a large relative error. The 10^{-3} plate combines separated colonies with a more suitable count, balancing distinguishability and sampling reliability.

b(iv)(2); original allocation: 2 mark(s)

The plate has 37 colonies. Under the calculation conditions implicit in the marking reference, the original sushi sample contains 3.7×10^5 CFU/g.

First correct for the 10^{-3} dilution, then relate the 500 mL stock suspension to 50 g of sushi. The calculation assumes 1 mL plated per dish. The original procedure does not separately specify the plated volume; the marking reference's numerical result corresponds to this assumption.

Stock concentration = $37 \div 10^{-3} = 37{,}000$ CFU/mL.

Total in stock = $37{,}000 \times 500 = 18{,}500{,}000$ CFU.

Count per gram = $18{,}500{,}000 \div 50 = 370{,}000$ CFU/g.

For another experiment plating 0.1 mL, the first step must additionally divide by 0.1; do not copy this result unchanged. CFU denotes colony-forming units, not necessarily individual cells.

b(iv)(3); original allocation: 2 mark(s)

Open the Petri-dish lid only slightly during inoculation and minimise the time open, reducing entry of airborne microorganisms onto the medium.

This aseptic technique applies directly while spreading solution on the agar plate. Contaminants could form additional colonies, inflate the sample count and bias the result upwards. Connect the action to contamination prevention. “Keep clean” or “disinfect the whole room beforehand” does not specify the inoculation procedure.

Data note

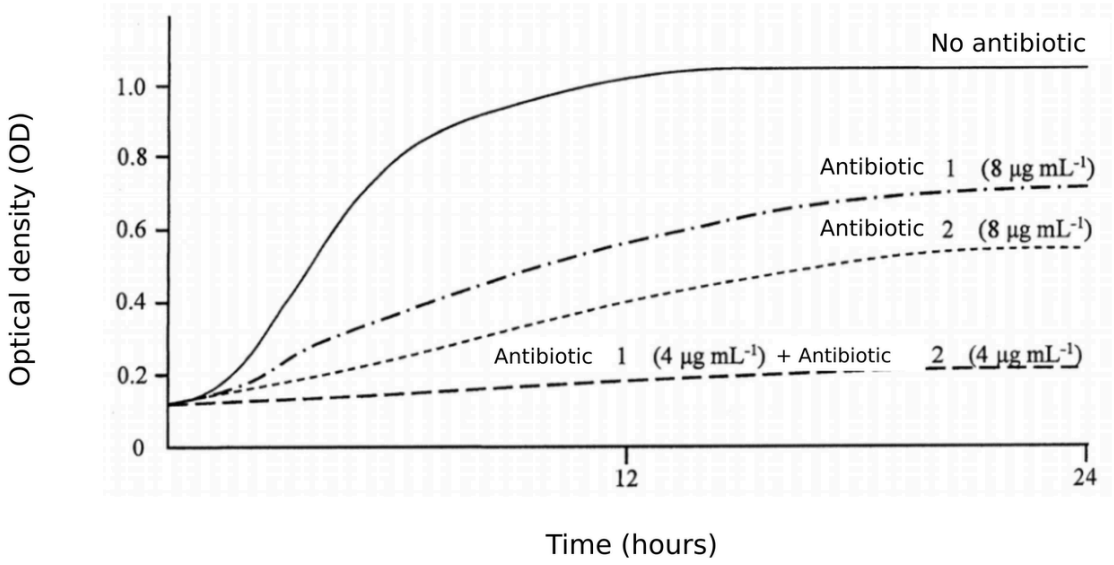
The original question does not explicitly state plated volume. The official calculation 37÷10^-3÷50×500 corresponds to 1 mL per plate. This explanation states that assumption and gives the correction for other volumes.

Marking reference: HKDSE Biology 2020 Paper 2 Q3, official marking scheme, source page(s) 11.

2. [HKDSE Biology 2025 Paper 2 Q3 (English translation)]

← *Hint: Check the scale; distinguish optical density, cell number and colonies.*

(a) Infection by Salmonella can cause diarrhoea and even death. Current treatment usually involves the use of antibiotics. To identify antibiotics that can act against Salmonella, one or more antibiotics were added to Salmonella cultures. The cultures were then incubated at 37 °C and their optical density was monitored over 24 hours. The results are shown below:



(i) A plate containing several colonies of Salmonella is available. Describe the preparatory steps needed for the above experiment to ensure that every culture has the same initial optical density. (3 marks)

(ii) Compare the data shown. What can you conclude about the effectiveness of antibiotics 1 and 2 when each is used alone? (4 marks)

(iii) In terms of the actions of antibiotics, suggest why using antibiotics 1 and 2 together can inhibit the growth of Salmonella more effectively. (1 mark)

(iv) An experiment was carried out to investigate the effects of probiotics in protecting mice against Salmonella infection. The table below shows the three treatments used and the results:

Set-up	Treatment	Result
A	Mice were fed Salmonella.	The mice became ill.
B	Mice were fed probiotics and, after a period of time, were fed Salmonella.	The mice remained healthy.
C	Mice were fed probiotics and, after a period of time, were fed antibiotics. After a further period of time, they were fed Salmonella.	The mice became ill.

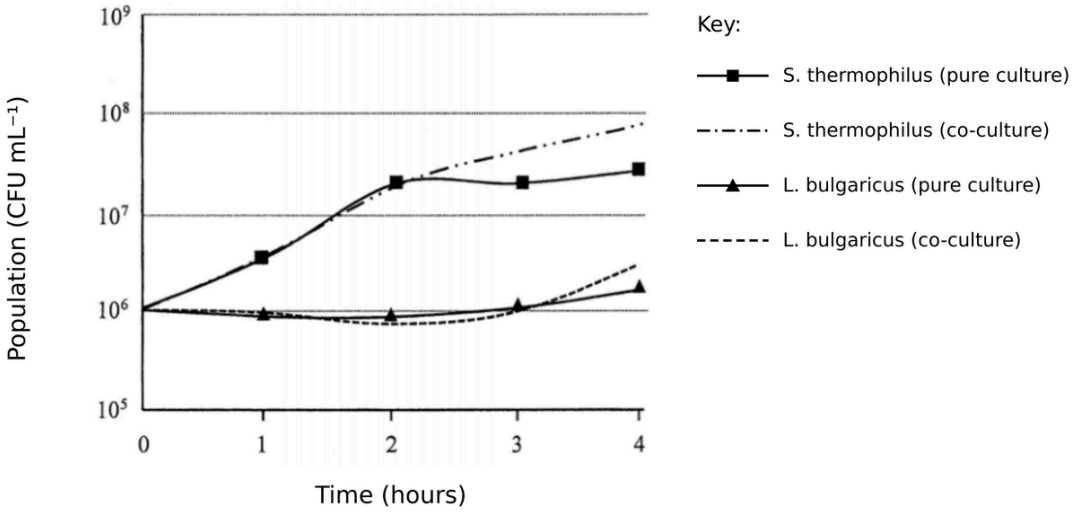
(1) What can you conclude about the effects of probiotics? (1 mark)

(2) Suggest one way in which probiotics could produce the effect stated in (1). (1 mark)

(3) Suggest why the mice in set-up C became ill. (1 mark)

(b) The two bacteria Streptococcus thermophilus and Lactobacillus bulgaricus are commonly used together in yoghurt fermentation. During fermentation, lactose in milk is converted into lactic acid. In an experiment, the two bacteria were added to milk either separately (pure cultures) or together (co-culture). The growth of each bacterial population was then measured using the spread-plate method and expressed as colony-forming units per millilitre (CFU mL^{-1}). Figure I below shows the results:

Figure I

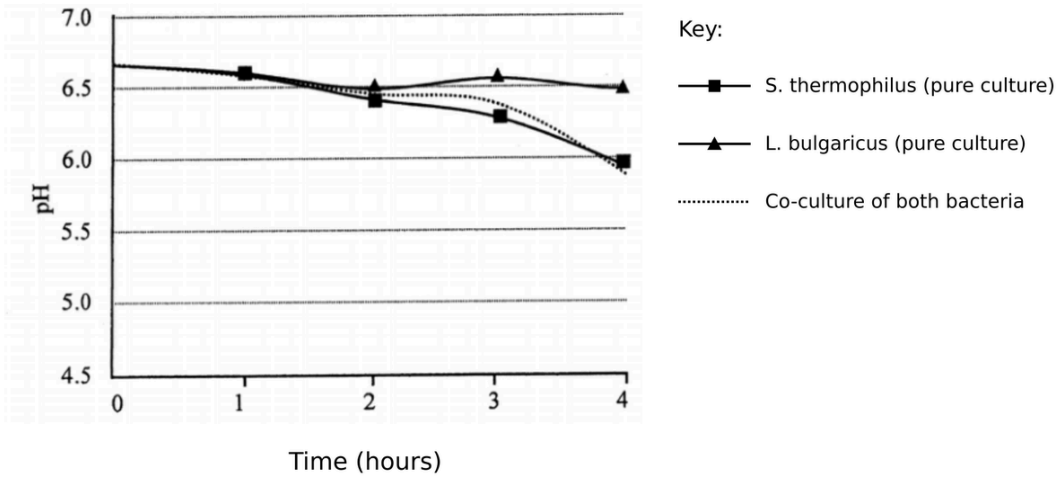


(i) With reference to Figure I, suggest the relationship between the two bacteria during yoghurt fermentation. Give evidence to support your answer. (2 marks)

(ii) With reference to Figure I, compare the growth trends of the two bacterial populations in the co-culture. (3 marks)

(iii) In the same experiment, changes in the pH of the cultures were recorded. Figure II and the table below show the experimental results and the optimum pH for the growth of the two bacteria:

Figure II



Bacterium	Optimum pH
S. thermophilus	6.5
L. bulgaricus	5.8–6.3

Using the information in Figure II and the table above, explain the growth of L. bulgaricus in the co-culture shown in Figure I. (2 marks)

(iv) Apart from the spread-plate method used in this experiment, briefly describe one other method that can be used to estimate the viable bacterial population in a culture. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **(a)(i); original allocation: 3 mark(s)**

A fair comparison requires a common starting point: use the same cell source, comparable initial populations and equal sample quantities in all four groups, giving the same optical density before different treatments are added.

This part specifically concerns equal initial optical density. A common source reduces population differences; a uniform starting suspension and equal portions prevent initial differences in cell quantity. Subsequent graphical differences can then be used to compare treatment effects. Generic equipment preparation or endpoint counts do not address the question.

(a)(ii); original allocation: 4 mark(s)

Used separately, both antibiotics produce lower optical densities than the no-antibiotic control, supporting growth inhibition by both. At the same individual-treatment doses shown, antibiotic 2 gives lower optical density than antibiotic 1, supporting stronger inhibition by 2.

Compare each individual treatment with the untreated control first, then compare 1 with 2. Do not use the combined-treatment curve to decide which is more effective alone. Lower optical density also does not prove that every cell is dead.

(a)(iii); original allocation: 1 mark(s)

The antibiotics may affect different cellular biological processes, giving a stronger inhibitory effect when combined.

The required mechanism concerns different processes being targeted. Numbered drugs in the graph do not identify actual drug names or establish clinical effectiveness.

(a)(iv)(1); original allocation: 1 mark(s)

The data support probiotics helping reduce infection in mice in this comparison.

Compare A with B: prior probiotic treatment differs, and so does the outcome. Restrict the conclusion to the experimental situation shown.

(a)(iv)(2); original allocation: 1 mark(s)

Probiotics can compete with pathogens for intestinal nutrients or attachment sites, reducing pathogen establishment.

Explain one concrete mechanism completely. “Probiotics are beneficial” is insufficient, and probiotics are not antibodies.

(a)(iv)(3); original allocation: 1 mark(s)

Antibiotics can reduce protective probiotic bacteria and weaken their protective effect, so group C still becomes ill.

Link reduced probiotics to reduced protection to explain the result. Do not reverse the relationship and claim that probiotics themselves cause the disease.

(b)(i); original allocation: 2 mark(s)

The relationship is mutualistic. Under joint culture conditions, both species' later populations exceed their respective populations when grown alone, supporting benefit to both.

Compare each bacterium with its own separate-culture curve. An increase in only one does not establish mutual benefit.

(b)(ii); original allocation: 3 mark(s)

In joint culture, S. thermophilus increases throughout the four hours. L. bulgaricus remains approximately constant for the first three hours and increases markedly only after hour 3.

The turning point is hour 3, not hour 2. The vertical axis is logarithmic, so equal vertical distances represent equal fold changes, not equal absolute increases in cell number.

(b)(iii); original allocation: 2 mark(s)

During the first three hours, S. thermophilus activity lowers pH. Conditions then approach the optimum pH range of L. bulgaricus, allowing its population to increase markedly.

Match the growth intervals in Figure I with the pH decline in Figure II, then explain using the optimum-pH table. "The bacteria help each other" does not specify the changed environmental condition.

(b)(iv); original allocation: 2 mark(s)

Combine staining that distinguishes live from dead cells with microscopic counting to estimate viable-cell numbers. A counting chamber of known volume allows conversion to viable-cell concentration.

First establish a basis for distinguishing viability, then count. Directly viewing undifferentiated cells cannot identify which remain alive. Since the original question already uses spread-plate culture, repeating it does not provide an alternative method.

Examiner's comment (source page(s) 77)

Use the appropriate controls for individual treatments. The second bacterium's turning point in joint culture is hour 3. The final part requires another viability-discriminating method, not simply direct observation of unstained cells.

Marking reference: HKDSE Biology 2025 Paper 2 Q3, official marking scheme, source page(s) 63, 64.

Practice VII.b Uses of microorganisms

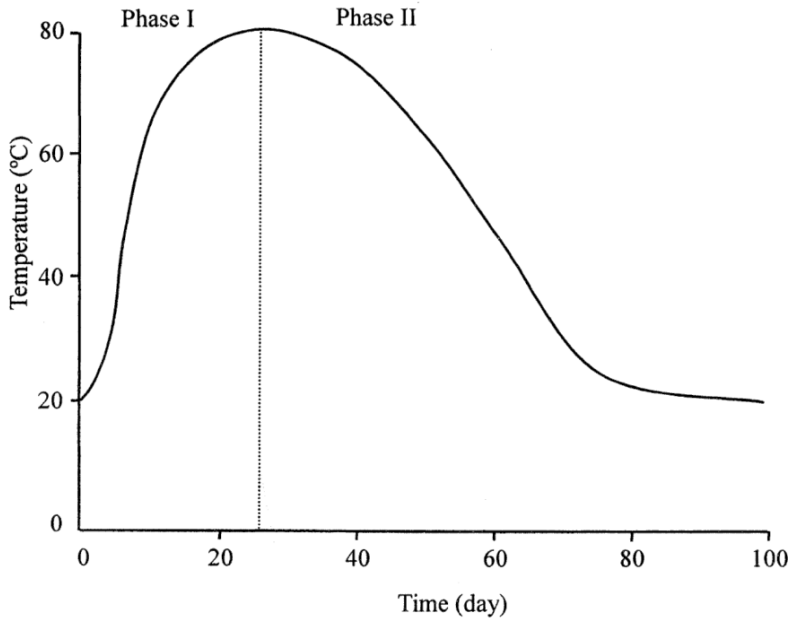
Related type 1 Track microbial substrates, products and population changes

Degree of integration ★★★★☆

Practice

1. [HKDSE Biology 2013 Paper 2 Q3]
Hint: Explain each interval through substrate, microorganism and product.

(a) Garden composting can effectively turn food waste into fertilizer. This is done by mixing the right amounts of food waste with soil containing various types of active microorganisms. The graph below shows the change in temperature inside a compost pile over 100 days:



(i) Describe and explain the changes in temperature inside the compost during Phase I and II. (5 marks)

(ii) It was noted that the diversity of microorganisms decreased during Phase I of the composting. With reference to the graph, suggest an explanation for this observation. (2 marks)

(iii) (1) Food waste should be chopped into small pieces before they are mixed with soil. What is the purpose of doing this? (1 mark)

(2) Adequate and continuous stirring of the food waste-soil mixture is essential for effective composting. Explain why this is important. (3 marks)

3(b) *Aspergillus niger* is a fungus that spoils fruit. An enzyme called pectinase is secreted from the fungus during its colonisation on the fruit surface. This enzyme can be used in fruit juice production.

(i) With reference to the features of fungi, explain how *Aspergillus niger* makes use of pectinase to obtain nutrients from the inside of the fruit. (4 marks)

(ii) With reference to the action of pectinase, suggest why fruit juice produced by using pectinase

(1) has a higher yield, and (1 mark)

(2) is more nutritious. (2 marks)

(iii) People consuming food contaminated with microorganisms may suffer from food borne infection or food poisoning. Distinguish between food borne infection and food poisoning. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation a(i); original allocation: 5 mark(s)

In stage I, temperature rises from approximately 20°C to 80°C because microorganisms decompose organic matter and respire, releasing substantial heat. In stage II, temperature gradually returns to approximately 20°C as nutrients decline and metabolic waste accumulates. Microbial activity and respiration decrease, reducing heat production.

For each stage, describe the change before explaining its mechanism. Falling temperature does not automatically mean that decomposition has stopped completely; net heat production decreases and gradually balances environmental heat loss.

a(ii); original allocation: 2 mark(s)

Microorganisms differ in heat tolerance. Rising temperature eliminates less tolerant species, leaving more heat-tolerant species and reducing species diversity.

This selects among existing differences; every microorganism does not instantly become a new heat-tolerant species because it needs to.

a(iii)(1); original allocation: 1 mark(s)

Cutting material into small pieces increases total surface area, giving microorganisms and their secreted enzymes more sites to contact and decompose.

For the same total volume, fragmentation increases surface-area-to-volume ratio.

a(iii)(2); original allocation: 3 mark(s)

Stirring continually introduces oxygen into the compost, enabling aerobic respiration and greater energy release to support faster microbial activity and organic-matter decomposition.

"Mix evenly" alone omits the physiological reason for faster composting. Air supply is central here.

b(i); original allocation: 4 mark(s)

Aspergillus niger secretes pectinase, which digests pectin outside its cells and loosens plant cell walls and the adhesive material between cells. Hyphae can penetrate deeper into fruit, secrete further digestive enzymes, break organic matter into small soluble molecules and absorb them.

Fungi digest extracellularly; they do not engulf whole pieces of food like animals. Explain external enzyme action, opening of tissues, deeper hyphal penetration and nutrient absorption.

b(ii)(1); original allocation: 1 mark(s)

Pectin breakdown releases cell contents more readily, increasing juice extraction.

Greater yield reflects more effective release of existing juice, not creation of new fruit cells by enzymes.

b(ii)(2); original allocation: 2 mark(s)

Enzyme treatment releases nutrients enclosed by plant cell walls that humans otherwise access less readily. Human digestion lacks enzymes that effectively break down these wall structures, so treated juice can contain more available nutrients.

Pectinase is not a vitamin and does not directly add large quantities of new nutrients. It increases release of intracellular materials.

b(iii); original allocation: 2 mark(s)

Food-borne infection occurs when ingested pathogens multiply inside the body and cause disease. Food poisoning can instead result from microbial toxins already formed in food, even without pathogen multiplication in the body.

Distinguish the main cause: infection by living pathogens versus action of pre-existing toxins.

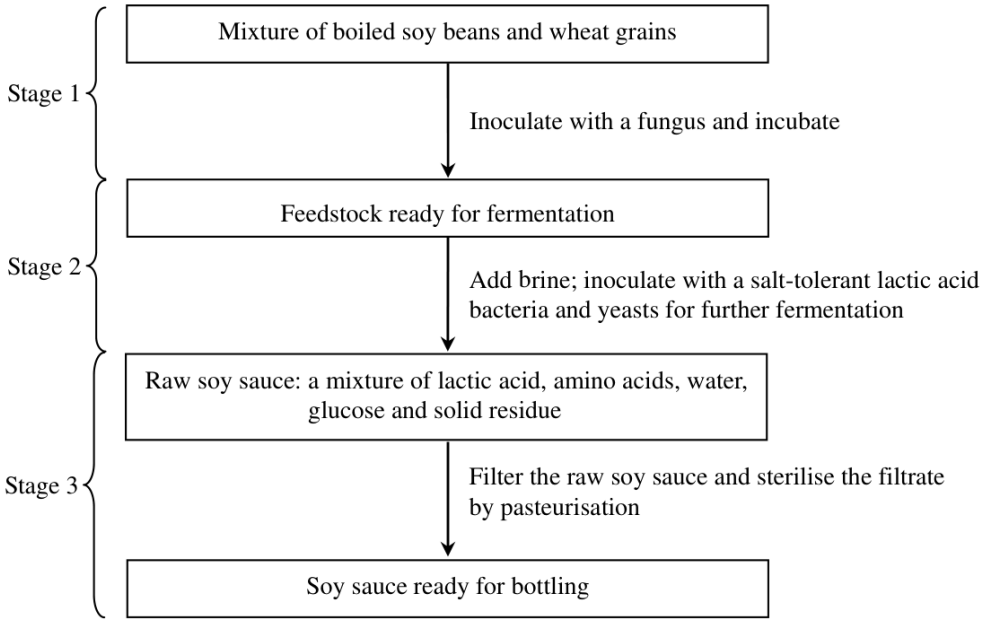
Examiner's comment (source page(s) 4)

The compost graph shows temperature, not population growth. Stirring supplies oxygen for aerobic respiration and decomposition. Connect pectinase to extracellular digestion and release of cell contents.

Marking reference: HKDSE Biology 2013 Paper 2 Q3, official marking scheme, source page(s) 9.

2. [HKDSE Biology Practice Paper Paper 2 Q3]
Hint: Explain each interval through substrate, microorganism and product.

(a) Soy sauce is made by fermentation of a combination of soy beans, wheat grains, water and salt. The production of soy sauce consists of three stages: incubating boiled soy beans and wheat grains with a fungus to produce the feedstock, fermenting the feedstock in the brine added, and pasteurising the raw soy sauce for bottling. An outline of the manufacturing process is given in the flow chart below:



(i) Explain why Stage 1 is an essential preparatory step for Stage 2. (2 marks)

(ii) Explain the importance of adding brine to the mixture in Stage 2. (2 marks)

(iii) Why is it necessary to use pasteurisation in Stage 3 to sterilise the raw soy sauce? (2 marks)

(iv) Aseptic techniques are used in food manufacturing processes involving microbes. State the principles of aseptic techniques. (3 marks)

3. (b) The bacterium *E. coli* is a natural inhabitant of the human intestine.

(i) Figure 1 shows an *E. coli* infected by microbe M. Figure 2 is the magnified image of a part of Figure 1.

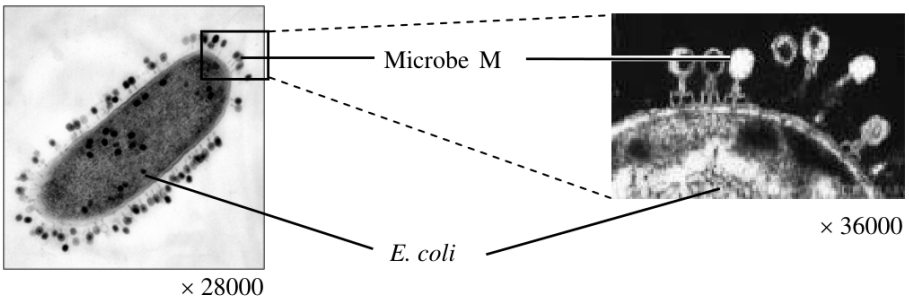


Figure 1 **Figure 2**

(1) Identify microbe M. With reference to the above figures, state **one** observable difference between *E. coli* and microbe M. (2 marks)

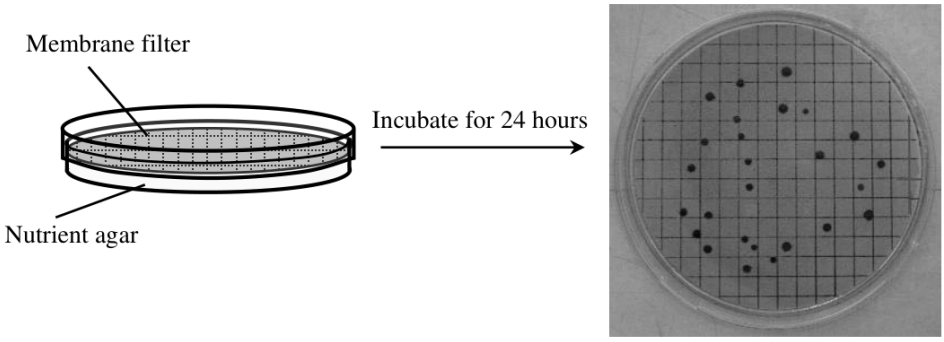
(2) Describe briefly the events shown in Figure 2. (2 marks)

(3) In the mass production of human insulin, *E. coli* is genetically modified to carry the human insulin gene. Explain why bacteria (e.g. *E. coli*) are suitable for use in recombinant DNA technology. (2 marks)

(ii) *E. coli* is used as a water pollution indicating organism. A beach with an *E. coli* count exceeding 1600 per 100 cm^3 seawater is graded 'Very Poor' by the Environmental Protection Department. A 'Very Poor' beach is not suitable for swimming.

(1) Suggest why there is a health risk to people swimming in a 'Very Poor' beach. (1 mark)

(2) A student collects a water sample from a beach to determine its *E. coli* count. He filters 50 cm^3 of the water sample through a membrane filter, on which bacteria are retained. The membrane filter is then placed on a nutrient agar plate suitable for the growth of *E. coli* and incubated for 24 hours. This step and the result after incubation are shown in the figure below:



(I) Explain how this method can be used to determine the *E. coli* count of the water sample. (2 marks)

(II) Calculate the *E. coli* count per 100 cm^3 of the water sample. Based on your calculation, determine if this beach is 'Very Poor'. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation a(i); original allocation: 2 mark(s)

In stage 1, fungi secrete enzymes that break down large food molecules in soya beans and wheat into smaller usable substances, for example proteins into amino acids and starch into sugars. These supply fermentation substrates and growth nutrients for bacteria and yeast in stage 2.

Extracellular digestion of the raw materials first provides suitable substrates for later microorganisms. Stage 1 does not exhaust all nutrients.

a(ii); original allocation: 2 mark(s)

High salinity creates selective conditions, inhibiting undesirable salt-sensitive microorganisms while allowing the specified salt-tolerant lactic acid bacteria and yeast to carry out the required fermentation.

Salt does not kill every microorganism; otherwise stage 2 fermentation would be impossible.

a(iii); original allocation: 2 mark(s)

Pasteurisation reduces viable microorganisms and inactivates some residual enzymes, slowing continued fermentation and other changes to maintain stable flavour and quality. Moderate treatment also limits changes to product constituents caused by excessive heat.

The main reason is control of microorganisms and subsequent reactions. Do not claim complete sterilisation or that amino acids themselves are "denatured" like enzymes.

a(iv); original allocation: 3 mark(s)

Keep the workspace and equipment clean and appropriately disinfected. Prevent non-target microorganisms entering ingredients or products. Handlers should use suitable hygiene measures, including handwashing, clean work clothing and covering the mouth and nose to reduce contamination.

The three marks concern operational principles, not repetition of every experimental step.

b(i)(1); original allocation: 2 mark(s)

M is a virus/bacteriophage. It is much smaller than E. coli and has a head-and-tail structure, whereas E. coli is rod-shaped.

One visible difference is sufficient. Metabolic properties not visible in the photograph cannot serve as visual evidence.

b(i)(2); original allocation: 2 mark(s)

The bacteriophage first attaches to the bacterial surface, then injects its nucleic acid.

Most of the protein coat remains outside; the entire head-and-tail structure does not enter.

b(i)(3); original allocation: 2 mark(s)

Bacteria have plasmids that can act as vectors for introducing foreign genes. They also reproduce rapidly, allowing many cells to be cultured quickly to produce the desired product.

Well-understood, controllable culture conditions are another acceptable advantage. Engineering uses selected strains with known safety characteristics; not every E. coli strain is non-pathogenic.

b(ii)(1); original allocation: 1 mark(s)

High E. coli counts suggest more severe faecal contamination and possible presence of other pathogens. Ingestion or contact while swimming can therefore pose an infection risk.

Indicator counts provide evidence of contamination; not all E. coli necessarily cause disease directly.

b(ii)(2)(I); original allocation: 2 mark(s)

The membrane traps bacteria from the water. During incubation, each viable cell or cluster capable of colony formation multiplies into a visible colony. Counting colonies estimates culturable viable organisms in the original sample.

Use colony-forming units as an approximation. The many descendants inside one colony are not separate organisms from the original sample.

b(ii)(2)(II); original allocation: 2 mark(s)

The 26 colonies come from 50 cm^3 of water. Per 100 cm^3:

$26 \times 100/50 = 52$.

52 is far below the stated 1600 per 100 cm^3 threshold, so the water is not "very poor" under this historical criterion.

Match the denominator to sampled volume. This classification uses only the given indicator and does not exclude every other pollutant.

Marking reference: HKDSE Biology Practice Paper Paper 2 Q3, official marking scheme, source page(s) 13.

3. [HKDSE Biology 2017 Paper 2 Q3]
Hint: Explain each interval through substrate, microorganism and product.

(a) Beer brewing includes some steps that involve biological activities, as follows:

Step	Description
Malting	Barley grains are soaked in water for 2 days and a sugar solution is collected at the end of the process.
Yeast culturing	A small amount of yeast is mixed with the sugar solution and air is continuously pumped into the mixture for several hours.
Yeast fermentation	The pumping of air is stopped and the mixture is allowed to ferment for several days.

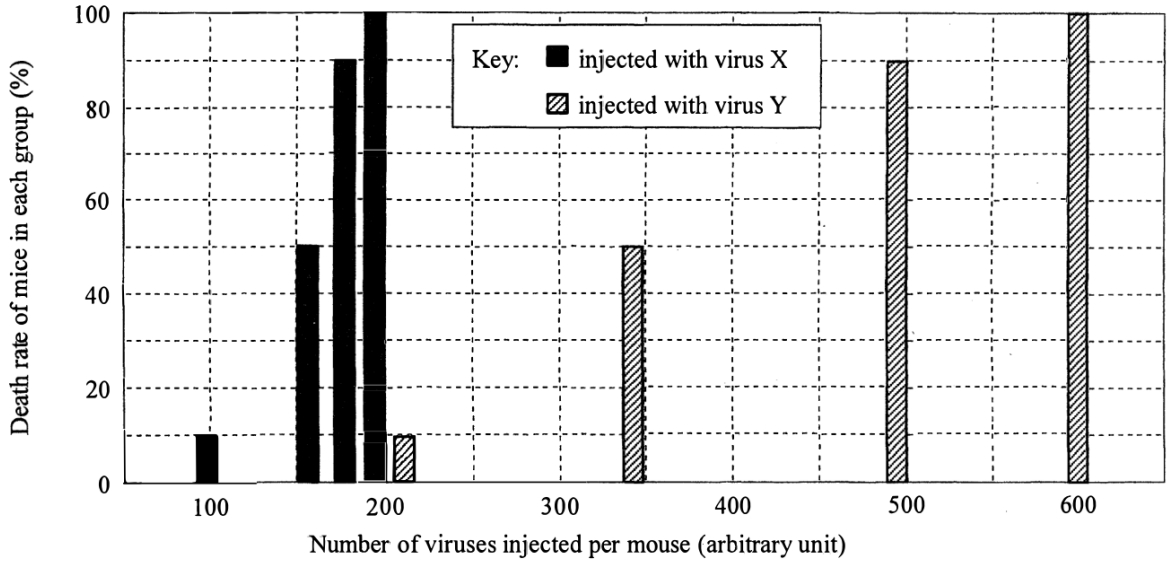
(i) Write a simple word equation of yeast fermentation. (2 marks)

(ii) Describe what happens to barley grains during malting. Why is this process necessary for the later fermentation? (4 marks)

(iii) Explain the importance of continuously pumping air into the mixture during yeast culturing. (3 marks)

(iv) How do the fermentation products contribute to the characteristics of beer? (2 marks)

3(b) An experiment was conducted to study the lethal effect of viruses X and Y on their host. Different amounts of viruses were injected into different groups of mice. The graph below indicates the relationship between the number of viruses injected per mouse and the death rate of mice in each group after 24 hours:



(i) With reference to the above graph, compare and contrast the lethal effect of viruses X and Y on the mice. Deduce which virus was more lethal to the mice. (3 marks)

(ii) Suggest why some viruses are more lethal than others. (2 marks)

(iii) Briefly describe how viruses reproduce in the mice. (4 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **(a)(i); original allocation: 2 mark(s)**

Simple sugar, such as glucose → ethanol + carbon dioxide.

Under oxygen-poor conditions, yeast obtains a small amount of energy through alcoholic fermentation, producing ethanol and CO_2. Give the simple word equation requested; lactic acid is not the main fermentation product of brewing yeast.

(a)(ii); original allocation: 4 mark(s)

Barley absorbs water and germinates, inducing enzyme production or activation. Enzymes break down starch and other reserves into simple sugars, supplying subsequent yeast growth and fermentation.

Barley's main reserve is starch, which yeast cannot efficiently access directly within intact grains. Malting converts reserves into usable sugars and must precede fermentation. Merely saying water softens the grains is insufficient.

(a)(iii); original allocation: 3 mark(s)

Early oxygen supply allows aerobic respiration, giving yeast more energy for reproduction. A larger population then supports faster subsequent fermentation.

Aerobic population growth and later anaerobic fermentation serve different purposes. First increase the number of active yeast cells; then stop aeration to direct more sugar towards the desired fermentation products. Alcoholic fermentation itself does not require oxygen.

(a)(iv); original allocation: 2 mark(s)

Ethanol provides the alcohol content and associated aroma; carbon dioxide produces bubbles.

Link each product to one beer characteristic. Yeast produces other flavour compounds too, but the two main marking points here are ethanol and CO_2.

(b)(i); original allocation: 3 mark(s)

For both viruses, increasing the injected amount increases mortality after 24 hours. X produces the same mortality at a lower amount, so its lethal effect on mice is stronger.

For example, X reaches approximately 100% mortality at 200 relative units, while Y requires approximately 600. X also requires much less for 50% mortality. Restrict the conclusion to these mice and this observation period; it does not directly establish human effects.

(b)(ii); original allocation: 2 mark(s)

Different viruses may attack different cell types. Infection of vital organs or systems may cause more serious consequences.

This is a mechanistic inference; develop one complete reason. Alternatively, different replication rates may cause different tissue damage within the same period. Replacing "stronger" with "more toxic" adds no explanation.

(b)(iii); original allocation: 4 mark(s)

A virus attaches to and enters a suitable host cell, releasing its genetic material. It uses host materials and synthetic machinery to replicate its genome and produce viral proteins. Components assemble, and new viruses are released to infect other cells.

Viruses cannot reproduce through independent cellular metabolism and must use a host. Follow attachment/entry → genome replication and protein synthesis → assembly → release. Release may involve lysis or budding. Do not describe all animal viruses as injecting only DNA while leaving their coats outside, as in a typical bacteriophage example.

Examiner's comment (source page(s) 6)

Water uptake initiates germination during malting. Enzymes convert reserves into sugars for later fermentation. Compare viral pathogenicity by linking replication rate to host-tissue damage, not merely copying separate mortality values.

Marking reference: HKDSE Biology 2017 Paper 2 Q3, official marking scheme, source page(s) 10.

Practice VII.c Microbial genetics

Related type 1

Interpret viruses and microbial communities using host specificity and controls

Degree of integration ★★★☆☆

Practice

1. [HKDSE Biology 2021 Paper 2 Q3]
Hint: Identify the host and controls, then limit the conclusion accordingly.

(a) *Listeria monocytogenes* is a foodborne pathogen which is commonly found in various food products. Ready-to-eat food products that require refrigeration such as fresh milk and yoghurt are at high risk of spreading *Listeria monocytogenes*. The table below lists the temperature range for the growth of this bacterium:

	Lower limit	Optimum	Upper limit
Temperature (°C)	-1.5	30 - 37	45

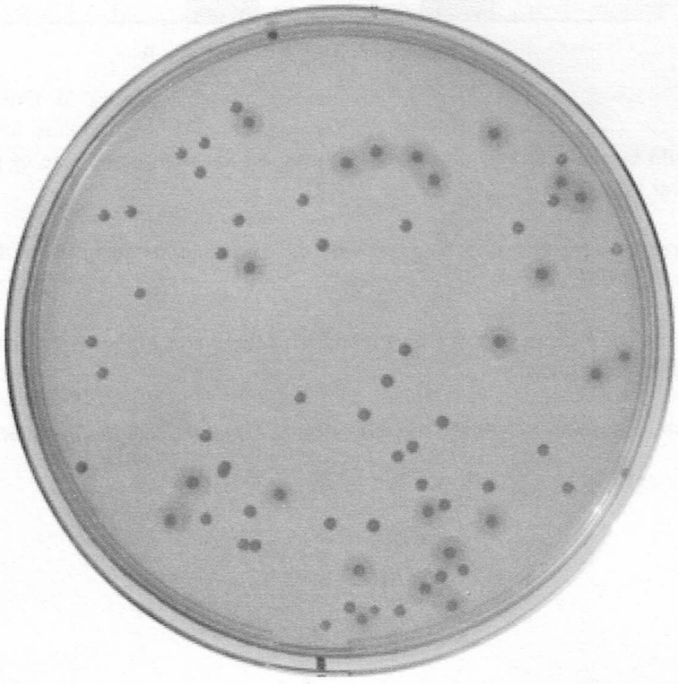
(i) With reference to the above information, explain why this bacterium is often spread via fresh milk and yoghurt and causes infections to humans. (4 marks)

(ii) Pasteurisation is a common method for preserving fresh milk.

(1) Describe the process of pasteurisation of fresh milk. (2 marks)

(2) Explain why pasteurisation can kill this bacterium. (1 mark)

(iii) To detect *Listeria monocytogenes* in food, agar plates with selective medium can be used. The selective medium allows only the species of *Listeria* genus to grow on it. For *Listeria monocytogenes*, an opaque area will be formed around the colony. The photograph below shows the colonies formed on an agar plate with selective medium:



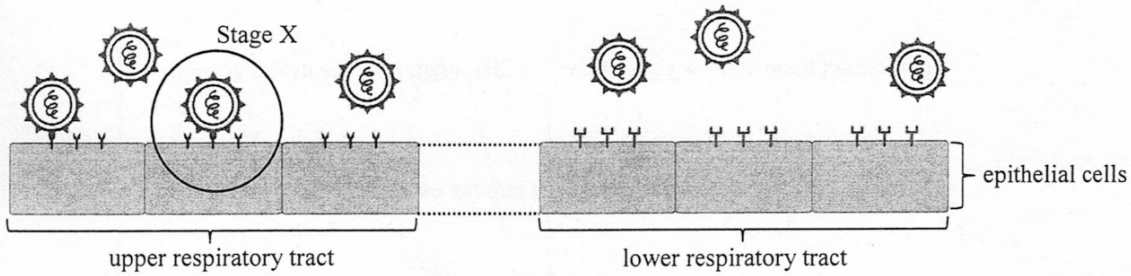
(1) Count the number of *Listeria monocytogenes* colonies. (1 mark)

(2) Suggest ***one*** safety measure that should be taken to dispose of the agar plates after the experiment in order to avoid contamination of the environment. (1 mark)

3(b) Viruses are host-specific because they can only infect certain types of cells. The following diagram shows a virus which infects epithelial cells in the upper but not the lower respiratory tract in human:

Key:

protein on the viral surface
protein on the surface of epithelial cells in the upper respiratory tract
protein on the surface of epithelial cells in the lower respiratory tract



(i) With reference to the life cycle of viruses, describe what happens in stage X. (1 mark)

(ii) Using your understanding of protein structure, explain why this virus can only infect the epithelial cells of the upper respiratory tract but not the lower respiratory tract. (2 marks)

(iii) Fish and pigs may carry viruses of their own. Viruses of one host species can go through a series of mutations and become infectious to other host species. Explain which viruses, from fish or pigs, have a higher potential to infect humans when present in the food products. (3 marks)

(iv) Foodborne illnesses of microbial origin, especially bacterial infections, is a serious food safety problem. Scientists have proposed the use of bacteriophages to eliminate bacterial pathogens present in food.

(1) Describe how a bacteriophage could eliminate the whole population of a bacterial pathogen in food even if only one bacterial pathogen is infected by the bacteriophage. (4 marks)

(2) With reference to the host-specificity of viral infection, state ***one*** advantage of using bacteriophages in this approach to eliminate bacterial pathogens in food. (1 mark)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 4 mark(s)**

This bacterium can grow at refrigeration temperatures, and ready-to-eat milk products or yoghurt may be consumed without cooking.

The minimum growth temperature of −1.5°C is below a typical refrigerator temperature near 4°C, so chilling does not guarantee prevention of multiplication. Contaminated food without adequate heating before consumption may still carry bacteria into the body. Link the temperature evidence and ready-to-eat handling separately to transmission.

a(ii)(1); original allocation: 2 mark(s)

Heat milk briefly to approximately 75°C, then cool rapidly to approximately 10°C.

This paper's marking reference uses short high-temperature exposure followed by rapid cooling. Pasteurisation reduces harmful microorganisms while preserving food quality as far as possible, unlike prolonged boiling or complete sterilisation.

a(ii)(2); original allocation: 1 mark(s)

Heat damages essential bacterial proteins and cellular functions, inactivating or killing the bacteria.

75°C is well above the stated growth maximum and, with suitable exposure time, can kill the pathogen. Distinguish growth inhibition by refrigeration from heat killing. Being below optimum growth temperature alone does not mean bacteria are dead.

a(iii)(1); original allocation: 1 mark(s)

22.

Listeria is identified by the opaque zone surrounding its colonies, so count only colonies with that feature, not every coloured spot. Count systematically by regions or rows to avoid duplication or omission.

a(iii)(2); original allocation: 1 mark(s)

Autoclave the culture dishes before disposal.

Inactivate cultured microorganisms first to prevent release of living bacteria into the environment. This is post-experiment safe treatment, not disposal of live colonies in ordinary rubbish.

b(i); original allocation: 1 mark(s)

The virus recognises and attaches to the host-cell surface, initiating infection.

The virus circled at X contacts an upper-respiratory-tract cell, corresponding to host recognition through surface proteins.

b(ii); original allocation: 2 mark(s)

Upper- and lower-respiratory-tract cells have differently shaped surface proteins. The viral surface proteins fit the upper-tract receptors appropriately.

Protein three-dimensional structure makes binding specific. Both cell types being epithelial does not imply identical receptors. Effective attachment is required before entry and use of the cell.

b(iii); original allocation: 3 mark(s)

The pig virus is more likely to infect humans.

Pigs and humans are mammals and more closely related, so their cell-surface proteins may be more similar. A pig virus may therefore need fewer changes to recognise human cells. This is a relative likelihood, not a claim that every pig virus infects humans.

b(iv)(1); original allocation: 4 mark(s)

Bacteriophages replicate extensively inside an infected bacterium, lyse it and are released to infect other target bacteria. A phage introduces nucleic acid and redirects host metabolism to make new phage components. After assembly, the host lyses. Multiple released phages infect other bacteria of the same target type in the food. Repeated spread can therefore reduce the whole target population even if only one cell is infected initially.

b(iv)(2); original allocation: 1 mark(s)

Host specificity allows phages mainly to remove the target pathogen while affecting other microorganisms less.

Recognition of particular bacterial surface receptors does not confer the ability to infect human cells. The advantage concerns restricted recognition, not killing every food-borne microorganism.

Examiner's comment (source page(s) 9, 10)

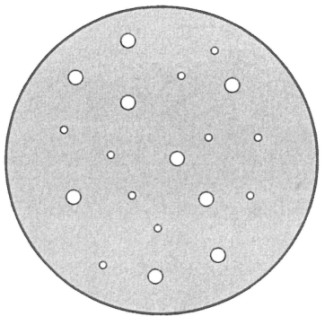
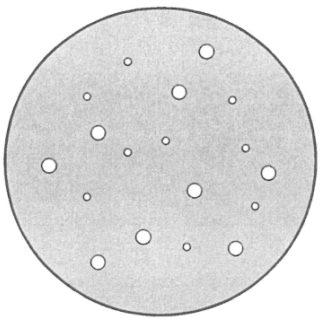
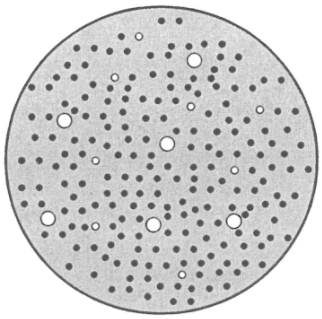
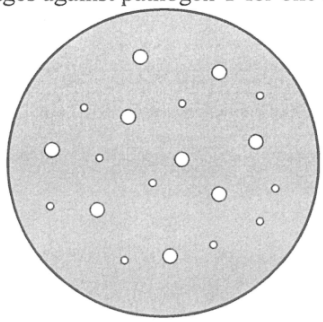
Count Listeria colonies by the stated distinguishing feature, not all colonies. Refrigeration is not sterilisation. Connect viral surface proteins to host-receptor recognition. A bacteriophage is a virus infecting bacteria, not a cell that engulfs them.

Marking reference: HKDSE Biology 2021 Paper 2 Q3, official marking scheme, source page(s) 9, 10.

2. [HKDSE Biology 2024 Paper 2 Q3]
Hint: Identify the host and controls, then limit the conclusion accordingly.

(a) An experiment was conducted to illustrate how bacteriophages can be used to fight against pathogenic bacteria. This phage therapy may be an alternative way to combat bacteria with multiple anitbiotic resistance. In the experiment, leaf surfaces of a plant were subjected to different treatments. After that, bacterial samples were taken from the leaf surface and transferred to an agar plate for incubation. Colonies of bacterial pathogen Y in the agar plate would stain red. Below are the four different treatments and the results obtained:

Key:
- ● red colony
- ○ white colony

Treatment I: A leaf was allowed to stand for one hour without any treatment. 	Treatment II: A leaf was immersed in a mixture containing different phages against pathogen Y for one hour. 
Treatment III: A leaf was exposed to pathogen Y for 10 minutes and then allowed to stand for one hour. 	Treatment IV: A leaf was exposed to pathogen Y for 10 minutes and then immersed in a mixture containing different phages against pathogen Y for one hour. 

(i) By comparing the results of the following pairs of treatment, what can be deduced? Tabulate your answer in the answer book according to the following format. (4 marks)

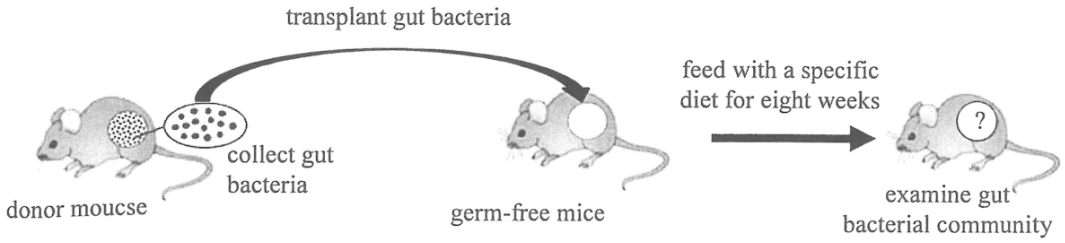
Treatments	*Comparison of results*	*Deduction*
I and II		
III and IV		

(ii) According to the results of the experiment, suggest the advantage of using phage therapy to treat bacterial infection over traditional antibiotic treatment. (2 marks)

(iii) Describe how the bacterial sample from the surface of the leaf can be obtained. (2 marks)

(iv) Describe ***two*** aseptic techniques that should be adopted during plate spreading in order to avoid contamination from the environment. (2 marks)

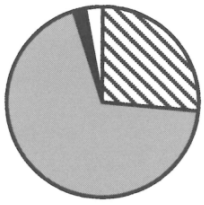
3(b) Recently, increasing evidence linking obesity to the gut bacterial community has been reported. To investigate the interrelationship between diet, gut bacterial community and energy balance, a group of germ-free mouse siblings received a transplant of gut bacterial community from a donor mouse, followed by a specific diet for eight weeks. After that, their gut bacterial communities were examined. The diagram below shows the workflow:



(i) (1) Suggest why mouse siblings were used in the investigation. (1 mark)

(2) Suggest and explain ***two*** experimental procedures which are necessary for producing germ-free mice. (2 marks)

(ii) The following table shows a summary of the treatments and the results obtained:

	Experimental group	***Control group***
Diet of the donor mouse	Diet rich in plant polysaccharides	
Diet used to feed the recipient mice for eight weeks	High-fat and high-sugar diet	Diet rich in plant polysaccharides
Average body mass gained (g)	5.3	1.5
Average body fat (%)	3.7	1.7
Composition of gut bacterial community Key: P Q R S		

(1) Which group of mice represented the obese group? (1 mark)

(2) Describe how the high-fat and high-sugar diet has modified the composition of the gut bacterial community of the experimental group. (2 marks)

(3) It is known that a certain group of the gut bacteria in the experimental group can convert some indigestible materials into fatty acids which are then absorbed by the mice. Which group of bacteria is likely to be capable of such conversion? Explain your answer. (2 marks)

(iii) To test if the gut bacterial community induced by the 'high-fat high-sugar' diet increases nutrient absorption as stated in (ii)(3), another experiment was conducted as shown in the table below:

	Experimental group	*Control group*
Diet of the donor mouse	High-fat and high-sugar diet	Diet rich in plant polysaccharides
Diet used to feed the recipient mice for eight weeks	Diet rich in plant polysaccharides	

After eight weeks, the experimental group had an average body mass and body fat percentage which were significantly higher than the control group. What conclusion can be drawn? Explain your answer. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 4 mark(s)**

I versus II: colony types and numbers are similar, suggesting no appreciable killing of non-target leaf-surface bacteria by the phage. III versus IV: III has many red Y colonies, whereas IV has none, showing that phage treatment kills pathogen Y.

For each pair, compare visible colonies before drawing the inference. I/II tests effects on existing non-target bacteria. Both III and IV receive Y, but differ in phage treatment, allowing disappearance of Y colonies to be attributed to it.

a(ii); original allocation: 2 mark(s)

Bacteriophages are host-specific and can target pathogenic bacteria while affecting beneficial or other non-target bacteria less.

A phage must match specific host-cell surface receptors to infect. The results show removal of Y while other colonies remain, indicating less disruption of normal microbiota than a relatively broad-spectrum antibiotic.

a(iii); original allocation: 2 mark(s)

Wipe the leaf surface with a moistened sterile swab, then place it in sterile culture broth to transfer the sample into the liquid.

The swab contacts an uneven leaf surface, and moisture helps remove bacteria. Transfer into broth disperses the sample for subsequent spread plating.

a(iv); original allocation: 2 mark(s)

For example, work in a suitable aseptic workspace and open the Petri-dish lid only slightly, replacing it promptly.

Both reduce airborne microorganisms falling onto the plate. Sterile equipment is also necessary. Give practical measures relevant to spread plating, not simply "keep clean".

b(i)(1); original allocation: 1 mark(s)

Mice from the same parents have more similar genetic backgrounds, reducing genetic confounding.

Genes can affect body mass, metabolism and responses to microbiota. Common parentage makes subsequent differences more attributable to transplanted microbiota and diet.

b(i)(2); original allocation: 2 mark(s)

Procedure 1: use appropriate antibiotics to remove existing intestinal bacteria. Procedure 2: keep mice in a sterilised environment isolated from external sources, preventing renewed bacterial exposure.

First remove existing gut bacteria, then prevent new entry; these serve different functions. Food and drinking water must also remain sterile during isolation. Cleaning body surfaces or cages alone does not remove bacteria already inside the intestine.

b(ii)(1); original allocation: 1 mark(s)

The experimental group.

Its mean mass gain is 5.3 g compared with the control's 1.5 g, and body fat is 3.7% compared with 1.7%. Both increased mass and fat accumulation support its greater obesity.

b(ii)(2); original allocation: 2 mark(s)

After the high-fat, high-sugar diet, Q increases greatly in proportion and becomes dominant, while P and R decrease proportionally.

The control pie chart is dominated by P with some R, whereas Q dominates the experimental group. These are relative compositions; without total bacterial counts, do not claim a particular fold increase in Q's absolute cell number.

b(ii)(3); original allocation: 2 mark(s)

Q.

Q shows the largest proportional increase and becomes dominant after the high-fat, high-sugar diet, accompanying greater mass gain and body fat. It is therefore the most likely candidate for converting otherwise poorly digested substances into absorbable fatty acids and increasing energy acquisition. This is an initial candidate inference; the subsequent transplantation experiment tests microbiota effects more directly.

b(iii); original allocation: 2 mark(s)

Microbiota induced by the high-fat, high-sugar diet can increase the host's ability to digest, absorb and obtain energy from food.

Both recipient groups in the new experiment receive the same plant-polysaccharide-rich diet, controlling current food intake. Recipients of microbiota from high-fat, high-sugar donors still gain more mass and fat. This supports an effect of the transplanted microbiota rather than merely greater fat and sugar consumption by recipients.

Examiner's comment (source page(s) 76, 77)

For phage conclusions, compare non-target colonies in I/II and red pathogen colonies in III/IV. The question does not compare bactericidal efficiency between therapies, so it cannot establish that phages are always more effective. Microbiota pie charts show proportions only. Transplant recipients eat the same diet and differ in donor-microbiota origin.

Marking reference: HKDSE Biology 2024 Paper 2 Q3, official marking scheme, source page(s) 63, 64.

Practice VII.d Harmful effects and control of microorganisms

Related type 1 Draw bounded conclusions from inhibition, survival and food data

Degree of integration ★★★★☆

Practice

1. [HKDSE Biology 2019 Paper 2 Q3]
   ⟵ Hint: Distinguish growth inhibition, survival and toxins.

(a) There is a diversity of foodborne pathogens which have different abilities in causing illness in humans. Investigations of their growth rate under different conditions can provide important information about the disease-causing risks of the pathogens. Below are the results of a series of experiments testing the doubling time of bacterial pathogens A, B and C under different conditions:

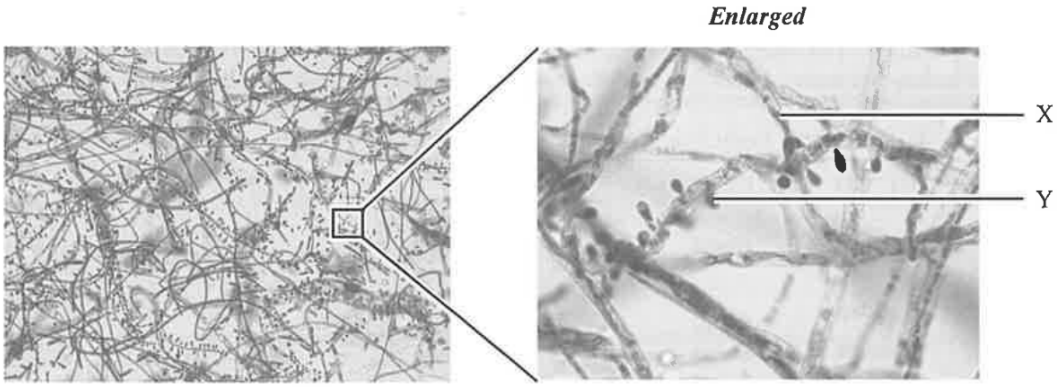
pH	Temperature (°C)	Doubling time (hour)		
		Pathogen A	Pathogen B	Pathogen C
4	4	100	5	10
	37	20	20	1
8	4	50	1	30
	37	1	10	10

(i) The doubling time indicates the time that a bacterial population requires to double its population size once under a particular growth condition. Using the data in the table, calculate the amount of time required by bacterial pathogens A and B respectively to increase their population size by 4 times at 37°C and pH 8. (2 marks)

(ii) Gastric juice in the human stomach has a pH value of around 2. However, when there is food inside the stomach, the pH value can be raised to 4–5. With reference to the above table, deduce which bacterial pathogen is most likely to cause stomach infection. (4 marks)

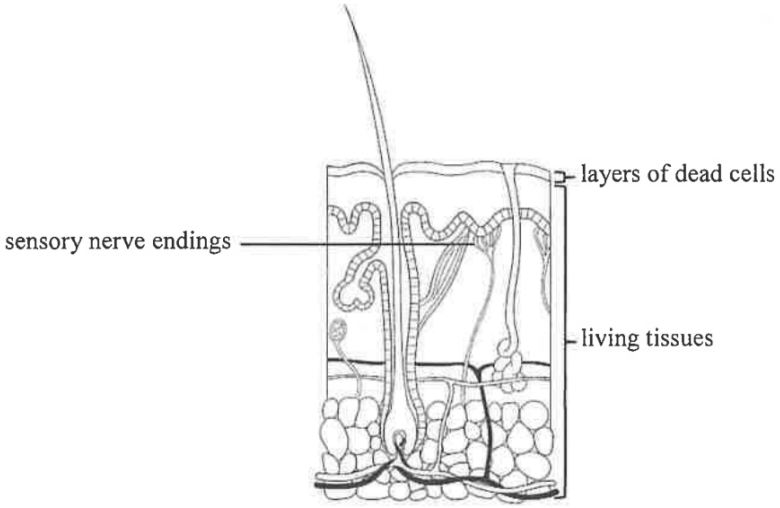
(iii) Bacterial pathogen B would lead to food safety concerns to the food stored in the chilling compartment of a refrigerator which is kept at around 4°C. Even though the food has been cooked thoroughly, people may still contract a foodborne illness after consuming food contaminated by bacterial pathogen B. With reference to the above table, explain if the illness is more likely to be food poisoning or a foodborne infection. (3 marks)

3(b) Athlete's foot is a common skin infection caused by a fungus. The disease can be spread by contact with infected skin or the fungus in the environment. The photomicrograph below shows the appearance of the fungus on an agar plate:



(i) Identify structures X and Y. (2 marks)

(ii) One of the common symptoms of athlete's foot is itching. The itchy feeling comes from the sensory nerve endings located in the skin. The diagram below shows a section of the skin:



With reference to the mode of nutrition of fungi, describe how structure X of the fungus can pass through layers of dead cells to reach and stimulate the sensory nerve endings. (4 marks)

(iii) Ken suffered from athlete's foot. He was infected while he was walking barefoot in the shower room of a fitness centre.

(1) Suggest ***one*** environmental condition that enables the shower room to be a breeding ground for the fungus. (1 mark)

(2) Ken's doctor instructed him to apply an antifungal cream to the infected area for two weeks. After several days, Ken did not feel itchy any more and stopped the treatment. However, he felt itchy again two days later. With reference to the life cycle of fungi, explain the changes in symptoms mentioned above. (4 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 2 mark(s)**

A: 2 hours. B: 20 hours.

At 37°C and pH 8, A doubles in 1 hour and B in 10 hours. A fourfold increase requires two doublings: N→2N→4N. Thus the times are 2×1 = 2 and 2×10 = 20 hours, not four times the doubling time.

a(ii); original allocation: 4 mark(s)

C.

After eating, stomach pH is approximately 4–5 and temperature near 37°C, so use the pH 4, 37°C row. C doubles in 1 hour there, while A and B both require 20 hours. C multiplies fastest and is most likely to establish infection in these stomach conditions. Do not choose the fastest entry anywhere in the table while ignoring its conditions.

a(iii); original allocation: 3 mark(s)

Food poisoning is more likely.

B can multiply relatively quickly at 4°C: 5 hours at pH 4 and 1 hour at pH 8. It can therefore increase and accumulate toxins in refrigerated food. Thorough cooking can kill bacteria, but some preformed toxins may remain and cause illness. Under the stated conditions, toxins better explain the symptoms than substantial multiplication of surviving live bacteria after cooking.

b(i); original allocation: 2 mark(s)

X: hyphae. Y: spores/sporangia.

X points to elongated, branching filaments; Y points to smaller reproductive or dispersal structures. Use visible shape and do not confuse hyphae with bacterial flagella.

b(ii); original allocation: 4 mark(s)

The fungus secretes enzymes externally, digests dead cell layers and absorbs the products for growth. Hyphae X extend through skin layers and stimulate deeper sensory nerve endings.

Fungi digest extracellularly, first converting surrounding organic matter into absorbable small molecules. These support gradual hyphal extension into nutrient-containing skin layers. In the question's model, approaching and stimulating sensory endings produces itching. The fungus does not swallow whole skin cells.

b(iii)(1); original allocation: 1 mark(s)

For example, the bathroom is damp.

Warmth, moisture and organic matter such as shed skin favour this fungus. Only one condition is required; choose dampness and connect it to fungal growth requirements.

b(iii)(2); original allocation: 4 mark(s)

The cream damages hyphae, reducing nerve stimulation and itching. Spores may persist and be more resistant to the cream. After treatment stops, they germinate into hyphae, causing renewed infection and itching.

Temporary symptom relief is not complete removal of every fungal structure. Inhibition of growing hyphae can rapidly reduce sensory stimulation without killing all spores. Surviving spores resume growth under suitable conditions, explaining recurrence after stopping treatment.

Examiner's comment (source page(s) 10)

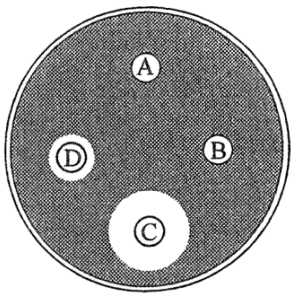
Include units for doubling times. Select C using stomach pH and body temperature, not the fastest cell in the table. Distinguish preformed toxins in food poisoning from live pathogens in infection. Explain itching and recurrence through extracellular digestion, hyphae and spores rather than unsupported evolution of resistance.

Marking reference: HKDSE Biology 2019 Paper 2 Q3, official marking scheme, source page(s) 9.

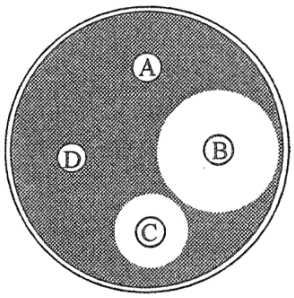
2. [HKDSE Biology 2015 Paper 2 Q3]
Hint: Distinguish growth inhibition, survival and toxins.

(a) To investigate the effectiveness of antibiotics on inhibiting the growth of different microorganisms, four agar plates, each with a different microorganism (1, 2, 3 and 4) were prepared. Circular paper discs soaked with the same concentration of antibiotics A, B, C and D respectively were placed onto the plates. Clear zones were formed on the agar plates after incubation as shown in the diagram below:

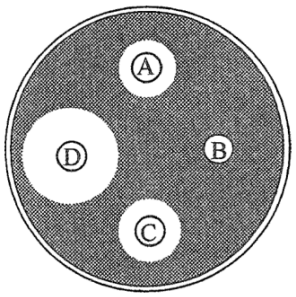
Agar plate with microorganism 1



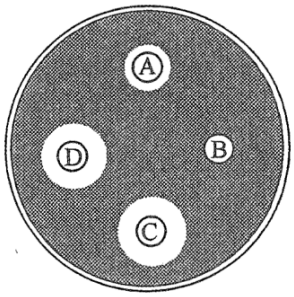
Agar plate with microorganism 2



Agar plate with microorganism 3



Agar plate with microorganism 4



Key:
- ○ circular paper disc soaked with different antibiotics
- ▒ region with the growth of microorganisms
- ▭ clear zones formed around the paper discs

(i) In this investigation, how can you compare the effectiveness of different antibiotics on inhibiting the growth of a microorganism? (1 mark)

(ii) If a doctor has not yet confirmed which microorganism is responsible for an infection, which antibiotics should he use? Explain your answer. (3 marks)

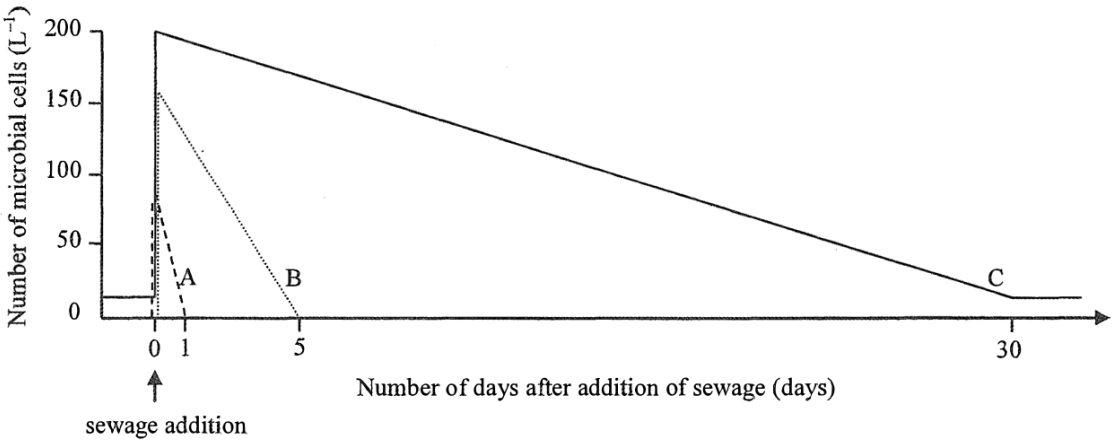
(iii) To find out the type of microorganism responsible for a certain infection, a microbial sample taken from a patient is cultured for identification.

(1) State *two* steps in the aseptic technique that should be used during the inoculation of microorganisms on the agar plate. (2 marks)

(2) What is the advantage of identifying the microorganism responsible for a certain infection? (2 marks)

(iv) Give *two* properties of antibiotics which can be generalized from the above results. (2 marks)

3(b) An experiment was conducted to investigate the change in the population size of sewage microorganisms by adding sewage to a beaker of unpolluted river water. The beaker was then placed outdoors under natural light. The graph below shows the change in population size of three kinds of sewage microorganisms A, B and C in the beaker during the course of the experiment:



(i) State *two* environmental factors that would cause the drop in the population of sewage microorganisms observed above. Explain your answer. (4 marks)

(ii) Suggest why the microbial populations dropped at different rates as shown above. (1 mark)

(iii) Some microorganisms can be used as indicating organisms of sewage pollution in river water. Discuss whether the microorganisms shown above are suitable for use as indicating organisms. (3 marks)

(iv) In the above case, both biomass measurement and optical measurement are ***not*** suitable for monitoring the population size of the microorganisms effectively. Explain why not. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **(a)(i); original allocation: 1 mark(s)**

Compare clear inhibition-zone diameters under the same conditions. A larger zone indicates stronger inhibition of the microorganism's growth.

The clear zone lacks visible growth. Measuring its diameter converts a qualitative observation into comparable data. Keep disc size, antibiotic concentration and incubation conditions consistent.

(a)(ii); original allocation: 3 mark(s)

C is suitable in these data because it produces an inhibition zone for all four microorganisms. Its broader activity gives a greater chance of effectiveness against an unidentified pathogen.

Check C on every plate rather than choosing the largest zone on a single plate. Broad-spectrum means activity against more types, not necessarily the strongest action against each.

(a)(iii)(1); original allocation: 2 mark(s)

Flame the inoculating loop until red-hot, then cool it before use. Flame the culture-tube mouth before and after use.

These steps reduce contamination from equipment and openings. The loop must cool to avoid killing the intended sample.

These illustrate the basic aseptic principles given in the question.

(a)(iii)(2); original allocation: 2 mark(s)

After identifying the pathogen, choose an antibiotic effective against it, improving specificity and reducing unnecessary effects on normal body microorganisms.

Identification allows that organism's inhibition results to guide selection. Continuing broad-spectrum treatment solely because the infection was initially unknown fails to use the identification evidence now available.

(a)(iv); original allocation: 2 mark(s)

Antibiotics differ in their range of activity and can also differ in inhibitory strength against the same microorganism.

B produces a clear zone only for microorganism 2, indicating a narrower range. For microorganism 3, D's zone is larger than A's or C's, showing different strengths against the same organism. Support each conclusion with its own comparison from the figure.

(b)(i); original allocation: 4 mark(s)

Outdoor sunlight contains ultraviolet radiation that damages microorganisms. Unpolluted river water also has fewer available organic nutrients, so microorganisms may die from nutrient shortage.

Adding sewage to cleaner river water outdoors introduces both light exposure and changed nutrient conditions. For each, explain the mechanism reducing numbers.

(b)(ii); original allocation: 1 mark(s)

The three microorganisms differ in tolerance of stresses such as light exposure and nutrient shortage, producing different death rates.

Compare different types in the same environment. Initial count differences alone do not explain all slope differences.

(b)(iii); original allocation: 3 mark(s)

B is the better indicator: it survives for a detectable period after addition but is not persistently abundant in unpolluted water. A disappears too quickly, so its absence cannot exclude earlier contamination. C was already present at low levels, so finding it alone cannot establish contamination.

An indicator needs both contamination specificity and a detectable time window. Choosing longest-surviving C ignores its pre-contamination presence; choosing fastest-disappearing A may miss the contamination signal.

(b)(iv); original allocation: 2 mark(s)

Cell numbers per litre are very low, producing changes in biomass and turbidity too small for reliable detection by these methods.

Cell presence does not guarantee a measurable mass or light-scattering signal. Compare the method's detectable signal with the sample's actual order of magnitude when choosing measurements.

Examiner's comment (source page(s) 5)

Measure inhibition-zone diameter when comparing activity. Describe contamination prevention during the actual inoculation. Choose indicator organisms using survival in both polluted and clean water.

Marking reference: HKDSE Biology 2015 Paper 2 Q3, official marking scheme, source page(s) 9.

Chapter 10

Biotechnology: question types and practice

VIII.a Modern biotechnology

Read the explanations and diagrams in Concept VIII.a first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Make testable deductions from DNA fragments and signals

Degree of integration ★★★☆☆

Key ideas

① If one DNA band in an offspring is explained by the father, compare the other with the relevant maternal data. Every offspring band need not match both parents simultaneously. After cutting linear DNA, fragment lengths sum to the original length. Equal-length fragments may overlap as one band.

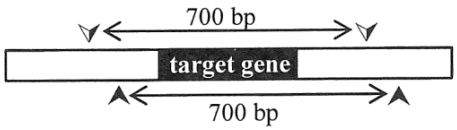
② DNA usually moves towards the positive electrode during electrophoresis, with shorter fragments travelling farther. PCR, restriction digestion and electrophoresis address different steps: whether a target is present, which lengths are produced, and how differences are observed.

How to recognise the problem List fragment numbers and lengths before predicting visible bands. Distinguish 'not detected' from 'absent', and check for suitable positive or negative controls.

Example 1 [HKDSE Biology 2020 Paper 2 Q4]

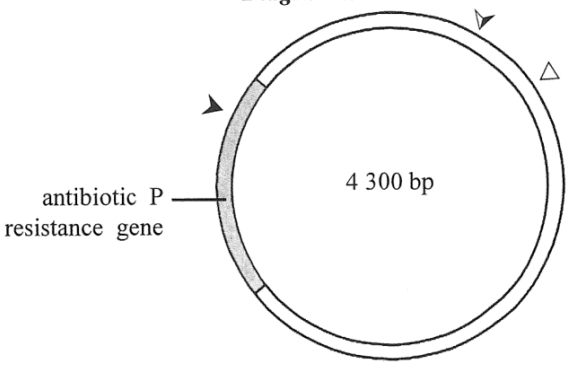
(a) Study the diagrams below. Diagram X shows a DNA segment with a target gene. Diagram Y shows a plasmid with a size of 4 300 base pairs (bp). The restriction sites for restriction enzymes EcoRI, PstI and HindIII are shown in both diagrams.

Diagram X



Key: ⩛ restriction site for EcoRI
⮟ restriction site for PstI
▽ restriction site for HindIII

Diagram Y

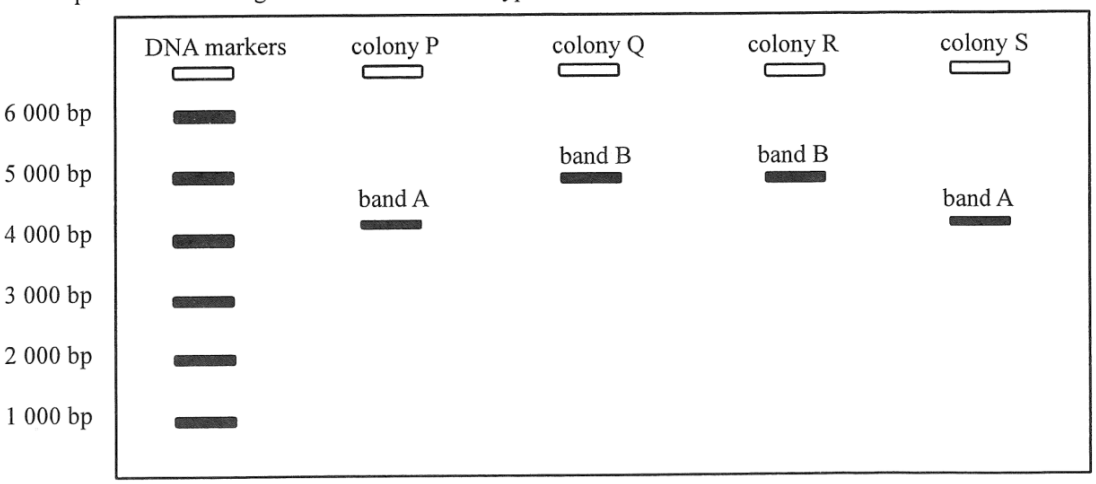


(i) A student plans to insert the target gene into the plasmid and screen the transformed bacteria with antibiotic P.

(1) Which restriction enzyme should be used for the insertion? Explain your answer. (3 marks)

(2) State another type of enzyme which is required to complete the insertion. (1 mark)

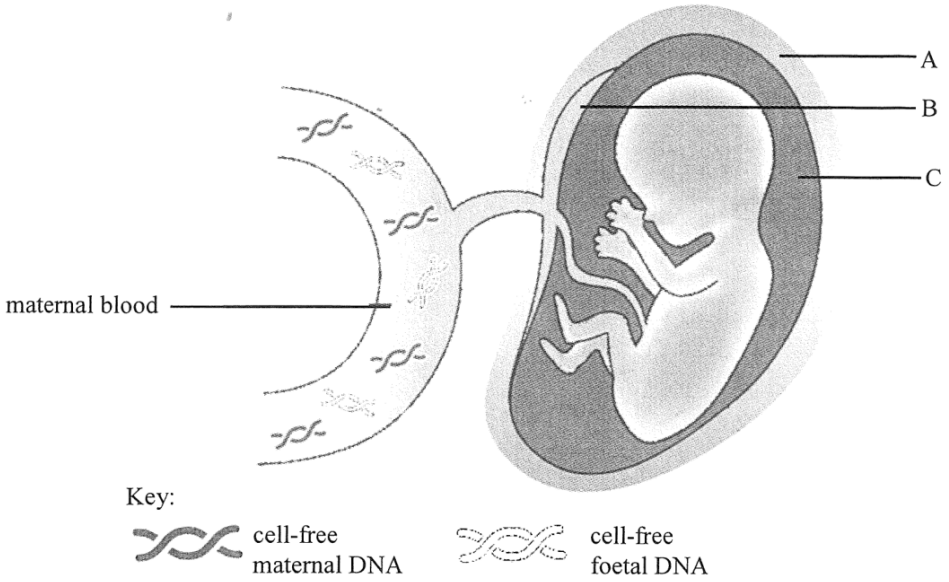
(ii) After transformation, screening and culture, the plasmids from different colonies were harvested and cut using enzyme HindIII. Gel electrophoresis was then conducted to check the size of the cut plasmids. The diagram below shows two types of DNA band obtained:



(1) Which band (A or B) represents the plasmids with the target gene? Explain your answer. (3 marks)

(2) Explain why two types of DNA band were detected in the cut plasmids after gel electrophoresis. (3 marks)

4(b) Traditional diagnosis of Down Syndrome requires the collection of foetal tissue. The recent discovery of cell-free foetal DNA in maternal blood has opened up new possibilities for diagnosis. Cell-free DNA are fragments of DNA released into the plasma from the degradation of normal cells. The diagram below shows a foetus and its associated structures inside the mother's body:



(i) With reference to the above diagram, which labelled structure does the cell-free foetal DNA come from? Explain your answer. (2 marks)

(ii) In the maternal plasma, cell-free foetal DNA is only a minor proportion of the total cell-free DNA. Suggest a technique for enhancing the detection of very small amounts of cell-free foetal DNA in maternal plasma. Explain your answer. (2 marks)

(iii) Nowadays, the identity of the genes in the cell-free DNA can be found through DNA sequencing and mapping against human genome databases.

(1) Scientists proposed that Down Syndrome could be diagnosed by comparing the frequency of occurrence of the genes found on chromosome 21 and another autosome in the cell-free foetal DNA. Based on your understanding about Down Syndrome, explain the biological principle involved in this method. What would be the expected results? (2 marks)

(2) Suggest ***two*** other possible applications of the results obtained from DNA sequencing. Explain the principle of each application. (4 marks)

Solution a(i)(1); original allocation: 3 mark(s)

Method: List fragments and signals, then use controls to check the inference.

EcoRI. It cuts at both ends of the target DNA and in the plasmid, obtaining the target-gene fragment while preserving the antibiotic P resistance gene.

Use both diagrams. EcoRI has sites flanking the target fragment in X and a corresponding plasmid site in Y, producing compatible ends. Its plasmid site lies outside the resistance gene. PstI can release the fragment but disrupts the resistance gene needed for antibiotic P selection. HindIII has no marked pair of sites flanking the target in X, so it is unsuitable for this step.

a(i)(2); original allocation: 1 mark(s)

DNA ligase.

Restriction enzymes cut DNA. Once fragment and opened-plasmid ends match, DNA ligase covalently joins their sugar–phosphate backbones. DNA polymerase mainly extends a new strand along a template and cannot replace this joining step.

a(ii)(1); original allocation: 3 mark(s)

Band B.

First calculate the post-insertion length:

Recombinant plasmid length = 4,300 + 700 = 5,000 bp.

After HindIII opens the plasmid, compare linear-DNA size with the marker. B lies near 5,000 bp, matching the 700 bp insertion, whereas A is near the smaller original 4,300 bp plasmid. “B is higher” alone is insufficient; connect the length calculation and marker.

a(ii)(2); original allocation: 3 mark(s)

Some opened plasmids join to the target fragment, while others recircularise without it, producing two plasmid sizes.

Insertion does not always succeed. An unmodified plasmid retaining antibiotic P resistance can still enter bacteria and allow survival during selection. Resistance supports possession of a plasmid expressing that trait, not guaranteed presence of the target gene. After HindIII cutting, approximately 5,000 bp plasmids form B and smaller original plasmids form A. Transformation is bacterial uptake of foreign DNA, distinct from recombinant-plasmid ligation.

b(i); original allocation: 2 mark(s)

B, the placenta.

The placenta contains fetal-derived cells and forms the maternal–fetal exchange interface. Normal breakdown of fetal-derived cells releases DNA fragments that may enter maternal plasma, making B most suitable. Maternal and fetal blood normally do not mix extensively; detection of small quantities of fetal-derived DNA does not imply complete continuity of the circulations.

b(ii); original allocation: 2 mark(s)

Polymerase chain reaction (PCR).

PCR uses suitable primers to copy selected DNA fragments repeatedly, increasing copy number from a very small sample and improving subsequent detection. It increases fragment numbers, not fragment length or fetal-cell size. The whole DNA-fingerprinting procedure is unnecessary here.

b(iii)(1); original allocation: 2 mark(s)

A fetus with Down syndrome usually has three chromosome 21 copies, so chromosome-21 sequences occur more frequently relative to sequences from another normal autosome.

Normal diploid chromosomes have two copies each, providing a relative copy-number baseline. An extra chromosome 21 increases the relative proportion of its DNA. The blood sample mixes maternal and fetal cell-free DNA, so do not assign an exact 3:2 ratio to the entire sample. The main prediction is increased relative frequency.

b(iii)(2); original allocation: 4 mark(s)

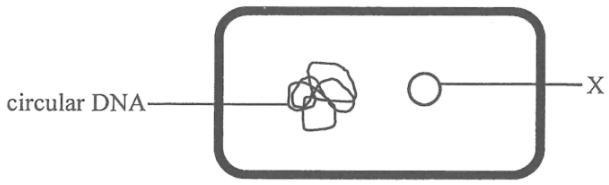
Y-chromosome-specific sequences can help identify fetal chromosomal sex. Comparing sequences with known pathogenic variants can also screen for some inherited-disease risks. First, align sequencing reads to the human genome. Reliable Y-specific sequences support the presence of Y-derived DNA. Second, inspect a disease-associated position for the relevant pathogenic allele and connect its presence to disease risk. For each, link the application to the sequence evidence being compared. Disease names alone are insufficient, and generic species-evolution analysis is not a direct application of these human fetal data. Screening and diagnosis still have different evidential requirements.

Marking reference: HKDSE Biology 2020 Paper 2 Q4, official marking scheme, source page(s) 12.

Example 2 [HKDSE Biology 2024 Paper 2 Q4]

(a) Fish oil contains specific types of polyunsaturated fatty acids (PUFAs) which are beneficial to human health. A biotechnology company successfully isolated a key gene encoding the enzyme responsible for the production of PUFAs. Using agrobacterium, the foreign gene was transferred to three different seed crops (A, B and C).

(i) The schematic diagram below shows the structures of an agrobacterium:



What is structure X? Describe the role of this structure in the transformation process. (2 marks)

(ii) In a greenhouse trial, the following data were obtained for the three different types of genetically modified (GM) crops.

GM crop	*Seed yield (g per crop)*	*Content of PUFAs (arbitrary unit per g of seed)*	*Time required to reach seed harvest stage (month)*
GM-A	50	9	6
GM-B	150	3	3
GM-C	100	4	8

(1) The three GM crops grow equally well. Based on the data shown, which one would you select for PUFAs production in the greenhouse? Show how you arrived at your answer. (3 marks)

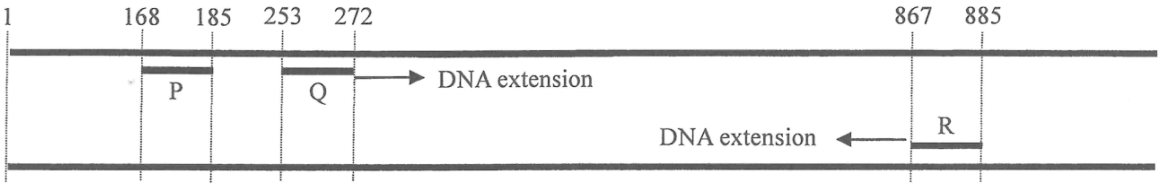
(2) Some people firmly believe that GM crops are not safe for consumption because they contain foreign genes which may have harmful effects on humans. How would you convince them that it is safe to consume PUFAs extracted from the GM seeds? (2 marks)

(iii) The biotechnology company selected a suitable GM crop for PUFAs production in the field. However, an environmental group strongly opposed to the cultivation in the field because there was a wild relative of such GM crop near the proposed area.

(1) What may be the major argument underlying the objection? (1 mark)

(2) Briefly describe an experiment to test whether the argument mentioned above is correct. (3 marks)

4(b) A gene fragment in the human genome is shown in the diagram below. A mutation at position 285 (nucleotide number) of this gene fragment has been found in 0.05% Asians. This mutation destroys the cut site of a restriction enzyme. Individuals with two copies of such mutation have an increased risk of developing a certain lung cancer.

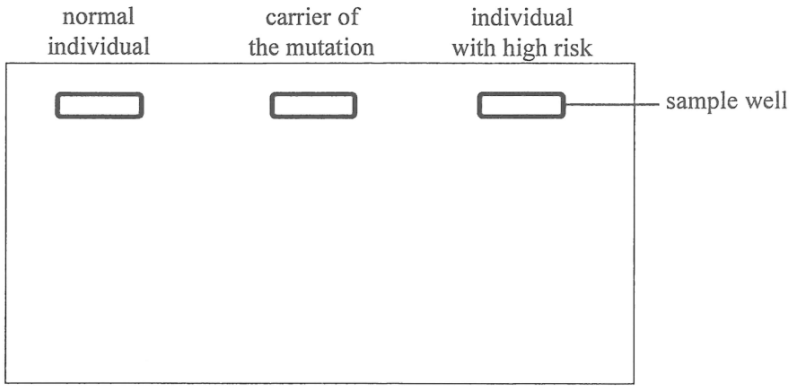


You were given three primers (P, Q and R) for polymerase chain reaction (PCR). The positions where they anneal are shown in the above diagram. With the help of gel electrophoresis, DNA samples obtained from individuals can be used to identify their genotypes for this gene.

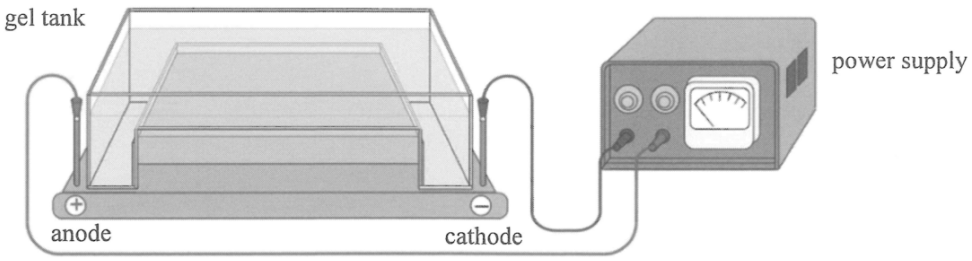
(i) Using the above information, briefly describe how you can identify their genotypes and state clearly the purposes of methods used. (3 marks)

(ii) Considering the annealing positions of the primers and the position of the mutation, explain which primer should ***not*** be used for this purpose. (1 mark)

(iii) Copy the following diagram of agarose gel onto your answer book. Draw the DNA bands that would be produced by samples from the three types of individuals. (3 marks)



(iv) The diagram below shows the equipment used to produce the results in (ii):



When the agarose gel is placed, on which side should the sample wells rest? Explain your answer. (2 marks)

Solution a(i); original allocation: 2 mark(s)

X is a plasmid acting as a vector to carry the foreign gene into plant cells.

Agrobacterium transfers an appropriate plasmid-DNA segment into plant cells. Placing the gene for a PUFA-producing enzyme in this vector allows transformed plants to express it.

The small ring in the diagram is separate from the main circular chromosomal DNA.

a(ii)(1); original allocation: 3 mark(s)

Choose GM-B. PUFA yields per plant per month are A: 75, B: 150 and C: 50 arbitrary units.

Calculate total yield per harvest, then divide by months required:

GM-A: 50×9÷6 = 75.

Method: List fragments and signals, then use controls to check the inference.

GM-B: 150×3÷3 = 150.
GM-C: 100×4÷8 = 50.
B gives the greatest yield per plant over equal time. Comparing content per gram alone would incorrectly select A by ignoring seed yield and harvest time.

a(ii)(2); original allocation: 2 mark(s)

The consumed product is purified PUFAs, not seed DNA containing the foreign gene. These PUFAs have the same molecular structures as the corresponding PUFAs in fish.
Genes are nucleic acids and PUFAs are fatty acids: different types of molecules. The extracted, purified fatty acids do not become "foreign genes" because their producer was genetically modified. Address this particular concern using the product's composition and molecular structure.

a(iii)(1); original allocation: 1 mark(s)

The foreign gene may pass to nearby wild relatives through cross-pollination.
If the GM crop can hybridise with wild relatives, pollen may introduce the gene into wild offspring and alter the population. Gene flow, not mere proximity, is the main concern.

a(iii)(2); original allocation: 3 mark(s)

Grow the GM crop and wild relatives together in a controlled environment with an opportunity for pollination. Collect seeds produced by the wild plants or grow their offspring, then test for the foreign gene or its expression product.
Include both conditions permitting gene exchange and a measurement verifying its outcome. Testing nearby soil does not establish hereditary transfer. Testing newly produced wild-parent seeds/offspring determines whether the gene entered the next generation.

b(i); original allocation: 3 mark(s)

First amplify the gene region containing the mutation by PCR, then digest it with the relevant restriction enzyme. Finally, separate fragments by size using gel electrophoresis and compare banding patterns.
PCR supplies enough DNA. The normal sequence retains the restriction site and yields shorter fragments, while the mutant sequence remains uncut. Electrophoresis converts this sequence difference into a visible size difference. Explain the purpose of all three steps.

b(ii); original allocation: 1 mark(s)

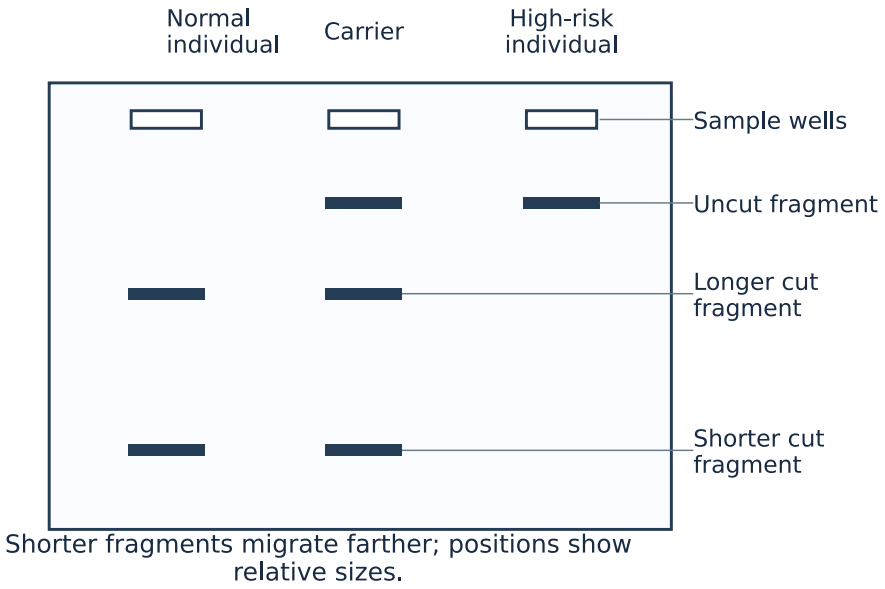
Primer Q is unsuitable.
Q occupies positions 253–272, too close to the mutation/restriction site near 285. Amplifying with Q and R would produce a very short fragment after digestion, which might run off the gel or be difficult to see. P is farther away and, with R, retains two more readily visible fragments.

b(iii); original allocation: 3 mark(s)

Normal individual: two lower digested-fragment bands. Carrier: one upper uncut band plus two digested bands. High-risk individual: only the upper uncut band.
Both normal alleles have the cutting site and produce two shorter fragment sizes. A carrier has normal and mutant alleles, producing three sizes. Both alleles in the high-risk individual are mutant, leaving only the uncut long fragment. Longer fragments remain nearer the wells; shorter ones travel farther, as illustrated. The precise cleaved bond is not given, so exact fragment base counts need not be invented.

b(iv); original allocation: 2 mark(s)

The wells are near the cathode/negative electrode, on the right of the diagram.
DNA's phosphate backbone is negatively charged, so DNA moves towards the anode/positive electrode. Wells belong at the negative end so fragments separate through the gel towards the positive end. Reversing the orientation may send DNA directly out of the gel.



Electrophoretic patterns for a normal individual, a carrier, and an individual with two mutant alleles.

Examiner's comment (source page(s) 77, 78, 79)

PUFAs are fatty acids, not proteins, DNA or genes. Standardise yield per plant per month. Test seeds/offspring of wild relatives for gene flow. Diagnosis proceeds through PCR, digestion and electrophoresis; Q's short fragment may run off the gel. Distinguish hollow wells from solid DNA bands. Negatively charged DNA migrates towards the positive electrode.

Marking reference: HKDSE Biology 2024 Paper 2 Q4, official marking scheme, source page(s) 64, 65.

Type 2 Infer insertion, expression and selection from vector structure

Degree of integration ★★★★☆

Key ideas

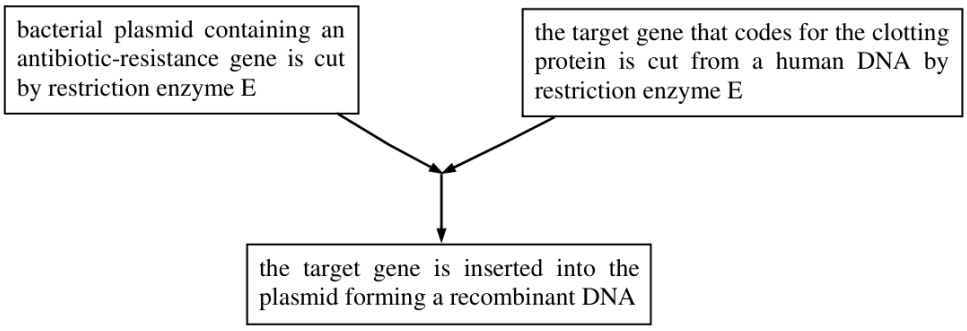
① A schematic circular vector of 4300 bp becomes 5000 bp after insertion of a 700 bp fragment. Correct length supports only part of the structural interpretation; it does not guarantee expression in every specified cell, location or period. First check that the target and regulatory regions are intact, then determine which candidates the markers can distinguish.

② When comparing modified lines, separate evidence for DNA presence, mRNA level and actual phenotype. A better value for one indicator does not mean that every trait is better.

How to recognise the problem Use the diagram and stated conditions to follow integrity → marker differences → expression → phenotype. Give evidence at every stage; 'successful modification' does not replace all these checks.

Example 1 [HKDSE Biology Sample Paper Paper 2 Q4]

4.(a) In some people, a gene mutation results in a failure to produce a blood clotting protein and this leads to the disease haemophilia. One way to treat this disease is by introducing an external source of the clotting protein into these people's blood. The clotting protein can be produced by recombinant DNA technology. The flow chart below outlines the major steps of producing the recombinant DNA:



(i) (1) What is a bacterial plasmid? (1 mark)

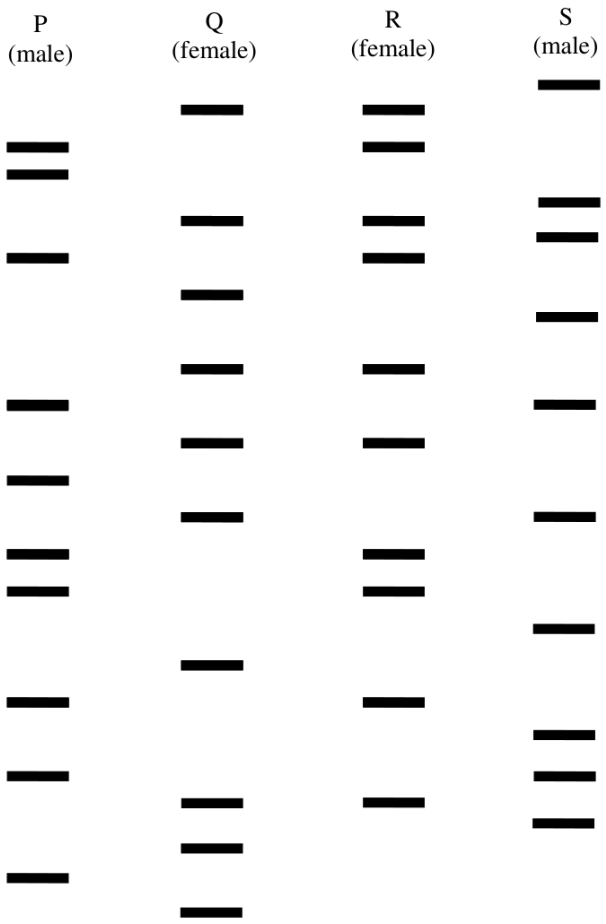
(2) Give ***two*** reasons why plasmids are commonly used as vectors in recombinant DNA technology. (2 marks)

(ii) In most cases, the restriction enzyme used to cut the target gene from the human DNA should be the same as that used for cutting the plasmid. Explain the importance of this in the formation of the recombinant DNA. (2 marks)

(iii) Outline how the clotting protein is produced on a large scale after obtaining the recombinant DNA. (4 marks)

(iv) Traditionally, the clotting protein is obtained by extraction from donated blood. Give ***two*** advantages of using the clotting proteins produced by recombinant DNA technology over that obtained from the traditional source. (2 marks)

4.(b) One application of DNA fingerprinting is to identify the relationship among members in a family. The diagram below shows the DNA fingerprints of four members in a family, which consists of a couple and their two children. One of the children is the couple's biological child, while the other child is adopted.



(i) Outline the DNA fingerprinting technique and state how it can be used to identify the relationship among members in a family. (4 marks)

(ii) Based on the information above, deduce, with reasons,

(1) the relationship of R with P. (2 marks)

(2) which one is the adopted child. (2 marks)

(iii) Suggest ***one*** other application of DNA fingerprinting. (1 mark)

Solution a(i)(1); original allocation: 1 mark(s)

A plasmid is a small circular DNA molecule separate from the main bacterial chromosome. It is genetic material, not a protein, organelle or another bacterium. Many plasmids replicate independently of the main chromosome within the host and carry additional genes.

Method: Use vector structure to verify insertion, expression and phenotype in order.

a(i)(2); original allocation: 2 mark(s)

Plasmids are small and relatively readily taken up by bacteria. They can carry selectable markers such as antibiotic resistance, allowing identification of transformed cells.

After placing target DNA in a vector, it must enter the host and successfully transformed cells must be found. Small DNA is easier to handle and introduce; resistance markers allow transformants to survive selective culture conditions. These address delivery and identification respectively, rather than vaguely saying plasmids are convenient.

a(ii); original allocation: 2 mark(s)

The same restriction enzyme usually produces complementary sticky ends in the target fragment and plasmid, enabling pairing and recombinant-DNA formation.

Restriction enzymes recognise specific sequences and cut DNA. The same cutting pattern can provide compatible ends. Complementary bases first pair, bringing fragment and plasmid together; DNA ligase then joins the sugar–phosphate backbones. Restriction enzymes cut and ligase seals: do not reverse these roles.

a(iii); original allocation: 4 mark(s)

Introduce the recombinant plasmid into suitable host cells and select transformants using the resistance marker. Culture cells that express the target gene in large quantities, then extract, purify and test the clotting protein produced.

The plasmid-based scheme can be explained using bacteria for transformation, selection and large-scale multiplication. Antibiotic selection removes cells lacking the resistance marker, but selected cells must still be confirmed to contain the target gene and produce the required protein. Transcription and translation express the gene; protein collection and purification then yield the product. Do not inject living transgenic bacteria directly into a patient's blood. Practical production of functional clotting proteins also requires hosts capable of necessary protein processing and verification of activity. This simplified scheme does not imply that arbitrary bacteria can produce every human protein in functional form.

a(iv); original allocation: 2 mark(s)

The required protein can be produced more consistently at scale, reducing dependence on donated blood. It can also reduce introduction of blood-borne pathogens from human blood sources.

Cultured production cells allow output to expand with demand instead of repeatedly extracting protein from large donations. Avoiding donated plasma as the protein source removes part of the pathogen risk associated with human blood. Purification and quality testing remain necessary; recombinant products are not automatically risk-free or exempt from testing.

b(i); original allocation: 4 mark(s)

Extract each person's DNA, cut it into fragments with restriction enzymes, separate by size through gel electrophoresis and visualise comparable banding patterns. Each child's band should have a corresponding source in one parent, allowing assessment of parentage.

Band position reflects fragment size. Appropriate probes can reveal fragments containing selected variable sequences after separation, producing each fingerprint. Trace every child's band: some originate from the mother and others from the father. The child's entire lane need not match the father's, and equal total band counts do not establish parentage.

b(ii)(1); original allocation: 2 mark(s)

R is P's biological daughter; P and Q are her parents.

Every R band matches P or Q at the same height. For example, R's highest band matches Q and the next matches P; the rest are also explained by their combined patterns. R is labelled female, making her specific relationship to P daughter. A few shared P/R bands are insufficient; check every R band.

b(ii)(2); original allocation: 2 mark(s)

S is the adopted child.

Several S bands are absent from both P and Q. For example, S's highest band lies above the corresponding upper bands in both prospective parents. Their combined patterns cannot explain these bands. Under the stated condition of one biological and one adopted child, S is adopted.

b(iii); original allocation: 1 mark(s)

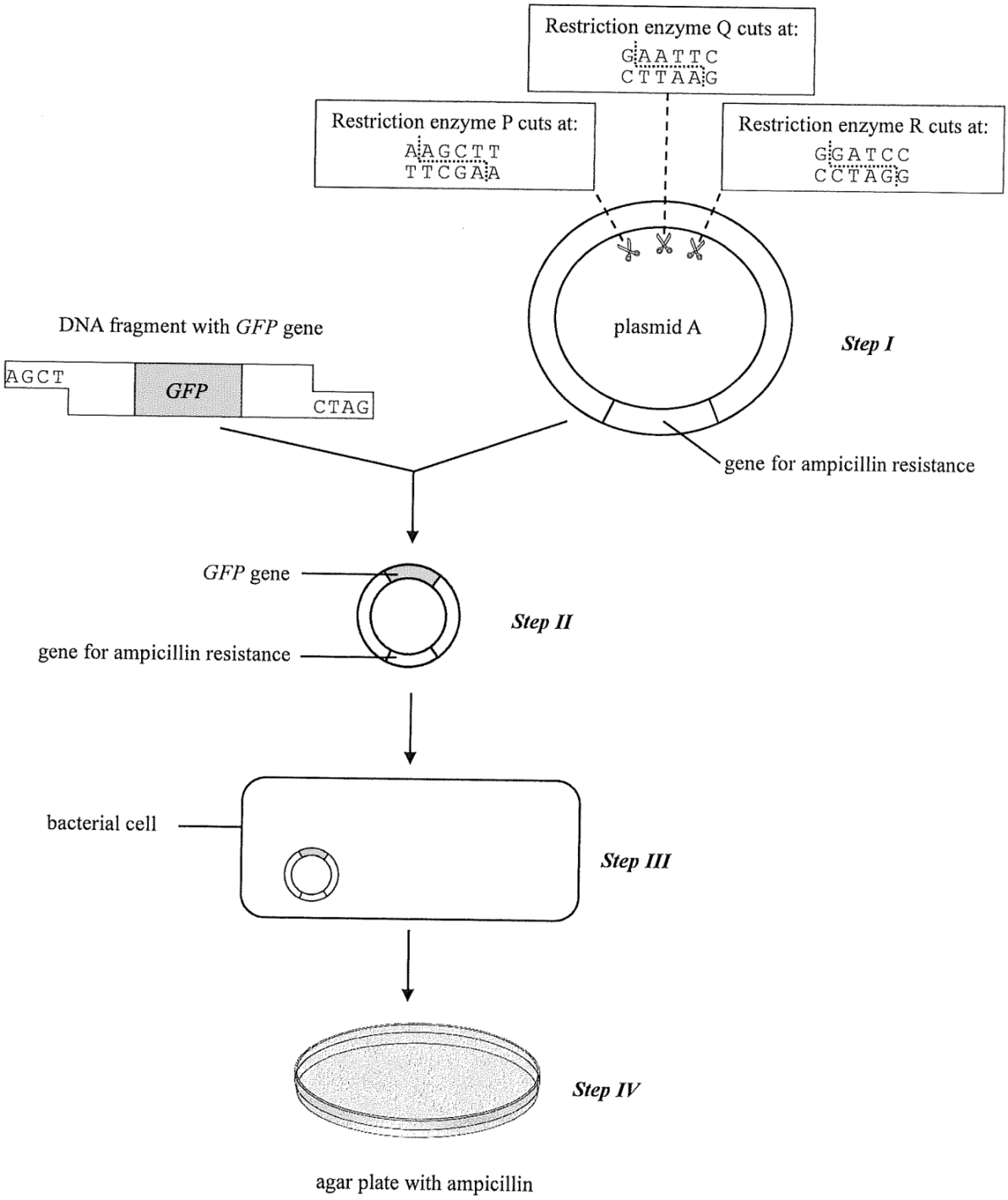
Compare DNA fingerprints from biological traces at a crime scene with suspects' fingerprints to assist identification.

For example, obtain a pattern from a bloodstain and compare it with candidate samples. This uses variation in DNA sequences between individuals, not visual examination of actual finger-ridge patterns.

Marking reference: HKDSE Biology Sample Paper Paper 2 Q4, official marking scheme, source page(s) 11.

Example 2 [HKDSE Biology 2023 Paper 2 Q4]

(a) *GFP* gene encodes a protein known as Green Fluorescent Protein (GFP) which naturally occurs in some jellyfish. This GFP emits green fluorescence when exposed to ultra-violet (UV) light. The diagram below shows the workflow of the insertion of the *GFP* gene into plasmid A and its transformation of bacterial cells:

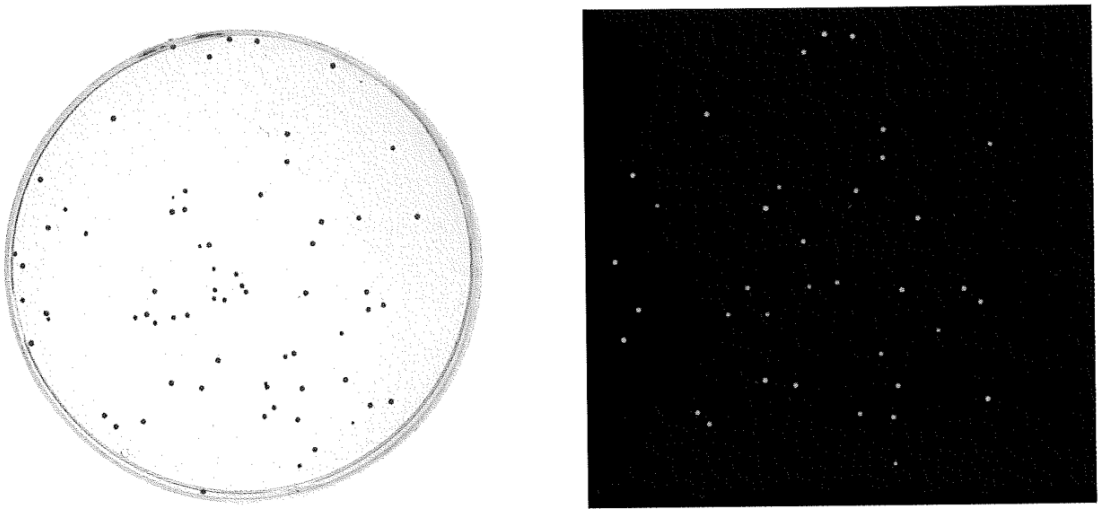


(i) State the restriction enzymes to be used for the insertion of the *GFP* gene into plasmid A. Explain your answer. (3 marks)

(ii) What is the purpose of Step IV? How can this step achieve its purpose? (2 marks)

(iii) The diagrams below show the appearance of bacterial colonies on the agar plate under normal light and UV light:

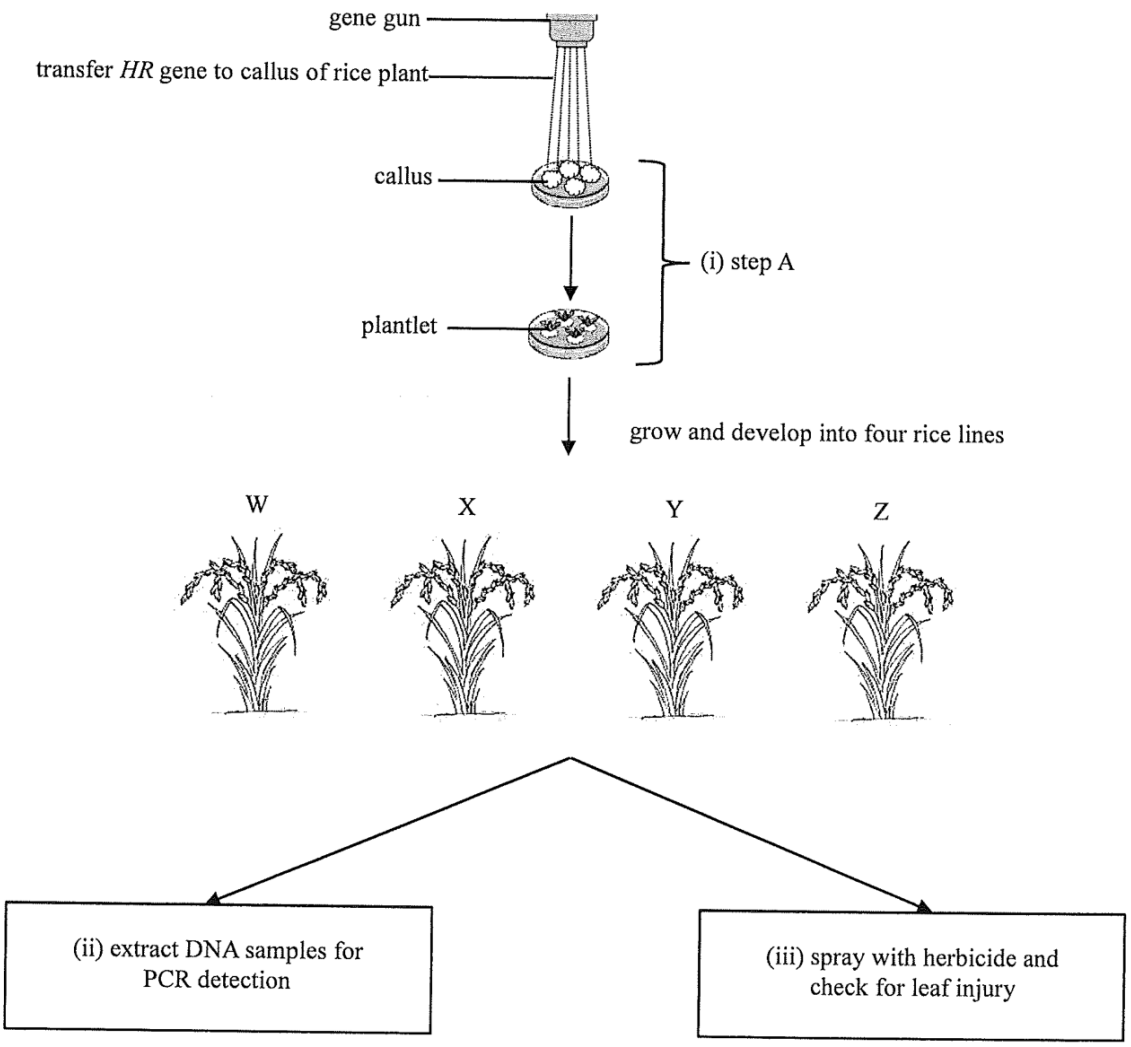
Under normal light ***Under UV light***



(1) Explain why only some bacterial colonies glow under UV light. (3 marks)

(2) Suggest ***one*** application of *GFP* gene. (1 mark)

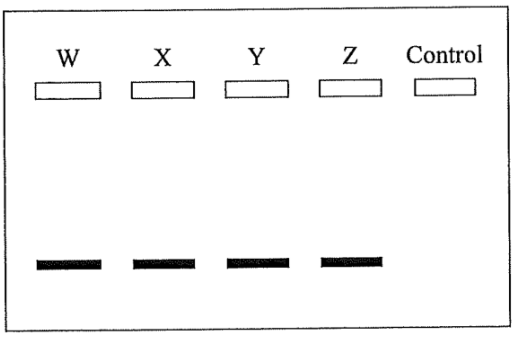
4(b) A company developed genetically modified (GM) rice plants with a herbicide resistant (*HR*) gene using the method shown below:



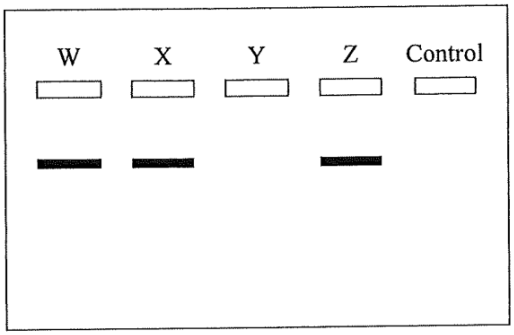
(i) State the technique used in step A. (1 mark)

(ii) PCR was performed on DNA samples of the rice lines W, X, Y and Z to detect the presence of the *ACTIN* gene which is found in all rice plants and the inserted *HR* gene. After completion, the PCR products were subject to gel electrophoresis. For the control, an equal volume of water was used in place of the DNA products. Results of the gel electrophoresis are shown below:

Gel I: PCR detection for *ACTIN* gene



Gel II: PCR detection for *HR* gene



(1) Determine which rice line samples contain the *HR* gene. (1 mark)

(2) If the DNA band in sample Y is absent in Gel I, how would you interpret the result for sample Y in Gel II? (2 marks)

(iii) Four groups (1 to 4) of rice plants were sprayed with a large amount of herbicide. Afterwards, the plants were grown under constant environmental conditions. After two weeks, the number of leaves with different degree of visible injury were recorded by scoring a total of 100 leaves in each group. A summary of herbicide induced injury in the GM rice lines is shown below.

Leaf area with visible injury (%)	Number of leaves			
	Group 1	Group 2	Group 3	Group 4
0	20	90	36	82
1 - 20	16	10	28	10
21 - 50	24	0	15	8
51 - 80	25	0	8	0
81 - 100	15	0	13	0

(1) Which group of rice plants shows the highest herbicide resistance? Explain your answer. (2 marks)

(2) Which rice line sample(s), W, X, Y or Z, is most likely to be represented by Group 1? Explain your answer. (3 marks)

(3) Despite the fact that there is more than one rice line with successful insertion of the *HR* gene, the results of herbicide induced injury still vary a lot. Suggest why this is so. (2 marks)

Solution a(i); original allocation: 3 mark(s)

Restriction enzymes P and R.

The insert has different sticky ends. P produces a plasmid end complementary to the insert's left AGCT end, while R produces the corresponding right-end match. Complementary bases pair, then ligase seals the backbone. Q's AATT end does not match the illustrated insert.

a(ii); original allocation: 2 mark(s)

Select bacteria carrying plasmid A. Its ampicillin-resistance gene allows plasmid-bearing bacteria to grow on ampicillin-containing plates.

Cells without the plasmid lack resistance and cannot form equivalent colonies. This selects the resistance marker but does not by itself establish that every plasmid contains GFP.

a(iii)(1); original allocation: 3 mark(s)

Not every resistance plasmid contains GFP. Only bacteria that successfully acquire and express GFP produce the protein that fluoresces under UV.

Method: Use vector structure to verify insertion, expression and phenotype in order.

Ampicillin selection permits growth of bacteria carrying resistance plasmids even if GFP insertion failed. Colonies containing and expressing GFP produce green fluorescent protein and become visible by fluorescence under UV, explaining different visible colony counts in the two photographs.

a(iii)(2); original allocation: 1 mark(s)

Use GFP as a reporter gene to indicate whether a gene is expressed in cells.

Linking GFP expression to the gene or regulatory region under study allows fluorescence to reveal which cells express it and where. Fluorescence provides a directly observable signal.

b(i); original allocation: 1 mark(s)

Plant tissue culture or micropropagation.

Small plant-tissue pieces divide and differentiate under suitable conditions, forming shoots, roots and plantlets before complete plants. This uses plant cells' potential to generate whole plants.

b(ii)(1); original allocation: 1 mark(s)

W, X and Z.

The HR PCR gel shows matching product bands in W, X and Z, but none in Y. The water negative control is also blank, supporting presence of the target HR sequence in those three samples.

b(ii)(2); original allocation: 2 mark(s)

If ACTIN also has no band, Y's negative HR result cannot establish absence of HR.

ACTIN is a positive internal reference expected in every rice sample. Its failure may indicate unsuccessful DNA extraction, insufficient rice DNA or PCR failure. The missing HR band could therefore be a false negative caused by the same technical problem.

b(iii)(1); original allocation: 2 mark(s)

Group 2: 90 leaves are undamaged, and only 10 have mild damage affecting 1–20%.

Each group contains 100 measured leaves, allowing direct comparison. Group 2 has the highest undamaged proportion and no leaves with more than 20% damage, indicating the least overall damage and greatest resistance.

b(iii)(2); original allocation: 3 mark(s)

Most likely Y.

Group 1 is most severely damaged: only 20 leaves are undamaged and 40 have 51–100% damage. With the original ACTIN control functioning normally, Y's absent HR band indicates lack of the resistance gene. It is therefore expected to have the lowest resistance, matching group 1.

b(iii)(3); original allocation: 2 mark(s)

Lines may contain different numbers of inserted HR copies or different insertion sites that alter expression.

PCR establishes sequence presence, not equal transcription or functional protein production. Gene dosage, insertion position or gene silencing may alter resistance-protein quantity, so HR-positive lines can still differ in damage.

Examiner's comment (source page(s) 12, 13)

Ampicillin selects resistance-plasmid carriers but does not guarantee GFP. The GFP protein fluoresces, not the gene itself. ACTIN checks sample and PCR function; if it fails, an HR-negative result cannot be accepted directly.

Marking reference: HKDSE Biology 2023 Paper 2 Q4, official marking scheme, source page(s) 14, 15.

VIII.b Applications of biotechnology

Read the explanations and diagrams in Concept VIII.b first, then work through the question types here. Each example retains the complete original question and explanation. Later exercises progressively integrate more information according to the demands of the whole question.

Type 1 Relate gene and cell therapies to effectiveness, inheritance and risk

Degree of integration ★★★★☆

Key ideas

① Adding a normal gene to a particular somatic cell type aims to make those cells produce the required product. It does not correct the DNA of every cell in the body or automatically pass the change to the next generation. Match stem cells' differentiation potential to the specific function required for treatment.

② Evaluate effectiveness, duration, immune responses, uncontrolled proliferation and environmental effects separately. Ethical judgement also considers beneficiaries, those bearing risks and acceptable alternatives.

How to recognise the problem Identify whether the intervention changes a gene, a cell type or a whole organism. Explain its physiological effect and limitations. Support ethical positions with specific affected groups and trade-offs.

Example 1 [HKDSE Biology 2015 Paper 2 Q4]

(a) Read the following passage and answer the questions that follow it.

'Bubble kid' success puts gene therapy back on track

When Nina was a 5-week-old baby, she had a condition called severe combined immunodeficiency (SCID) which is caused by a faulty *ADA* gene. This gene originally encodes the enzyme adenosine deaminase. The absence of this enzyme will lead to the accumulation of toxins in white blood cells and finally kill the cells. Affected kids have to live in a sterile environment and they are often called 'bubble kids'.

Today, Nina is a happy girl with a functioning immune system. She has gene therapy – and its latest improvements – to thank for this. In the therapy, cells were harvested from Nina's bone marrow and given a working version of the *ADA* gene, before being injected back into her bone marrow. After 5 months, her white blood cell count had nearly doubled, and today her immune system is fully functional.

(i) With reference to the roles of white blood cells, explain why children suffering from SCID have to live in a sterile environment. (2 marks)

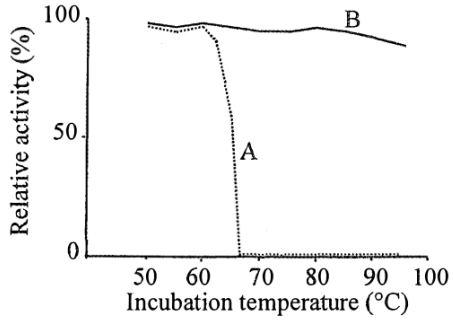
(ii) Why are cells from the bone marrow instead of white blood cells used in the gene therapy for SCID patients? (4 marks)

(iii) With reference to Nina's case, briefly describe how recombinant DNA technology is applied in gene therapy. (3 marks)

(iv) Suggest the potential hazards of gene therapy. (2 marks)

4(b) (i) Briefly describe the three major stages involved in a PCR cycle. (3 marks)

(ii) To test heat resistance of DNA polymerases A and B, the polymerases were incubated at different temperatures for 30 minutes. Their relative enzyme activities as compared to the maximum activity of the enzyme were measured. The results are shown in the graph below:



(1) Of the three stages mentioned in (i), which stage requires the action of DNA polymerase? (1 mark)

(2) Which DNA polymerase is more suitable for use in PCR? Explain your answer. (4 marks)

(iii) State ***one*** application of PCR. (1 mark)

Solution (a)(i); original allocation: 2 mark(s)

White blood cells recognise, resist and eliminate pathogens. Children with SCID lack normal defensive function and are more susceptible to infection, so environmental pathogen exposure must be reduced.

The stem states impaired white blood cells. Connect their missing immune function to infection risk rather than merely repeating the disease name.

Method: Identify the gene or cells changed, then discuss effects and risks.

(a)(ii); original allocation: 4 mark(s)

Bone-marrow haematopoietic stem cells continually divide and produce white blood cells. Introducing a functional gene into these stem cells can give their later white-cell descendants the function, producing longer-lasting effects. Mature white cells have limited lifespans, so directly correcting them loses effect as they die.

The key comparison is long-term self-renewal and production of descendants. Bone-marrow cells are not preferable because they lie deeper; stem-cell proliferation and differentiation sustain function.

(a)(iii); original allocation: 3 mark(s)

Place a normal ADA gene in a suitable vector and deliver it into the patient's bone-marrow cells. Integrate it or retain it as expressible DNA so the cells produce functional ADA enzyme, compensating for the original defect.

Trace gene delivery through to restored function. DNA entry alone is not the endpoint; expression must produce a functioning enzyme. The original marking scheme's approach inserts the functional gene into the patient's genome.

(a)(iv); original allocation: 2 mark(s)

Insertion may disrupt a normal gene or activate a nearby cancer-related gene. The patient may also mount an immune response to the vector or new product.

These arise at different stages: altered gene regulation after insertion versus the body's response to introduced materials or products. The therapeutic aim does not eliminate these possibilities.

(b)(i); original allocation: 3 mark(s)

Denaturation: heat separates double-stranded DNA. Annealing: cooling allows primers to pair with complementary sequences at the ends of the target. Extension: DNA polymerase adds complementary nucleotides from the primers to form new strands.

These form one cycle. Newly formed DNA becomes template in later cycles, allowing extensive amplification. Primers supply the starting points for extension.

(b)(ii)(1); original allocation: 1 mark(s)

The extension stage.

DNA polymerase joins free nucleotides to a growing new strand, so it acts directly during extension.

(b)(ii)(2); original allocation: 4 mark(s)

Choose DNA polymerase B. PCR repeatedly uses high temperatures to separate DNA strands. B retains high relative activity after high-temperature treatment, whereas A loses almost all activity above approximately 65°C. B can therefore continue catalysing extension over repeated cycles.

The curves measure residual activity after prior temperature treatment, so the issue is heat stability. Do not interpret every peak as an instantaneous optimum reaction temperature. Compare PCR's high-temperature requirement with both actual curves to justify B.

(b)(iii); original allocation: 1 mark(s)

Amplify small DNA samples for parentage testing, forensic analysis or detection of specific gene sequences; one application suffices.

PCR supplies more target DNA for subsequent analysis. It does not itself directly reveal a complete identity; it is the amplification step within a testing procedure.

Examiner's comment (source page(s) 5)

Long-term gene therapy requires cells that can continually generate functional descendants. PCR polymerase must withstand high-temperature denaturation in every cycle, not merely function at the extension temperature.

Marking reference: HKDSE Biology 2015 Paper 2 Q4, official marking scheme, source page(s) 10.

Example 2 [HKDSE Biology 2016 Paper 2 Q4]

(a) Parkinson's disease is a disorder of the central nervous system resulting from the degeneration of neurones and deficiency of a certain type of neurotransmitter in a particular area of the brain. Symptoms of this disease include tremor and muscular rigidity.

(i) With reference to the roles of neurones and neurotransmitters, suggest why patients with Parkinson's disease demonstrate the above symptoms. (2 marks)

(ii) In 1906, a drug was developed to relieve the symptoms of Parkinson's disease. This drug has a similar molecular structure to the neurotransmitter. Suggest how this drug works. (2 marks)

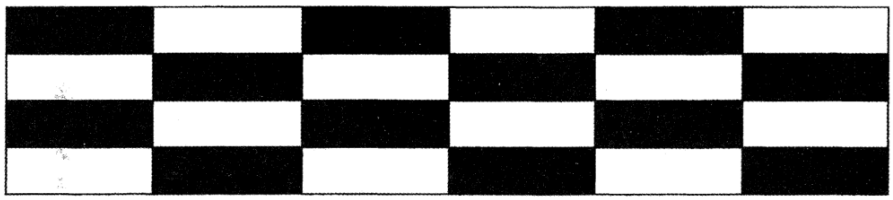
(iii) The benefits of this drug diminish after prolonged medication. In 2001, scientists attempted to use stem cell therapy to treat these patients by transplanting stem cells derived from aborted foetal tissues into their brains. Explain the principle behind this treatment. (2 marks)

(iv) A conceptual breakthrough came in 2007, when a team of biologists in Japan created stem cells from an adult's own tissue. They have 'reprogrammed' skin cells to a state like that of stem cells. Explain why this breakthrough will help overcome the problems associated with the use of stem cells from aborted foetuses for transplants. (3 marks)

4(b) *Bt* crops were one of the early transgenic plants produced to fight against pests. They contain a *Bt* gene that produces a chemical (*Bt* toxin) which is toxic to the larvae (caterpillars) of the pest.

(i) In the production of *Bt* crops, soil bacteria are used to infect and transform the original crops. Outline the steps that should be taken to modify the soil bacterium in preparation for the infection. (4 marks)

(ii) Many scientists are concerned that the overuse of *Bt* crops will produce pests resistant to the *Bt* toxin. *Bt* resistance is a result of a mutation in the pests. The mutated allele (b) is recessive to the wild type allele (B). After feeding on *Bt* crops, only the homozygous recessive (bb) caterpillars can survive. To minimise the emergence of *Bt* resistance, scientists divided farmland into small areas, some growing *Bt* crops and others growing normal crops, as shown in the diagram below:



Key: *Bt* areas growing *Bt* crops
Non- *Bt* areas growing normal crops

(1) It was found that the mutated allele is very rare in the pest population.

(I) Compare the population size of the adult pests found in the *Bt* areas and non-*Bt* areas. (1 mark)

(II) State the genotype(s) of the adult pests found in the *Bt* areas. (1 mark)

(III) State the genotype(s) of the adult pests that make up the largest proportion in the non-*Bt* areas. (1 mark)

(2) (I) Explain how this strategy (growing crops in the above pattern) works in minimising the emergence of *Bt* resistance in the offspring of adult pests. (3 marks)

(II) What is the assumption behind this strategy? (1 mark)

Solution (a)(i); original allocation: 2 mark(s)

Degeneration of specific neurones reduces the relevant neurotransmitter, impairing voluntary movement and muscular coordination and causing tremor, stiffness and related symptoms.

Method: Identify the gene or cells changed, then discuss effects and risks.

Connect the damaged cells to their signalling molecule and then neural-network function. "Brain damage" is too vague; neurotransmitter shortage is the key link supplied.

(a)(ii); original allocation: 2 mark(s)

A structurally similar drug can bind the corresponding receptors on the next neurone and mimic the neurotransmitter's action.

Receptors recognise molecular shape. A similar molecule that activates them triggers the appropriate postsynaptic response and partly compensates for the missing transmitter. The drug does not first become a neurone.

(a)(iii); original allocation: 2 mark(s)

Transplanted stem cells can differentiate into the required neurones and restore production of the relevant neurotransmitter.

Stem cells offer self-renewal and differentiation potential. If they form suitable functional nerve cells, they may replace the secretory capacity lost through degeneration. This is a treatment principle, not a guarantee that any transplantation succeeds.

(a)(iv); original allocation: 3 mark(s)

Using the patient's own cells lowers immune rejection. Skin cells are readily obtained and cultured, providing a more available source. The approach also reduces ethical concerns over fetal-tissue use.

The three aspects are compatibility, availability and ethics. Adult skin cells acquire differentiation potential through reprogramming; ordinary skin cells are not all naturally totipotent stem cells. Lower rejection does not remove every treatment risk.

(b)(i); original allocation: 4 mark(s)

Use restriction enzymes to obtain the Bt gene and open a plasmid. Join them with DNA ligase to form a recombinant plasmid, then introduce it into the soil bacterium.

Each step has a distinct role: restriction enzymes produce compatible ends, the plasmid carries the gene, ligase joins sugar–phosphate backbones, and the modified soil bacterium becomes the vector transferring the gene to plants. First modify the bacterium, then use it to transform plants; do not place the entire crop genome inside the bacterium.

(b)(ii)(1)(I); original allocation: 1 mark(s)

There are more adult pests in the non-Bt area.

Non-Bt plants lack the toxin, allowing sensitive pests to survive. In the Bt area, most sensitive larvae die and only a few resistant individuals reach adulthood.

(b)(ii)(1)(II); original allocation: 1 mark(s)

bb, the recessive homozygote.

The stem specifies b as the resistance mutation and resistance as recessive. Only bb therefore survives on Bt crops. Do not assume resistance is normally dominant.

(b)(ii)(1)(III); original allocation: 1 mark(s)

BB, the dominant homozygote.

b is rare in the original population, and non-Bt areas do not eliminate sensitive individuals, so most adults remain BB.

(b)(ii)(2)(I); original allocation: 3 mark(s)

The few surviving bb adults from Bt fields are more likely to mate with abundant BB adults from non-Bt fields, producing Bb offspring. Bb remains Bt-sensitive, reducing the proportion of resistant offspring.

The key cross is bb×BB → all Bb.

Refuges maintain many sensitive alleles, reducing the chance that resistant survivors mate with other bb individuals. This delays resistance accumulation; it does not eliminate every b allele.

(b)(ii)(2)(II); original allocation: 1 mark(s)

Adults from both areas must mix and mate, rather than Bt-field bb survivors mating only with one another.

If bb mates only with bb, all offspring remain resistant bb, and refuge BB cannot reduce their proportion. Cross-area mixing and suitable mating are therefore required.

Examiner's comment (source page(s) 5)

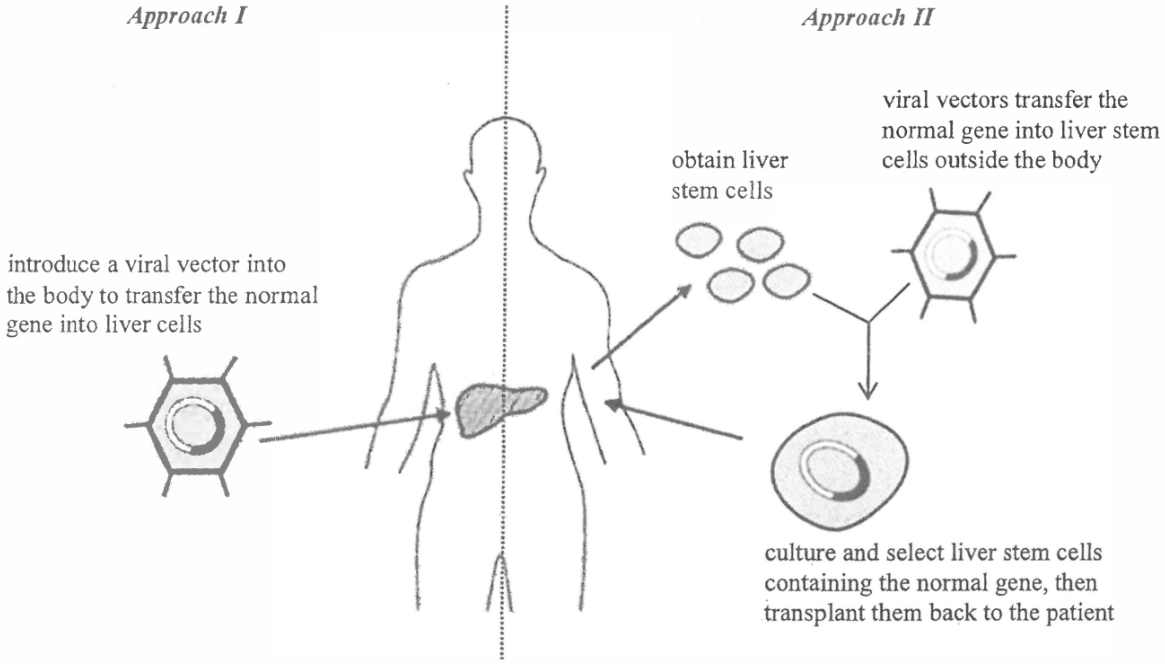
Distinguish receptors on brain neurones from neuromuscular junctions. Stem-cell properties arise after somatic-cell reprogramming; skin cells are not already pluripotent stem cells. Pest movement between fields affects changes in resistance alleles.

Marking reference: HKDSE Biology 2016 Paper 2 Q4, official marking scheme, source page(s) 11.

Example 3 [HKDSE Biology 2018 Paper 2 Q4]

(a) (i) John suffers from a genetic disorder that leads to difficulty in blood clotting. One way to treat John is infusing blood. Give ***two*** disadvantages of this treatment. (2 marks)

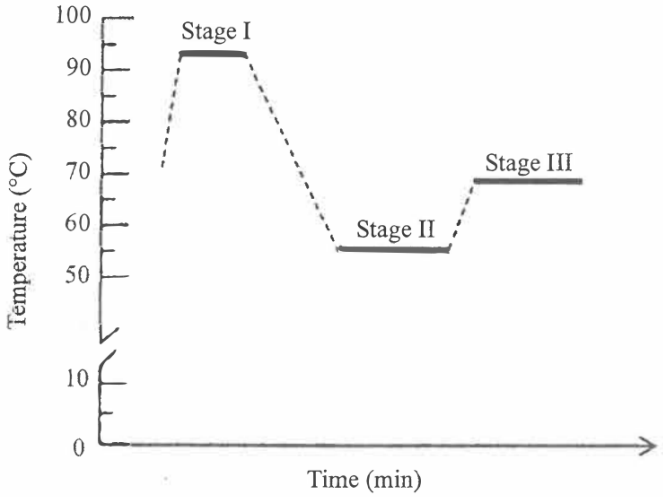
(ii) It was found that John's problem is caused by a defective gene that fails to produce a certain liver protein for blood clotting. Scientists have proposed gene therapy to treat this genetic disorder. The diagram below shows two possible gene therapy approaches:



(1) Explain why stem cells should be used for the gene therapy in Approach II. (2 marks)

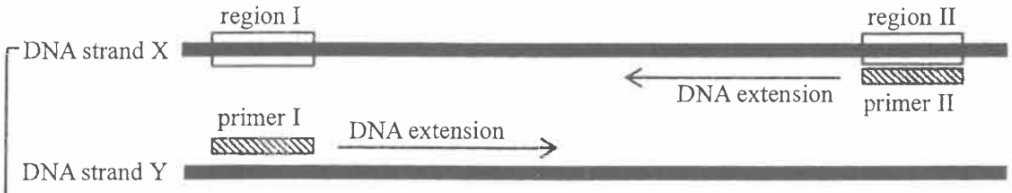
(2) With reference to the information in the diagram, explain why Approach II is a better option in terms of the potential hazards of gene therapy to John. (4 marks)

4(b) The diagram below shows the change in temperature during a polymerase chain reaction (PCR) cycle:



(i) Which stage corresponds to DNA denaturation? Explain your answer. (3 marks)

(ii) Mary planned to amplify a fragment of DNA using PCR. The following diagram shows the annealing of primers during PCR. The sequence of DNA strand X is shown below and the corresponding sequences of regions I and II are highlighted:



Sequence of DNA strand X:

					no. of bases
TGGCGCTGGG	CGCAATGCGC	GCCATTACCG	AGTCCGGGCT	GCGCGTTGGT	50
GCGGATATCT	CGGTAGTGGG	ATACGACGAT	ACCGAAGACA	GCTCATGTTA	100
TATCCCGCCG	TTAACCACCA	TCAAACAGGA	TTTTCGCCTG	CTGGGGCAAA	150
CCAGCGTGGA	CCGCTTGCTG	CAACTCTCTC	AGGGCCAGGC	GGTGAAGGGC	200
AATCAGCTGT	TGCCCGTCTC	ACTGGTGAAA	AGAAAACCA	CCCTGGCGCC	250
CAATACGCAA	ACCGCCTCTC	CCCGCGCGTT	GGCCGATTCA	TTAATGCAGC	300
TGGCACGACA	GGTTTCCCGA	CTGGAAAGCG	GGCAGTGAGC	GCAACGCAAT	350
TAATGTGAGT	TAGCTCACTC	ATTAGGCACC	CCAGGCTTTA	CACTTTATGC	400
TTCGGCTCG	TATGTTGTGT	GGAATTGTGA	GCGGATAACA	ATTTCACACA	450

region I

region II

Mary designed the following primers for the PCR:

Primer I: CGGUAGUGGG AUACGACGAU DNA extension →

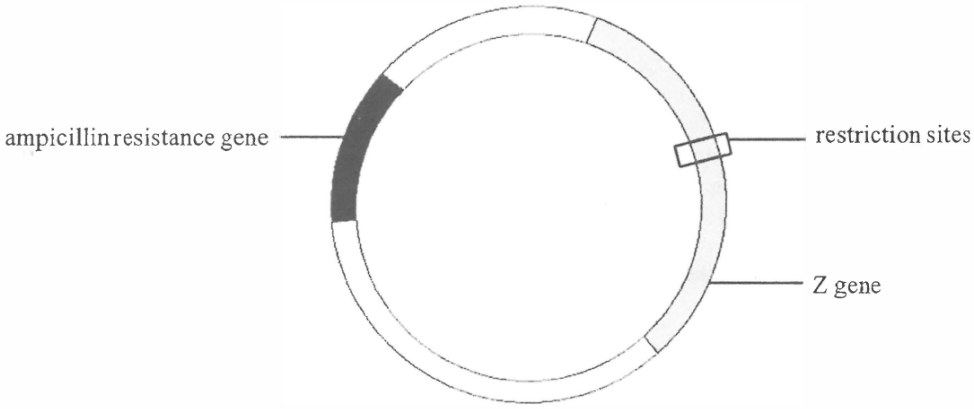
Primer II: CCTTAACACT CGCCTATTGT DNA extension →

(1) There is one type of mistake in each primer. Write the correct primers to be used. (2 marks)

(2) What is the predicted size (in terms of number of base pairs) of the PCR product? (1 mark)

(iii) Mary used the following plasmid as a vector to carry the PCR product to transform bacteria. The plasmid contained:

(I) an ampicillin resistance gene;
(II) a Z gene encoding an enzyme that converts substance X to blue compounds;
(III) some restriction sites within the Z gene.



After the transformation of the bacteria, Mary grew the bacteria on agar plates containing both ampicillin and substance X. Blue and white bacterial colonies were formed.

(1) What is the purpose of adding ampicillin to the agar plates? Explain your answer. (2 marks)

(2) Explain which type of colony (blue or white) contains non-recombinant plasmids, i.e. without DNA insert. (4 marks)

Solution a(i); original allocation: 2 mark(s)

Method: Identify the gene or cells changed, then discuss effects and risks.

Repeated transfusions are required regularly. Long-term repeated transfusion may cause iron accumulation and damage organs such as the liver.

Transfused normal blood components do not permanently correct the patient's own defective gene, so benefit is time-limited and treatment must recur. Repeated transfusions also add iron, whose accumulation can damage organs. Give limitations inherent to the treatment, not procedural errors such as transfusing the wrong blood group as though they were inevitable.

a(ii)(1); original allocation: 2 mark(s)

Liver stem cells can divide, self-renew and differentiate into normally functioning liver cells, forming a population able to produce the clotting protein.

Delivering a normal gene to one short-lived cell may not sustain treatment. Stem-cell division increases cell numbers; differentiation produces descendants capable of liver function. Expression in suitable liver cells can then maintain clotting-protein production. "Can divide" alone omits differentiation and protein function.

a(ii)(2); original allocation: 4 mark(s)

Method II transfers the gene using a viral vector outside the body, reducing risks of immune reactions from direct vector administration. During culture, transformed cells producing clotting protein without obvious abnormalities can be selected before return to the patient.

Compare the genuinely different steps. Method I delivers the viral vector directly; method II removes cells first and performs transfer, culture and selection externally, giving greater

control over returned cells. Checking expression and abnormalities can reduce risks of ineffective insertion, disruption of other genes or tumour formation. Method II still uses a virus, so it is not "completely virus-free". External processing and selection reduce some risks, not necessarily all.

b(i); original allocation: 3 mark(s)

Stage I. Its highest temperature breaks hydrogen bonds between complementary DNA strands, separating them.

PCR first separates template strands so primers can access their sequences in the next stage. I is each cycle's high-temperature stage and therefore corresponds to denaturation. Hydrogen bonds between base pairs break while the sugar–phosphate backbone remains intact. DNA is not cut into many small fragments, and protein-denaturation descriptions do not replace DNA-strand separation here.

b(ii)(1); original allocation: 2 mark(s)

Primer I: 5′-CGGTAGTGGGATACGACGAT-3′.

Primer II: 5′-TGTTATCCGCTCACAATTCC-3′.

The proposed primer I uses U, but PCR uses DNA primers, so those positions require T. Primer I pairs with strand Y and has the same sequence as positions 61–80 of X, with its 3′ end directed into the target region.

Primer II pairs with X positions 421–440, with its 3′ end directed leftwards into the target. Complement X's GGAATTGTGAGCGGATAACA, then read the primer in its own 5′→3′ direction to obtain TGTTATCCGCTCACAATTCC. The original proposed answer complements without reversing written direction. New strands extend only from each primer's 3′ end, so both extension arrows point towards the fragment centre.

b(ii)(2); original allocation: 1 mark(s)

380 base pairs.

The left endpoint includes position 61 corresponding to primer I, and the right includes position 440 corresponding to primer II.

Fragment length = 440 − 61 + 1 = 380 bp.

PCR products include both primer sequences, not only the intervening gap. Do not subtract the two primers' 20 bases each.

b(iii)(1); original allocation: 2 mark(s)

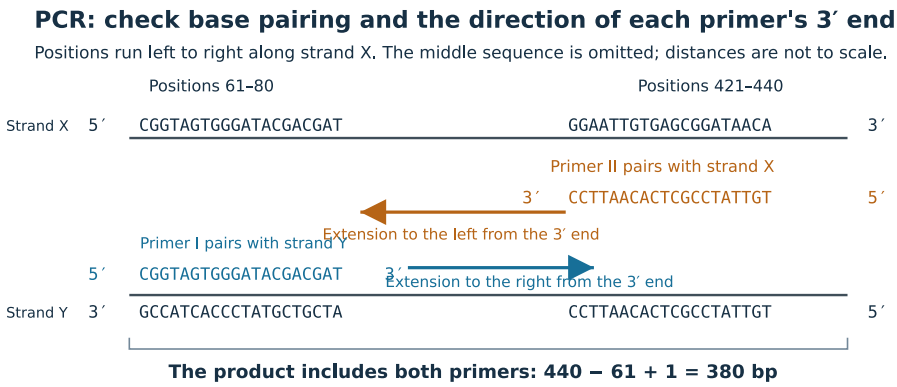
Select bacteria that have taken up plasmids. The plasmid's ampicillin-resistance gene allows transformed bacteria to survive on antibiotic-containing medium.

Bacteria without plasmids have not acquired resistance and are inhibited or killed by ampicillin. Plasmid uptake plus resistance-gene expression permits colony formation. This selects plasmid presence, not necessarily target-DNA insertion. Bacteria grow; free plasmids do not form colonies themselves.

b(iii)(2); original allocation: 4 mark(s)

Blue colonies. Plasmids without the DNA insert retain an intact Z gene, allowing bacteria to make functional enzyme that converts X into a blue compound.

Follow plasmid structure → gene expression → enzyme action → colour. Without insertion, intact Z produces enzyme that converts X, giving blue colonies. An insert within Z disrupts it, preventing normal enzyme production and giving white colonies. Both bacterial types can retain intact resistance genes and survive the plate, so blue–white colour distinguishes insertion after antibiotic selection.



Write each primer in its own 5′→3′ direction:
I CGGTAGTGGGATACGACGAT II TGTTATCCGCTCACAATTCC

Primers written in their own 5′→3′ directions, extending towards one another; positions 61–440 comprise 380 bp.

Marking reference: HKDSE Biology 2018 Paper 2 Q4, official marking scheme, source page(s) 9.

Practice VIII.a Modern biotechnology

Related type 1 Make testable deductions from DNA fragments and signals

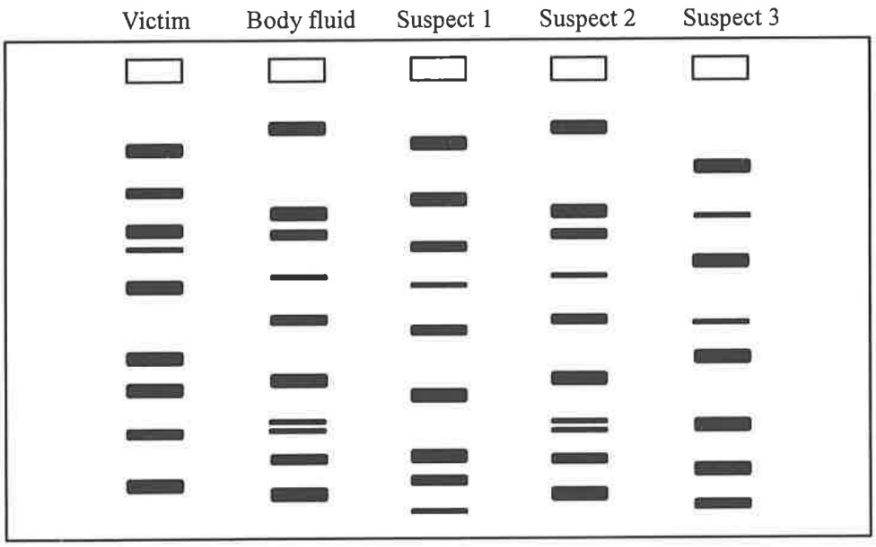
Degree of integration ★★★☆☆

Practice

1. [HKDSE Biology 2019 Paper 2 Q4]

Hint: List fragments and signals, then use controls to check the inference.

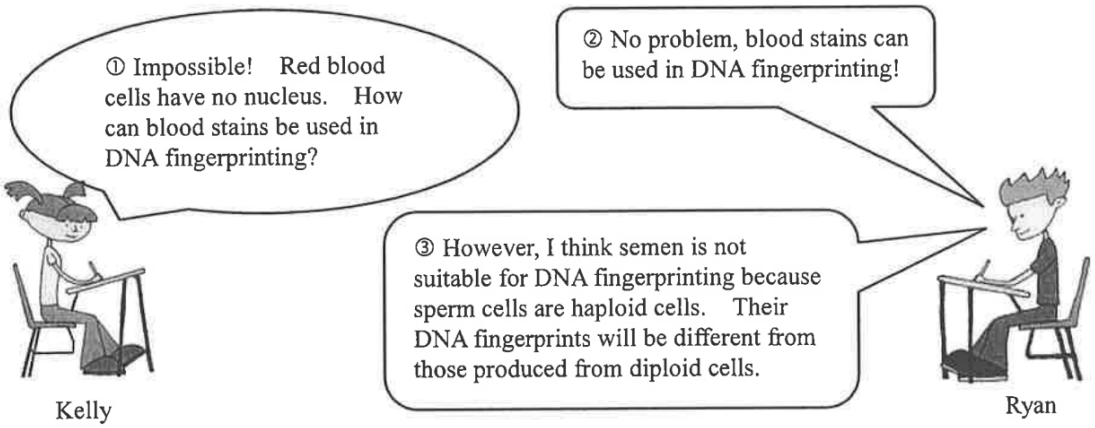
(a) Samples of body fluids or cells collected from crime scenes can be examined with VNTR-based DNA fingerprinting. The diagram below shows the DNA fingerprints of body fluid samples collected from a crime scene, and those of the victim and three suspects:



(i) Based on the above results, deduce who is most likely the criminal among the three suspects. (1 mark)

(ii) Explain why the DNA fingerprints of these suspects showed different patterns. (3 marks)

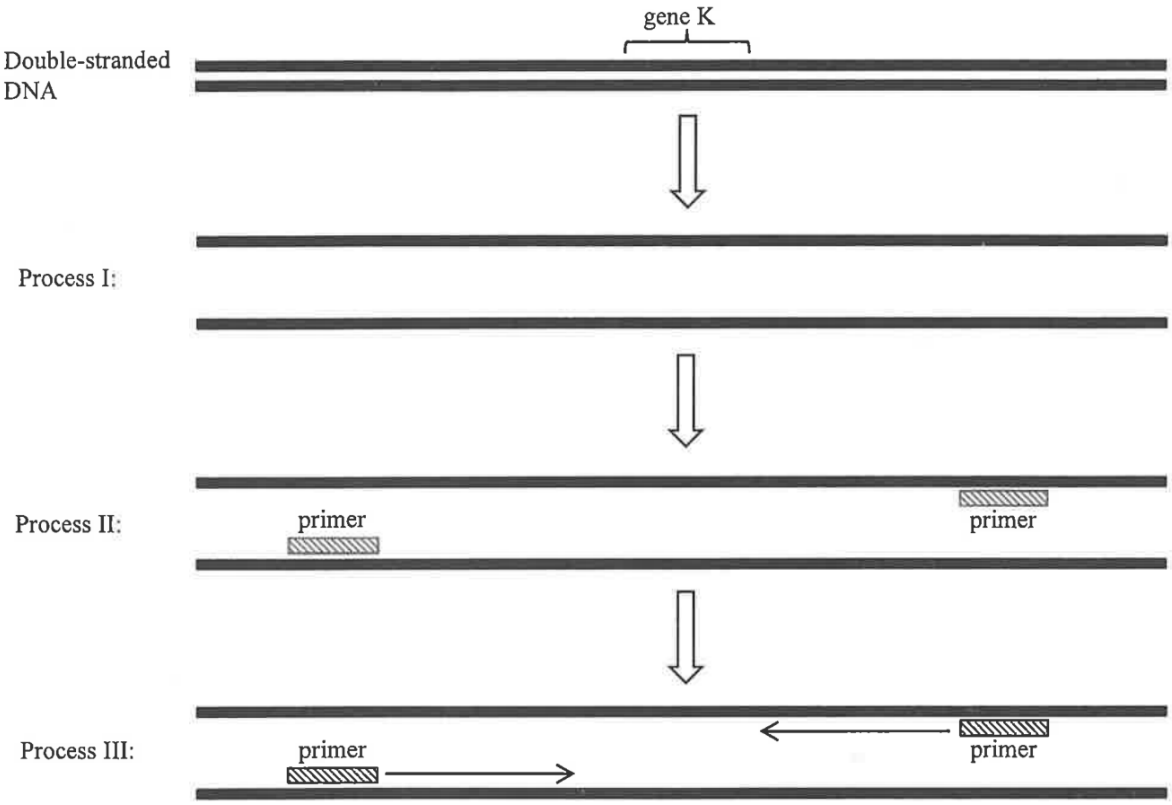
(iii) Semen and blood stains are body fluid samples which can be collected from crime scenes. Kelly and Ryan disagree about the suitability of using these body fluid samples for DNA fingerprinting. Below is their conversation:



(1) Is Kelly or Ryan right about whether blood stains can be used for DNA fingerprinting? Explain your answer. (2 marks)

(2) Do you agree with Ryan's comment about the suitability of using semen for DNA fingerprinting? Explain your answer. (4 marks)

4(b) A scientist discovered a bacterial species capable of secreting a protein. This protein is toxic to insects that damage the roots of a certain crop. The protein is encoded by gene K. The scientist used polymerase chain reaction (PCR) to amplify gene K. The diagram below shows some PCR processes:



(i) What is Process II? (1 mark)

(ii) Draw a simple labelled diagram to show the expected PCR product. (2 marks)

(iii) Primers often contain about 18–22 bases. If primers with fewer bases are used, PCR products of different sizes are obtained. Explain this phenomenon. (2 marks)

(iv) The scientist wanted to transfer gene K into the crop by using *Agrobacterium* (a soil bacterium). Explain why *Agrobacterium* is suitable for transferring the target gene into the crop. (2 marks)

(v) The cultivation of this transgenic crop was found to produce higher yields than its non-transgenic counterpart. Suggest an explanation for this. (3 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 1 mark(s)**

Suspect 2.

Compare every body-fluid DNA band with all three suspects. All body-fluid band positions match suspect 2. Check every position, not one shared band or overall appearance.

a(ii); original allocation: 3 mark(s)

Individuals differ in VNTR repeat number, producing DNA fragments of different lengths. Electrophoresis converts those length differences into bands at different positions.

A VNTR region can contain different numbers of tandem repeats, yielding different fragment lengths after appropriate processing. Shorter DNA generally migrates farther through the gel. Combining length patterns from several regions gives individual-identifying fingerprints. Individuals do not possess wholly different kinds of DNA molecules.

a(iii)(1); original allocation: 2 mark(s)

Ryan is correct: bloodstains can be used for DNA fingerprinting.

Mature red blood cells lack nuclei, but blood also contains white blood cells with nuclei and nuclear DNA. A property of red cells does not mean the whole blood sample lacks DNA.

a(iii)(2); original allocation: 4 mark(s)

Disagree with Ryan's claim that semen is unsuitable.

Each sperm is indeed haploid and carries one chromosome from each homologous pair. However, semen contains many sperm produced by different meiotic events. The mixed sample can contain both corresponding alleles and chromosome combinations from one individual, allowing reconstruction of the person's overall DNA fingerprint. The claim confuses a single sperm with a body-fluid sample containing many sperm.

b(i); original allocation: 1 mark(s)

Primer binding/annealing.

Process I separates DNA strands. In II, primers pair with complementary template sequences. Only in III does DNA polymerase extend from the primers. Thus II is not DNA replication or extension.

b(ii); original allocation: 2 mark(s)

Double-stranded DNA containing gene K forms, with the two primers at opposite ends on opposite strands, as shown.

The left primer on the lower template extends rightwards to form the upper new strand. The right primer on the upper template extends leftwards to form the lower new strand. After repeated PCR, abundant products have ends defined by the primers. Draw two complementary strands and correctly positioned primers at both ends, not a single strand.

b(iii); original allocation: 2 mark(s)

Shorter primers have fewer possible base combinations and are more likely to find complementary matches at non-target sites, producing non-specific products of different lengths.

A sequence of n bases has at most 4^n combinations. With smaller n, the same short sequence is more likely to occur elsewhere in DNA. Annealing at other sites changes extension distances, producing multiple sizes. Short primers do not merely shorten all normal products equally.

b(iv); original allocation: 2 mark(s)

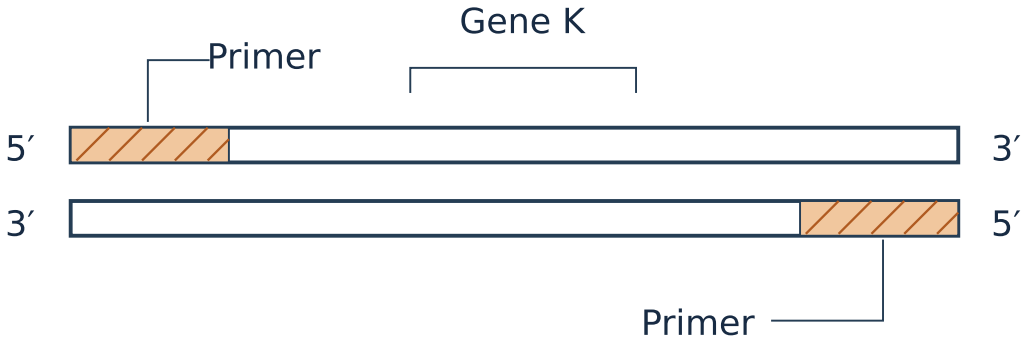
Agrobacterium has a plasmid into which gene K can be inserted and can transfer its DNA into plant cells/the plant genome.

The plasmid carries the target gene; interaction between Agrobacterium and plant cells supplies the transfer route. Together these make it a suitable vector. Rapid bacterial reproduction alone is not the explanation.

b(v); original allocation: 3 mark(s)

The protein expressed by K inhibits root-attacking insects, preserving a more intact root system. Plants absorb more water and minerals, grow better and store more material in fruits.

Follow gene → protein → protein action → organ function → yield. Reduced root damage may also improve anchorage and support. Do not jump from "foreign gene present" to inevitably higher yield; identify the protein's effect on root insects in this question.



Two complementary DNA strands; the primers lie at opposite ends.

Double strands and primer positions in the main PCR product, with gene K within the amplified region.

Examiner's comment (source page(s) 10, 11)

Explain how VNTR repeat counts produce fragment-length differences. One sperm is haploid, but many sperm in a mixed sample can represent both alleles of one individual. PCR diagrams need two strands and primers at opposite ends. Short primers cause non-specific annealing, not merely slightly shorter normal products.

Marking reference: HKDSE Biology 2019 Paper 2 Q4, official marking scheme, source page(s) 10.

2. [HKDSE Biology 2022 Paper 2 Q4]
← Hint: List fragments and signals, then use controls to check the inference.

(a) Sickle cell anemia is an autosomal genetic disorder caused by a recessive mutation in the gene coding for a polypeptide chain of haemoglobin. With this mutation, the seventh amino acid of the polypeptide is changed to another amino acid. Part of the DNA sequence of the mutated allele is shown below:

primer 1 →

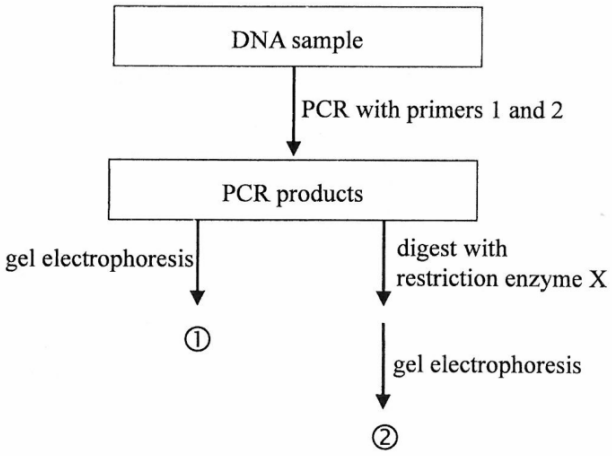
	number of bases
AGTCAGGGCA GAGCCATCTA TTGCTTACAT TTGCTTCTGA CACAACTGTG	50
TTCACTAGCA ACCTCAAACA GACACCATGG TGCATCTGAC TCCTGTGGAG	100
AAGTCTGCCG TTACTGCCCT GTGGGGCAAG GTGAACGTGG ATGAAGTTGG	150
TGGTGAGGCC CTGGGCAGGT TGGTATCAAG GTTACAAGAC AGGTTTAAGG	200
AGACCAATAG AAACTGGGCA TGTGGAGACA GAGAAGACTC TTGGGTTTCT	250
GATAGGCACT GACTCTCTCT GCCTATTGGT CTATTTTCCC ACCCTTAGGC	300
TGCTGGTGGT CTACCCTTGG ACCCAGAGGT TCTTTGAGTC CTTTGGGGAT	350
CTGTCCACTC CTGATGCTGT TATGGGCAAC CCTAAGGTGA AGGCTCATGG	400
CAAGAAAGTG CTCGGTGCCT TTAGTGATGG CCTGGCTC	438

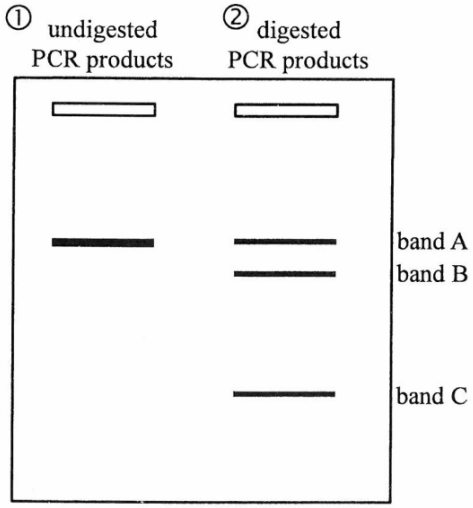
← primer 2

(i) The start of the polypeptide is encoded by genetic code ATG underlined in the above diagram. Write down the genetic code in which the mutation has occurred. (1 mark)

(ii) With reference to the above diagram, what is the expected size of the PCR product when primers 1 and 2 are used to amplify the mutated allele? Give the unit for the size of the PCR product. (2 marks)

(iii) It is known that the recognition site of restriction enzyme X is present in the normal allele but has been destroyed in the mutated allele. A DNA sample was obtained from an individual who is a carrier of sickle cell anemia for the following analysis:





(1) Explain why three DNA bands were detected in the gel electrophoresis of digested PCR products. (3 marks)

(2) Which band(s) correspond(s) to the mutated allele? Explain your answer. (3 marks)

(iv) Based on a DNA sequencing project, the table below shows the frequencies of this mutation in two human populations:

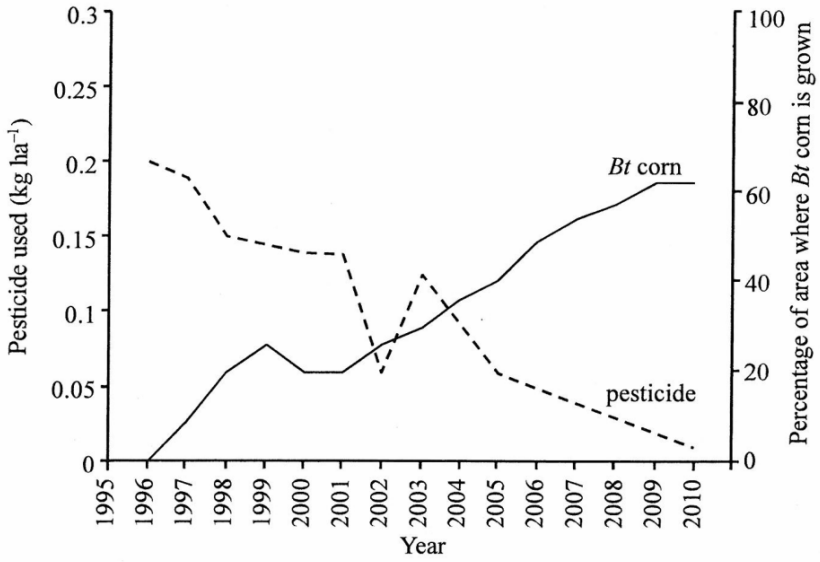
Population	Number of people	Number of mutations	Number of people who are homozygotes
African	12 482	1 121	4
East Asian	9 961	0	0

From which population was the DNA sample in (iii) most likely taken from? (1 mark)

4(b) *Bt* corn is a transgenic crop which contains a *Bt* gene obtained from a certain bacterium. This *Bt* gene can produce a protein which is toxic to certain insect pests.

(i) Agrobacterium transformation is commonly used in the production of transgenic plants. State ***two*** limitations of this method. (2 marks)

(ii) The graphs below show the adoption rate of *Bt* corn and the use of pesticides in the farms of the United States:

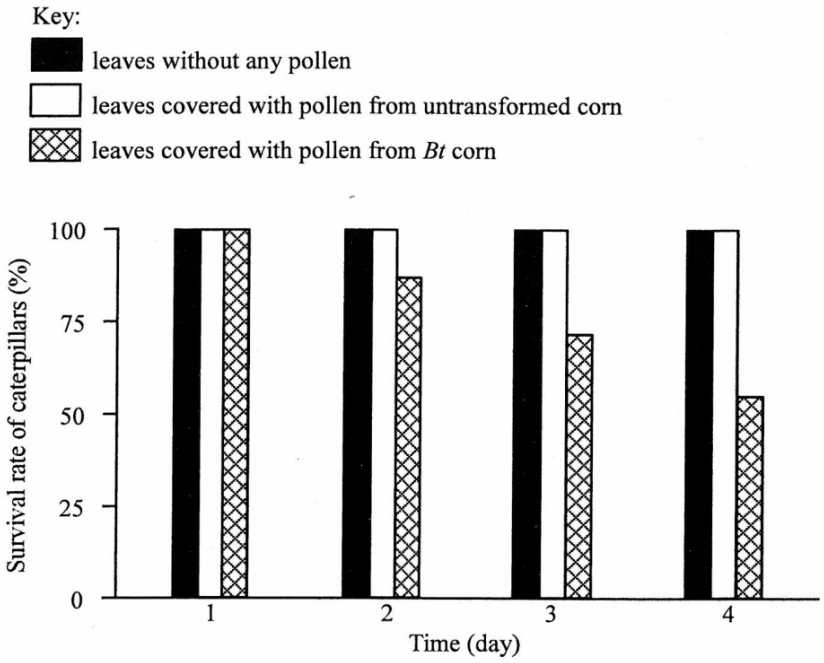


(1) Explain the relationship between the adoption rate of *Bt* corn and the use of pesticides. (3 marks)

(2) State ***one*** advantage for farmers on adopting *Bt* corn. (1 mark)

(iii) A rapid decline in the population of a butterfly species M was noted after *Bt* corn was introduced to the field. *Bt* corn was blamed for the decline.

(1) In a laboratory study, caterpillars of M were divided into three groups, each fed with a different type of leaves. The survival rates of the three groups of caterpillars are shown below:



Based on the results of this study, suggest a possible explanation for the decline of the population of M. (2 marks)

(2) Later, large-scale field studies were conducted by placing caterpillars of M at different distances away from the edge of *Bt* corn fields and untransformed corn fields. It was discovered that there was no significant difference in the survival rates of these caterpillars.

Scientists cast doubt on the validity of the laboratory findings in (1). Suggest ***two*** conditions in the fields which may affect the validity of the laboratory findings. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 1 mark(s)**

GTG.

Starting at the underlined ATG, read in triplets: ATG/GTG/CAT/CTG/ACT/CCT/GTG. The seventh is GTG. Do not restart grouping from the leftmost end of the entire DNA sequence.

a(ii); original allocation: 2 mark(s)

421 base pairs (bp).

The PCR fragment includes the entire primer-defined region. From base 11 to base 431 inclusive:

Length = 431−11+1 = 421.

PCR produces double-stranded DNA, so express length in base pairs, not merely bases.

a(iii)(1); original allocation: 3 mark(s)

A carrier is heterozygous. The normal-allele product is cut into two fragments, while the mutant-allele product remains uncut, giving three fragment lengths.

Somatic cells contain one normal and one mutant allele, both amplified by PCR. The normal fragment retains enzyme X's recognition site and becomes two shorter fragments. The mutation disrupts the site, leaving that fragment intact. Size separation therefore produces three bands.

a(iii)(2); original allocation: 3 mark(s)

Band A.

The mutant fragment is not cut by X and remains the longest, 421 bp. It moves slowest and stays nearest the well, at A. A also matches the undigested PCR product, providing independent control evidence. B and C are shorter products of normal-fragment digestion.

a(iv); original allocation: 1 mark(s)

The African population.

The table records 1121 mutations in the African sample and 0 in the East Asian sample. Given that the tested individual is a carrier, the data make origin from the African sample population where the mutation was observed more likely. This is a probabilistic inference from the stated frequencies.

b(i); original allocation: 2 mark(s)

Foreign-DNA insertion is relatively random and may disrupt host genes. Transformation success may also be low.

Agrobacterium-transferred DNA may not enter a predetermined site, and multiple copies may be inserted, affecting existing gene expression. Not all plant cells are infected and transformed efficiently, so successful transformants must be identified.

b(ii)(1); original allocation: 3 mark(s)

As the proportion of Bt maize planted increases, insecticide use generally decreases because the maize itself produces Bt protein that kills certain pests.

Bt-maize coverage rises from near zero to above approximately 60%, while insecticide use falls from approximately 0.2 kg/ha to near zero. The Bt gene produces a protein toxic to particular pests. Feeding pests are killed or inhibited, reducing farmers' need for insecticide.

b(ii)(2); original allocation: 1 mark(s)

Lower purchasing and application costs for insecticide.

Reduced use saves chemicals, application time and labour. Higher yield through reduced pest damage is another direct benefit.

b(iii)(1); original allocation: 2 mark(s)

Bt-maize pollen may land on leaves eaten by caterpillars. Its Bt protein can kill them and reduce species M's population. Survival remains near 100% on leaves without pollen or with ordinary-maize pollen, but falls to approximately 50% by day 4 with Bt pollen. This supports toxicity of a Bt-pollen component to these non-target caterpillars and a possible reduction in future adults.

b(iii)(2); original allocation: 2 mark(s)

Actual pollen quantities on field leaves may be lower. Rain or wind can remove deposited pollen.

Laboratory caterpillars may receive sustained, relatively high, fixed Bt-pollen exposure. In the field, pollen disperses with distance and is washed or blown away, potentially reducing ingested doses below those producing equivalent mortality. Alternative foods may also reduce exposure. Laboratory results therefore do not directly equal field-population outcomes.

Examiner's comment (source page(s) 11, 12)

PCR products are double-stranded DNA, measured in base pairs. Explain three bands through the carrier's two alleles and different digestion outcomes. Differences between Bt laboratory and field results require conditions that alter actual pollen exposure.

Marking reference: HKDSE Biology 2022 Paper 2 Q4, official marking scheme, source page(s) 12, 13.

Related type 2 Infer insertion, expression and selection from vector structure

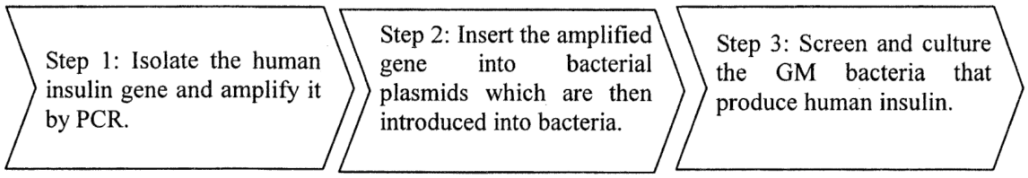
Degree of integration ★★★★☆

Practice

1. [HKDSE Biology 2013 Paper 2 Q4]

Hint: Use vector structure to verify insertion, expression and phenotype in order.

(a) In the past, diabetic patients were treated with insulin obtained from animal pancreases. With advances in recombinant DNA technology, insulin is now derived from genetically modified (GM) bacteria. Below shows a possible scheme for developing such a GM bacterium:



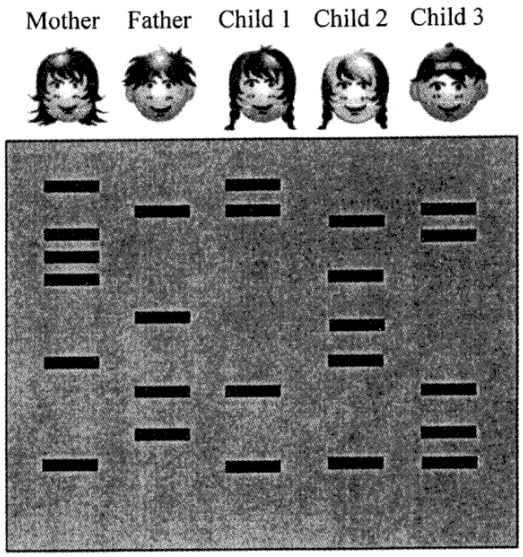
(i) Give ***two*** raw materials which are necessary in Step 1 for the amplification of the human insulin gene from a DNA template. (2 marks)

(ii) With reference to the immune response, explain why insulin from GM bacteria is used instead of that extracted from animal pancreas. (4 marks)

(iii) Explain why producing insulin from GM bacteria is cheaper than extracting it from animal pancreas. (2 marks)

(iv) To further cut the cost of insulin production, a GM crop which produces human insulin has recently been developed. However, an environmental group is worried that growing such GM crops may cause genetic pollution. Explain why growing the GM crops may cause genetic pollution. (2 marks)

4(b) The diagram below shows the DNA fingerprints of five members of a family, which consists of a couple and three children. One of the children is from the mother's previous marriage.



(i) Based on the information above, deduce which child is from the mother's previous marriage. (3 marks)

(ii) Although the other two children are the biological children of the parents shown, their DNA fingerprints display different patterns. Explain why this is so. (3 marks)

(iii) The different patterns shown in the DNA fingerprinting are due to the presence of variable number tandem repeats (VNTRs) on human chromosomes. VNTRs are short sequences of repeated DNA on the non-coding region of chromosomes and the number of VNTRs varies greatly from person to person. Explain why a large number of variations can exist in VNTRs but fewer variations are found in functional genes. (4 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 2 mark(s)**

Primers and the four deoxyribonucleoside triphosphates (dNTPs).

Primers provide starting points for DNA-polymerase extension, while dNTPs supply monomers for the new strand. The question asks for two required materials, not merely the catalytic enzyme.

a(ii); original allocation: 4 mark(s)

Insulin produced by genetically modified bacteria using the human insulin gene has the same amino-acid sequence as human insulin. It is less likely to be recognised as foreign and trigger antibodies. Animal insulin may differ in sequence, causing antibodies in some patients that remove or inactivate it and reduce effectiveness.

Connect sequence similarity to immune recognition and drug effect. "Human insulin is better suited to humans" is insufficient.

a(iii); original allocation: 2 mark(s)

Bacteria grow and reproduce rapidly, enabling sustained large-scale insulin production. Each animal pancreas supplies only a limited amount, and animal rearing is generally more costly.

Give two distinct causal cost explanations. Easier purification from suitable bacterial cultures is another option. Do not count "high yield makes it cheap" twice in different words.

a(iv); original allocation: 2 mark(s)

GM crops grown outdoors can release pollen to compatible non-GM plants. Following pollination and fertilisation, the introduced gene may pass through seeds into other plant populations.

Gene flow requires a viable reproductive route, not mere physical contact between plants.

b(i); original allocation: 3 mark(s)

Child 2. Some bands match the mother, but the remaining bands cannot be explained by the illustrated father's pattern, indicating that he is not the biological father.

The original stem describes a child from the mother's previous marriage, but (i) mistakenly says the father's. The official answer and diagram both support child 2 as the mother's child from her previous marriage. Check every remaining non-maternal band rather than merely counting shared paternal bands.

b(ii); original allocation: 3 mark(s)

The children arise from different combinations of gametes. Independent assortment of homologous chromosomes and possible crossing over during meiosis produce different parental-chromosome combinations in gametes. Random fertilisation recombines them, so children of the same parents have different DNA fingerprints.

Shared parents do not mean identical inherited halves of DNA. The sources are the same, but the combinations differ.

b(iii); original allocation: 4 mark(s)

The stated VNTRs lie in non-coding regions. Repeat-number changes are generally less likely to disrupt protein function directly and are therefore more likely to persist and be inherited, accumulating diversity across generations. In contrast, some functional-gene mutations disable proteins or their expression, impairing survival and reproduction, so natural selection more readily removes them.

Do not explain the difference solely by claiming VNTRs necessarily mutate faster. The focus is whether variants are retained by selection. Non-coding does not mean every sequence is functionless; this comparison uses the question's VNTR model.

Question wording and scope

The original (b) stem says "from the mother's previous marriage", whereas (b)(i) mistakenly says "father". Following the diagram and official marking reference, identify the child not biologically fathered by the illustrated father: child 2.

Examiner's comment (source page(s) 5)

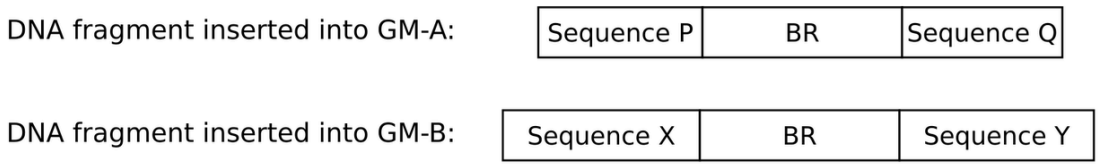
PCR materials are not the reusable enzyme. Explain insulin immune reactions through amino-acid sequence differences and genetic contamination through pollen transfer.

Marking reference: HKDSE Biology 2013 Paper 2 Q4, official marking scheme, source page(s) 10.

2. [HKDSE Biology 2025 Paper 2 Q4 (English translation)]

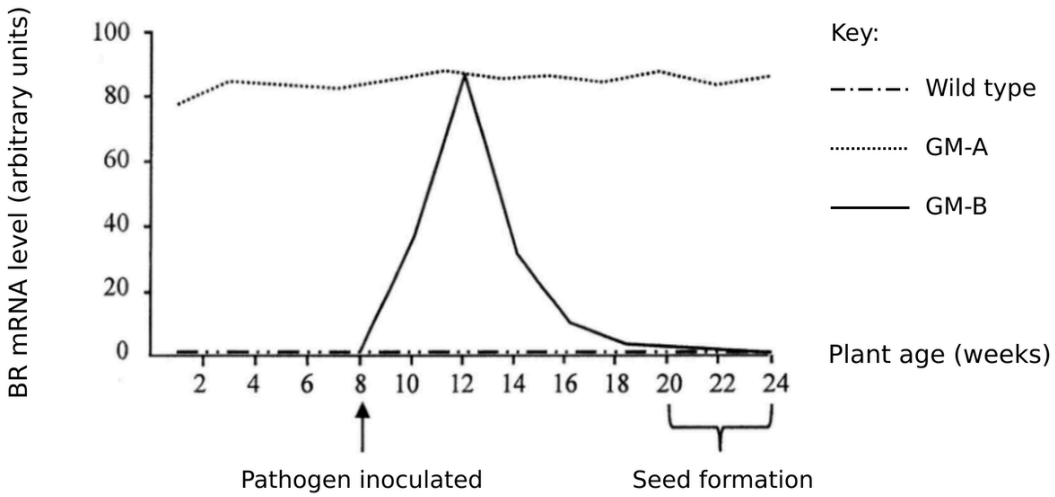
← *Hint: Use vector structure to verify insertion, expression and phenotype in order.*

(a) Pathogen Z poses a serious threat to a cereal crop grown on farmland. A research institute investigated different biotechnology strategies to improve the resistance of the crop to pathogen Z. A bacterial gene, BR, encoding a protein highly toxic to pathogen Z was isolated. The institute produced two types of genetically modified plants, GM-A and GM-B. Two DNA fragments of different lengths, each carrying the BR gene, were inserted into GM-A and GM-B respectively, as shown below:



(i) A student has mixed up the two types of GM plants and cannot distinguish between them. Briefly describe how polymerase chain reaction (PCR) can be used to identify a particular type of GM plant. (3 marks)

(ii) The amounts of BR mRNA in wild-type plants and GM plants were measured over a period of time. The plants were inoculated with pathogen Z at week 8. The graph below shows the results of the experiment:



In the above experiment, the seed yields before and after infection by pathogen Z, and the severity of disease after infection, were recorded as follows:

Plant type	Seed yield before infection (arbitrary units)	Seed yield after infection (arbitrary units)	Disease severity (percentage leaf damage)
Wild type	120	50	75
GM-A	60	60	5
GM-B	120	100	20

(1) In terms of energy expenditure, suggest one reason for the difference in seed yield between GM-A and GM-B. (2 marks)

(2) With reference to the graph and table above, explain the difference in disease severity between GM-A and GM-B. (5 marks)

(b) Gene therapy can treat, or potentially cure, certain diseases.

(i) Suggest one approach by which gene therapy can be used to treat a disease. (1 mark)

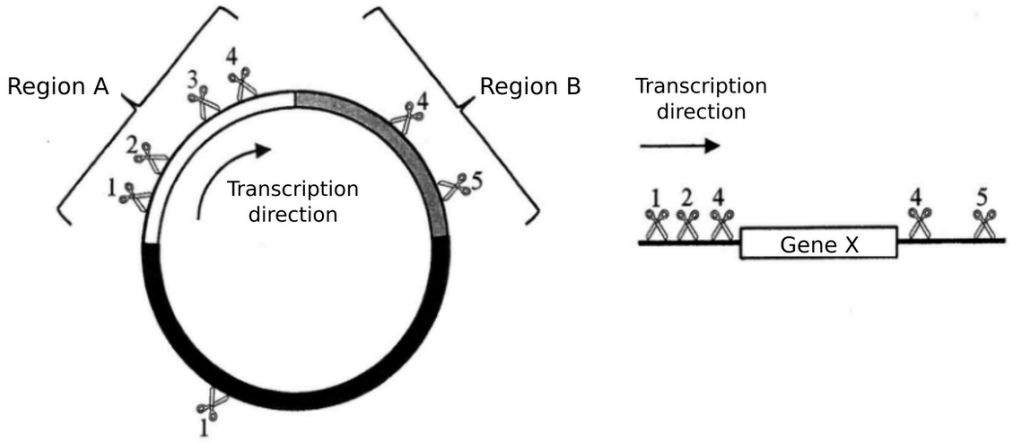
(ii) A lack of certain proteins can cause different diseases. Three such diseases are shown below:

Disease	Type of protein lacking	Number of amino acids making up the protein
P	I	533
Q	II	230
R	III	2479

(1) Based on the numbers of amino acids in these proteins, calculate the numbers of nitrogenous bases in the mRNA molecules encoding these proteins. (2 marks)

(2) A genetically modified virus has been constructed to deliver single-stranded DNA into target cells. This virus can carry only DNA that is less than 4700 bases long. Which disease(s) in the table above could be treated by gene therapy using this genetically modified virus? Explain your answer. (3 marks)

(iii) In another study, a target gene X and a plasmid are to be joined to produce a recombinant plasmid. The diagram below shows a plasmid and a DNA fragment carrying gene X. The numbers indicate the recognition sites of the corresponding restriction enzymes (1–5), which produce sticky ends:



To produce the desired recombinant plasmid, the beginning and end of gene X must be joined to any part of regions A and B of the plasmid respectively.

(1) Which restriction enzymes should be used to cut the plasmid and the DNA fragment carrying gene X to obtain the highest yield of the desired recombinant plasmid? (1 mark)

(2) Explain why the other restriction enzymes cannot be used to prepare the desired recombinant plasmid. (3 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **(a)(i); original allocation: 3 mark(s)**

Analyse DNA from each plant using specific primers recognising the flanking P/Q or X/Y sequences, obtain the corresponding PCR products and compare gel bands. GM-A's corresponding fragment is shorter in the original diagram, so its band should migrate farther than GM-B's.

BR is shared by both, so BR presence alone cannot distinguish the plants. Flanking sequences and fragment lengths supply discrimination. Connect the DNA analysed, primer specificity and observable band difference.

(a)(ii)(1); original allocation: 2 mark(s)

GM-A expresses BR strongly throughout the observation period, using more energy. GM-B mainly increases BR expression after stimulation, using less energy overall and leaving more for seed production, so seed yield is higher.

The question specifies an energy explanation. The table measures seed yield, not seed size or germination rate. Compare the costs of continuous versus induced expression, then connect them to reproductive output.

(a)(ii)(2); original allocation: 5 mark(s)

GM-A already has high BR mRNA before week 8 and maintains it throughout, providing sufficient protective BR protein earlier. GM-B's BR mRNA rises only after week 8, requiring time to produce sufficient protein. Different protection-onset times explain only 5% leaf damage in GM-A compared with 20% in GM-B.

Compare mRNA level, expression timing, timing of sufficient protein and disease damage in order. mRNA is not itself the protective protein; translation connects them. Greater seed yield and lower disease damage are different measures, so GM-B's higher yield does not imply less leaf damage.

(b)(i); original allocation: 1 mark(s)

For example, provide a normal gene to compensate for deficient function caused by a missing or defective gene.

This is one gene-therapy principle. The question asks for a therapeutic approach, not mutation types such as substitution, insertion or deletion.

(b)(ii)(1); original allocation: 2 mark(s)

Bases directly encoding amino acids:

I = 533×3 = 1599.

II = 230×3 = 690.
III = 2479×3 = 7437.
Including one stop codon as in this paper's marking table gives 1602, 693 and 7440 respectively.
Each amino acid corresponds to a three-base codon; a stop codon does not encode an amino acid. Real mRNA can also contain untranslated regions, so protein amino-acid count alone cannot determine total transcript length. These are the original question's simplified calculations. The marking table and examiner's report disagree over inclusion of the stop codon; see the data note.

(b)(ii)(2); original allocation: 3 mark(s)

Diseases P and Q. Under the simplified correspondence, the required single-stranded DNA length matches the corresponding mRNA coding length. I and II are below 4700 bases, while III exceeds the limit.
The question asks about diseases P, Q and R, not species. Using 1599, 690 and 7437 without the stop codon, or adding 3 as in the marking table, gives the same conclusion. The length limit excludes III; it provides no basis for arbitrarily excluding either I or II.

(b)(iii)(1); original allocation: 1 mark(s)

Restriction enzymes 2 and 5.
According to the original schematic's positions and orientation, the fragment's start must connect towards region A and its end towards region B. Pairing two different endpoints ensures the required directional connection.

(b)(iii)(2); original allocation: 3 mark(s)

Enzyme 1 also has a plasmid site outside region A, potentially allowing insertion at an inappropriate position. Enzyme 3 has no corresponding site on the illustrated target fragment. Enzyme 4 produces the same type of end at A, B and both fragment ends, allowing reverse insertion.
Identify the three distinct diagram-based problems: unsuitable location, no corresponding site, and uncontrolled orientation. Enzyme 3 does not cut gene X in the diagram, and enzyme 4 does not necessarily disrupt the gene. Vague "mismatching" cannot replace specific interpretation.

Data note

The supplied sources contain a contradiction. The marking table on page 65 of the 2025 compilation first gives 1599, 690 and 7437 without a stop codon, then awards another mark for 1602, 693 and 7440 after adding 3. However, the examiner's report on pages 77–78 describes including a stop codon as an error. This answer distinguishes the quantities counted and retains both numerical steps from the table without claiming that the sources agree. The discrepancy does not affect the subsequent choice of diseases P and Q.

Examiner's comment (source page(s) 77, 78)

Expression has an energy cost; compare seed yield separately from the timing of protection. Read each DNA cutting site, region and orientation. Producing a fragment does not ensure that every ligation has the required orientation.

Marking reference: HKDSE Biology 2025 Paper 2 Q4, official marking scheme, source page(s) 65, 66.

3. [HKDSE Biology 2017 Paper 2 Q4]
Hint: Use vector structure to verify insertion, expression and phenotype in order.

(a) A genetically modified (GM) salmon was approved for consumption in the United States in 2015. The flowchart below shows some simplified steps involved in the production of the GM salmon:

Step 1	Preparation of recombinant DNA with a growth hormone gene from salmon species A
↓	
Step 2	Microinjection of recombinant DNA into fertilised eggs of salmon species B
↓	
Step 3	Selection of homozygous transgenic salmon for breeding over several generations
↓	
Step 4	Collection of diploid fertilised eggs from the selected transgenic salmon
↓	
Step 5	Production of triploid fertilised eggs by certain treatments
↓	
Step 6	Culturing of sterile GM salmon developed from triploid fertilised eggs in isolated tanks

(i) What is the advantage of culturing this type of GM salmon over non-GM salmon? Explain your answer. (2 marks)

(ii) Microinjection method is used in step 2.

(1) Give ***one*** advantage of using the microinjection method instead of viral vectors for producing the GM salmon. (1 mark)

(2) Give ***one*** disadvantage of using the microinjection method. (1 mark)

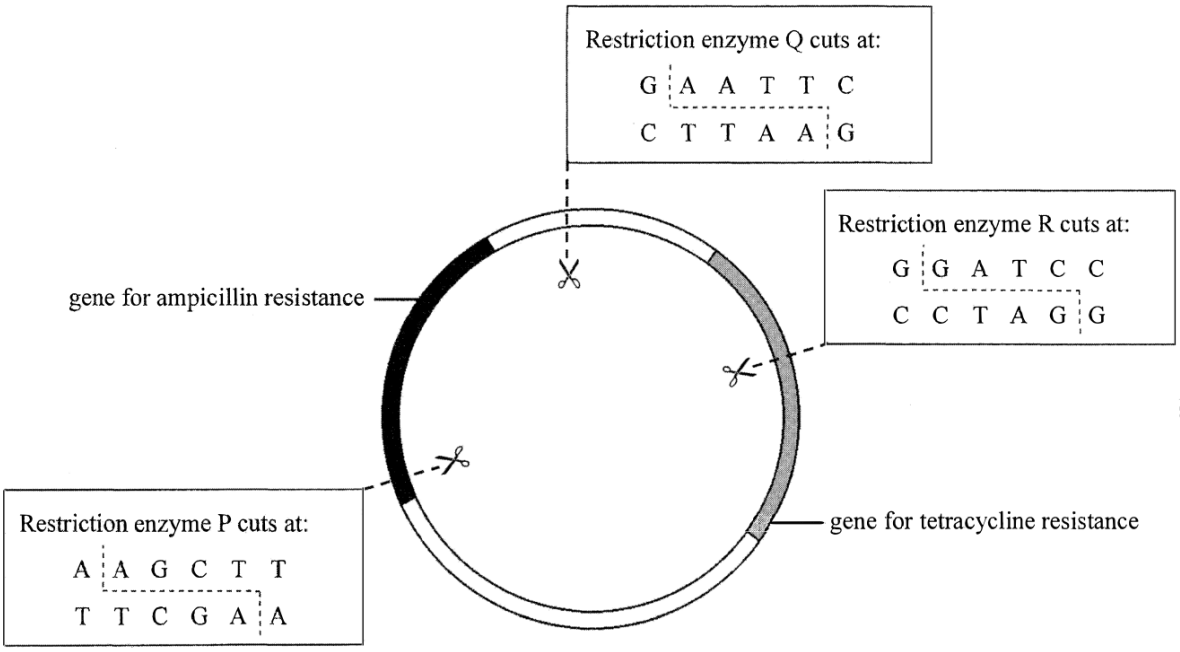
(iii) With reference to step 3, suggest ***two*** reasons why the selection was made over several generations rather than one generation. (2 marks)

(iv) In step 5, the triploid fertilised eggs produced contain three sets of chromosomes.

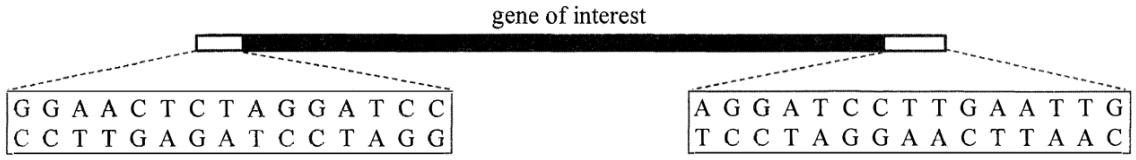
(1) With reference to the process of gamete formation, explain why the GM salmon developed from triploid eggs are sterile. (2 marks)

(2) Explain why this can act as a safety precaution for protection of wild lifes. (2 marks)

4(b) The diagram below shows a plasmid with two antibiotic resistance genes and three cut sites for restriction enzymes P, Q and R:



A student wanted to insert the following DNA fragment into this plasmid which was then used to transform bacteria. Only part of the nucleotide sequence of the two ends of the DNA fragment is shown:



(i) Based on the information above, choose one restriction enzyme to cut both the plasmid and the DNA fragment so that they can be successfully recombined together. Explain your answer. (4 marks)

(ii) After transformation, the bacteria were transferred to an agar plate containing ampicillin for selection. Explain the importance of this step. (3 marks)

(iii) For the bacterial colonies formed on the agar plate containing ampicillin, some of them have tetracycline resistance while some do not. Explain this phenomenon. (3 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **(a)(i); original allocation: 2 mark(s)**

Adding an expressible growth-hormone gene can promote faster growth, allowing fish to reach a larger size sooner.

Link gene function to the farming outcome: altered growth-hormone production changes growth rate. Not every foreign gene enlarges organisms; identify the growth-hormone-related gene.

(a)(ii)(1); original allocation: 1 mark(s)

No viral vector is required, avoiding introduction of its associated viral components.

Microinjection places recombinant DNA directly in the fertilised egg, bypassing viral-vector components or infection-related issues. This advantage does not establish absence of every other risk in the product.

(a)(ii)(2); original allocation: 1 mark(s)

Integration success may be low, and handling may damage the fertilised egg.

One disadvantage suffices. Working individually on each egg can also be time-consuming. Do not repeat absence of a virus as a disadvantage.

(a)(iii); original allocation: 2 mark(s)

Confirm stable inheritance of the transgene and continued normal expression of the desired trait over several generations.

Detecting DNA in the first generation does not establish entry into heritable cells or long-term function. Continued breeding and selection test both inheritance and expression stability and may help establish homozygous lines.

(a)(iv)(1); original allocation: 2 mark(s)

Three homologous chromosome sets cannot readily pair and segregate evenly during meiosis, making functional gametes with balanced chromosome complements difficult to form and causing sterility.

Diploid homologues pair one-to-one. The odd number of sets in triploids causes pairing and segregation problems. The issue is gamete formation, not an inability of every triploid somatic cell to undergo mitosis.

(a)(iv)(2); original allocation: 2 mark(s)

Even if fish escape, impaired reproduction with wild salmon reduces transgene flow into wild populations.

Link sterility to gene flow: few functional gametes → difficulty producing viable offspring → reduced reproductive spread of the foreign gene. This targets inheritance-mediated spread and does not alone exclude all ecological competition effects.

(b)(i); original allocation: 4 mark(s)

Choose restriction enzyme R. Both target-DNA ends contain its recognition sequence GGATCC, and cutting the plasmid and fragment with R produces complementary sticky ends.

Both target flanks must be released and vector ends compatible. R has one plasmid site and a site at each fragment end. The resulting single-stranded overhangs pair through complementary bases and facilitate joining. Q having a plasmid site alone is insufficient; check both target ends.

(b)(ii); original allocation: 3 mark(s)

Ampicillin selects bacteria that acquired plasmids. Their resistance gene allows growth on the antibiotic plate; cells lacking plasmids cannot grow.

This selects plasmid presence. Because the target insert does not disrupt ampicillin resistance, both empty-vector and recombinant-vector bacteria may survive. This step alone cannot establish successful target-gene insertion in every colony.

(b)(iii); original allocation: 3 mark(s)

Successful insertion disrupts the tetracycline-resistance gene and removes resistance. Empty or self-ligated plasmids without an insert can retain it.

First use ampicillin to select vector-bearing cells, then tetracycline resistance to distinguish insertional inactivation. Both types grow on the first plate. Mixed tetracycline-resistant and sensitive colonies therefore reflect the two plasmid outcomes.

Examiner's comment (source page(s) 6)

GM salmon have an additional growth-hormone-gene copy; ordinary salmon do not completely lack that gene. Ampicillin resistance only supports possible plasmid presence. Identify recombinants through insertional disruption of the other resistance gene.

Marking reference: HKDSE Biology 2017 Paper 2 Q4, official marking scheme, source page(s) 11.

Practice VIII.b Applications of biotechnology

Related type 1 Relate gene and cell therapies to effectiveness, inheritance and risk

Degree of integration ★★★★☆

Practice

1. [HKDSE Biology 2014 Paper 2 Q4]

Hint: Identify the gene or cells changed, then discuss effects and risks.

(a) In the past, animals with certain desirable traits were selected to breed for several generations and the offspring produced would be domesticated. This technique is known as selective breeding. Nowadays, animals with desirable traits can be produced by animal cloning or transgenic technology.

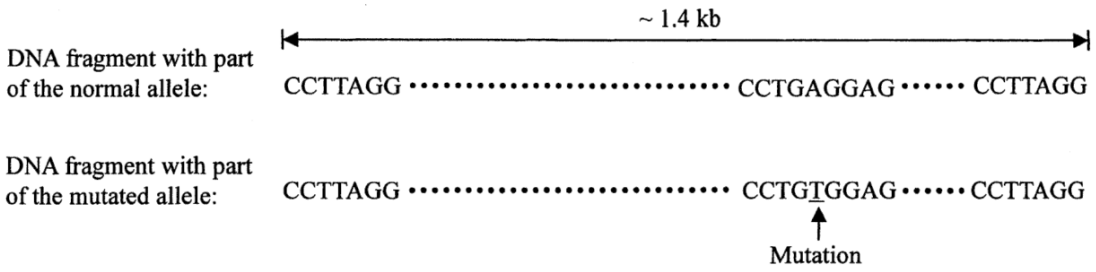
(i) With reference to the principles involved in selective breeding and animal cloning, explain why animal cloning is a better method of preserving the desirable traits of an animal than selective breeding. (5 marks)

(ii) Both selective breeding and transgenic technology affect the gene pool of a species. Transgenic technology is more controversial than selective breeding.

(1) How would selective breeding affect the gene pool of a species? (2 marks)

(2) Production of transgenic organisms is more controversial due to its effect on the gene pool of a species. Explain why. (3 marks)

4(b) DNA fingerprinting is used in the screening of a genetic disease known as sickle-cell anaemia. The disease is a result of a gene mutation which leads to the production of defective haemoglobin. To prepare the DNA fingerprint, copies of DNA fragments containing the gene associated with sickle cell anaemia are first produced by a polymerase chain reaction (PCR). The fragments are then treated by a restriction enzyme which cuts DNA at the middle of CCTNAGG, where N can be any nucleotide. The diagram below shows some nucleotide sequences of the DNA fragment containing the normal allele and the mutated allele for sickle-cell anaemia:



(i) How many restriction sites are found in the DNA fragment with the normal allele and that with the mutated allele respectively? (1 mark)

(ii) Based on the principle of gel electrophoresis, explain how the cutting of the two DNA fragments shown above would produce different DNA fingerprint patterns in a gel. (4 marks)

(iii) How many DNA bands would be observed in the DNA fingerprint of a carrier of sickle-cell anaemia? Explain your answer. (2 marks)

(iv) Explain why the gene mutation will result in the production of defective haemoglobin. (3 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation a(i); original allocation: 5 mark(s)

Selective breeding involves sexual reproduction and gamete fusion. Offspring vary genetically, so desirable allele combinations may separate or recombine with other traits and may not fully retain the donor's desired characteristics. Cloning develops mainly through mitotic divisions from the selected donor cell. Offspring have essentially the same nuclear genetic information as the nuclear donor and therefore retain its genetic combination more reliably.

Compare reproductive principle, variation and trait retention. Environment still affects phenotype, so identical genes do not guarantee identical traits under every condition.

a(ii)(1); original allocation: 2 mark(s)

Allowing only individuals with desired traits to reproduce increases relevant allele frequencies. Alleles in unselected individuals are transmitted less often and some may disappear from the gene pool.

Gene-pool change concerns population alleles and their frequencies; it does not instantly delete genes within each unselected individual.

a(ii)(2); original allocation: 3 mark(s)

Transgenic techniques can introduce genes previously absent from a species, including genes from another species, into its gene pool. If the trait spreads through reproduction or gene flow, it may alter ecological interactions. Uncertain long-term effects generate greater controversy.

Explain the new gene source, addition to the gene pool and possible consequences. Transgenesis does not automatically create a new species, and ecological harm should not be asserted as inevitable without evidence.

b(i); original allocation: 1 mark(s)

Normal fragment: 3 restriction sites. Mutant fragment: 2.

The recognition sequence is CCTNAGG, where N is any base. CCTTAGG at both ends matches. Normal central CCTGAGG also matches, but mutant CCTGGAG does not, eliminating the central site.

b(ii); original allocation: 4 mark(s)

The normal fragment is cut centrally, producing two shorter principal fragments. The mutant lacks that site and produces one longer principal fragment. Negatively charged DNA moves towards the positive electrode during gel electrophoresis. Shorter fragments pass through the gel faster and farther, giving different band positions.

Use the shared outer cutting sites as the studied fragment's boundaries; do not add unknown flanking fragments beyond the diagram. Identify digestion products before explaining separation through charge and size.

b(iii); original allocation: 2 mark(s)

Three bands. A carrier is heterozygous, possessing normal and mutant alleles, so both normal short bands and the mutant long band appear.

Two alleles do not mechanically mean two bands. Band number depends on each allele's digestion products.

b(iv); original allocation: 3 mark(s)

A single-base change can alter an mRNA codon and replace an amino acid in a haemoglobin polypeptide. Altered amino-acid interactions and protein conformation/polymerisation properties then produce abnormal haemoglobin function.

Follow DNA sequence → amino acid → protein properties. Not every single-base change alters a protein, but this sickle-cell mutation has functional consequences.

Question wording and scope

Both Chinese Paper 2 scans omit the whole final question 4. This item uses physical page 7 of the same year's English Paper 2; the Chinese official marking reference is on physical page 10. Question outline: (a)(i) compare selective breeding and cloning and explain why cloning better preserves desired traits; (ii)(1) explain gene-pool changes through selective breeding; (2) explain greater controversy over transgenic effects on gene pools. (b) The diagram shows normal and sickle-cell-anaemia mutant alleles; the restriction enzyme recognises CCTNAGG. (i) Count sites; (ii) explain band differences using electrophoresis; (iii) identify the carrier's band count and explain; (iv) explain production of abnormal haemoglobin by the mutation.

Examiner's comment (source page(s) 5)

Discuss alleles and their frequencies for gene-pool questions. Before reciting electrophoresis principles, identify two short normal fragments, one long mutant fragment and both patterns in carriers. The common small outer fragments are very short and are excluded from the principal bands in this question.

Marking reference: HKDSE Biology 2014 Paper 2 Q4, official marking scheme, source page(s) 10.

2. [HKDSE Biology 2021 Paper 2 Q4]
Hint: Identify the gene or cells changed, then discuss effects and risks.

(a) X-linked severe combined immunodeficiency (X-SCID) is a recessive genetic disorder which is due to a defective gene located on the X chromosome. X-SCID infants fail to produce T-lymphocytes. As a result, infections become life-threatening to X-SCID infants. In recent years, a new gene therapy has been developed for treating X-SCID patients. A simplified flowchart of the gene therapy is shown below:

(1) Extract bone marrow cells of the X-SCID infant with the defective gene

↓

(2) Insert a corrected version of the gene into the extracted bone marrow cells

↓

(3) Transplant bone marrow cells with the corrected gene into the X-SCID infant

↓

(4) Recovered X-SCID infant produces T-lymphocytes

(i) In gene therapy, a specific type of cells will be selected depending on the defective gene to be corrected. In the above case, bone marrow cells fulfill the criteria for the gene therapy. State ***two*** general criteria for selecting a suitable type of cells in gene therapy. (2 marks)

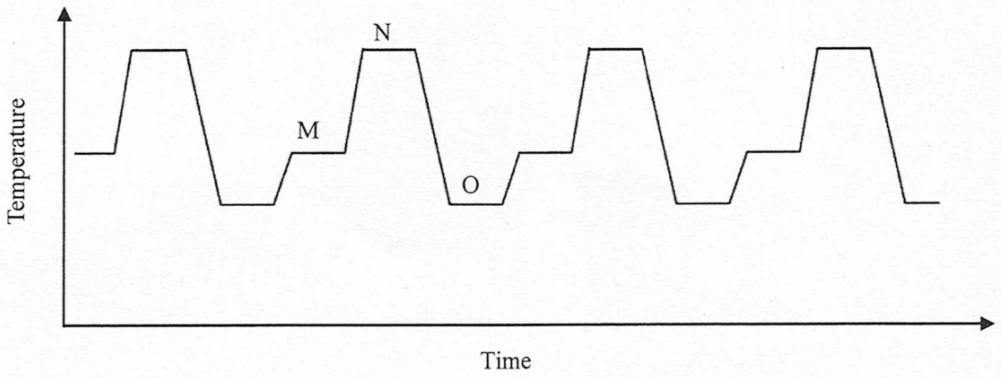
(ii) Suggest a suitable method for inserting the corrected version of the gene into the extracted bone marrow cells. State one advantage and one disadvantage of this method. (3 marks)

(iii) An X-SCID boy has recovered after receiving the gene therapy. If, after 20 years, he marries a healthy woman, will he pass the defective gene to his offspring in the future? Explain your answer. (3 marks)

(iv) Both gene therapy and production of transgenic animals involve recombinant DNA technology. Discuss why the gene therapy shown above is generally less controversial than the production of transgenic animals. (2 marks)

4(b) In the production of genetically modified organisms (GMO), antibiotic resistance genes are often used for the screening of successful transformation. A student collected some food products and wanted to determine if these food products are made from GMO. With primers corresponding to these antibiotic resistance genes, the student performed a polymerase chain reaction (PCR) on the food samples to check for the presence of the genes.

(i) The graph below shows the change in temperature during PCR cycles:



(1) Which stage (M, N or O) represents annealing? With reference to the events in a PCR cycle, explain your answer. (3 marks)

(2) Draw a simple labelled diagram to show what happens in the annealing stage. (2 marks)

(ii) The diagram below shows part of the DNA sequence of an antibiotic resistance gene X. The sequences shown are the corresponding regions for primer annealing:

Direction of primer extension →

... GGATCAGCTG ACTCGCCTGG ..... CACGCGGAGG AGCGTGCGCG ...

600 base pairs

Which of the following primers (I, II, III or IV) should be used for amplifying gene X? (2 marks)

Primer I: GGTCCGCTCA GTCGACTAGG
Primer II: CCTAGTCGAC TGAGCGGACC
Primer III: GTGCGCCTCC TCGCACGCGC
Primer IV: GCGCGTGCGA GGAGGCGCAC

(iii) Describe how you can use the principle of gel electrophoresis to confirm if the PCR product is gene X. (3 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 2 mark(s)**

Choose stem cells that continually produce new cells, whose descendants can express the corrected gene.

Modifying only short-lived, non-renewing cells gives limited persistence. Bone-marrow stem cells produce later blood cells. If descendants retain and express the normal gene, they can continuously generate normally functioning T lymphocytes.

a(ii); original allocation: 3 mark(s)

A viral vector can be used. Its advantage is delivery of the gene into the relevant host cells; disadvantages include residual-virus or immune-reaction risks.

The vector supplies a delivery tool, but successful transfer does not mean zero risk. Other reasonable methods are acceptable when accompanied by one method-specific advantage and disadvantage.

a(iii); original allocation: 3 mark(s)

The original defective gene can still pass to daughters, but this X-linked defect is not passed to sons through the father's Y chromosome.

Treatment changes bone-marrow somatic cells, not the genetic composition of germ cells. The male patient's defective X can still enter sperm. Daughters receive his X, while sons receive his Y. Improved health does not establish corrected germ cells.

a(iv); original allocation: 2 mark(s)

The normal gene is human-derived rather than transferred across species, and somatic-cell modification does not directly create a heritable new trait.

These features reduce concerns about crossing species boundaries or permanently changing descendants' inheritance. In contrast, transgenic animals released into nature may interact with ecosystems. Compare the stated somatic therapy; do not generalise that every gene therapy lacks ethical issues.

b(i)(1); original allocation: 3 mark(s)

O is the primer-annealing stage.

The preceding high-temperature N stage breaks interstrand hydrogen bonds and separates DNA into single strands. Cooling to O then allows short primers to bind complementary sequences. M's intermediate temperature corresponds to polymerase extension. The hottest stage is not the most favourable for primer binding.

b(i)(2); original allocation: 2 mark(s)

The two DNA strands are separated. Primers bind complementarily at opposite ends of opposite templates, with their extendable ends directed into the target.

Clearly show both single strands and both primer positions. Primers provide synthesis starting points. Inward extension from both ends defines and amplifies the target over repeated cycles.

b(ii); original allocation: 2 mark(s)

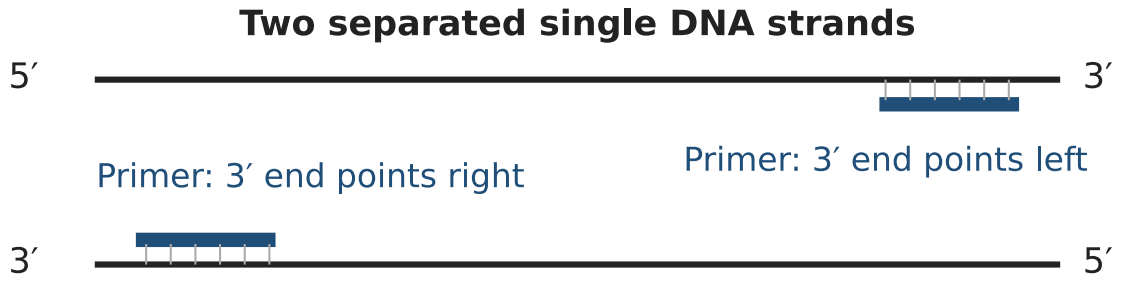
Primers II and IV.

Check complementarity at the left end first: II fits. The other primer must bind the right end of the opposite strand and extend inward, requiring IV. Literal complementarity at the right end alone ignores the strands' opposite directions.

b(iii); original allocation: 3 mark(s)

Separate PCR products by gel electrophoresis and compare them with known-length DNA markers for a band near 600 base pairs.

Negatively charged DNA moves towards the positive electrode. Shorter fragments pass through the gel more readily and migrate faster and farther. Comparing product position with known-length markers tests whether it matches gene X's stated 600 bp size. Equal length alone does not prove identical complete sequences, but it meets this question's size-verification requirement.



Each primer binds its complementary template, allowing DNA polymerase to extend into the target region.

Completed answer diagram for the conditions in this question.

Examiner's comment (source page(s) 10, 11)

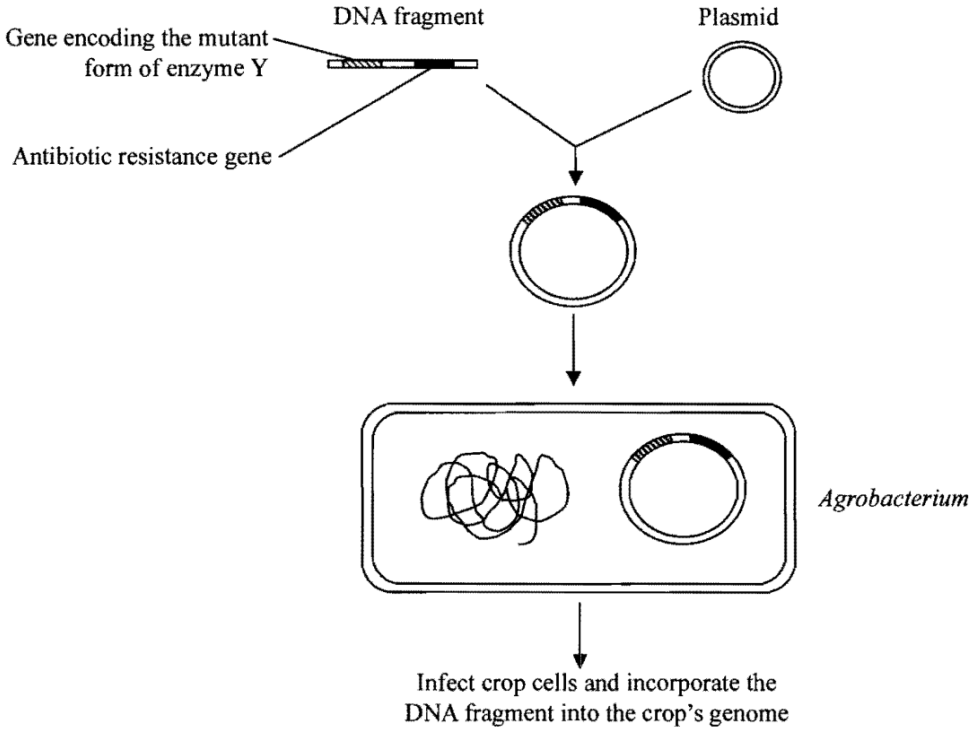
Gene-therapy cells must continue dividing and expressing the corrected gene. Somatic therapy does not correct gametes. Relate PCR temperatures to annealing and extension, and compare products with known-length DNA markers to judge size.

Marking reference: HKDSE Biology 2021 Paper 2 Q4, official marking scheme, source page(s) 10, 11.

3. [HKDSE Biology 2012 Paper 2 Q4]
Hint: Identify the gene or cells changed, then discuss effects and risks.

4.(a) Chemical X inhibits enzyme Y in photosynthesis and kills all plants. A scientist identified a mutant form of enzyme Y that works properly in photosynthesis and is not inhibited by chemical X. He cloned the gene encoding the mutant form of enzyme Y and put it into a plasmid so that the gene can be transferred into some cells of a crop species. Figure 4A below is a diagrammatic representation of the process:

Figure 4A



(i) Describe ***two*** steps taken to put the DNA fragment into the plasmid. (2 marks)

(ii) The scientist then transferred the plasmid into the cells of a crop plant using *Agrobacterium*. After the treatment, only some crop cells have incorporated with the DNA fragment.

(1) Explain why only some of the treated crop cells have incorporated with the DNA fragment. (2 marks)

(2) How can the scientist identify those crop cells that carried the DNA fragment? (3 marks)

(iii) The scientist suggests that growing this genetically modified crop together with chemical X applied to the field can greatly increase the crop yield. Explain the reasoning for this suggestion. (4 marks)

4.(b) A 25-year-old athlete, Keith, had nerve damage and became paralyzed. In view of the difficulties associated with tissue transplants, he proposed cloning himself, using the method used for Dolly the sheep, so as to get a nerve for transplantation. He discussed his idea with a scientist.

(i) Give ***two*** problems associated with conventional tissue transplants. (2 marks)

(ii) Keith's idea could not be put into practice. State ***one*** ethical reason to explain why not. (1 mark)

(iii) The scientist suggested that stem cell therapy may be a way of obtaining some nerve cells.

(1) State ***one*** place in Keith's body where stem cells can be obtained. (1 mark)

(2) Describe how stem cells may be used to cure Keith. (3 marks)

(3) Describe ***two*** limitations of using stem cell therapy in this case. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 2 mark(s)**

Use the same restriction enzyme to cut the target-gene DNA and plasmid, producing complementary sticky ends. Then use DNA ligase to join the fragment and plasmid sugar–phosphate backbones.

Restriction enzymes cut at specific sequences; ligase joins. These functions are not interchangeable. The same restriction enzyme gives compatible ends.

a(ii)(1); original allocation: 2 mark(s)

Some opened plasmids may recircularise without incorporating the target DNA. Some crop cells may also fail to receive the target-bearing plasmid/gene transfer.

Treating every cell does not make every step completely successful. These identify failures in recombinant-plasmid formation and cellular transfer respectively, explaining why only some cells contain the fragment.

a(ii)(2); original allocation: 3 mark(s)

Culture treated plant cells on agar containing the corresponding antibiotic. Cells that acquire the DNA fragment also acquire resistance and survive to form cell populations; other cells fail to grow normally.

Figure 4A places resistance and target genes on the same fragment, making resistance a selectable marker. State the selection condition, outcome for successful cells and marker function.

a(iii); original allocation: 4 mark(s)

Chemical X inhibits enzyme Y in ordinary plants, preventing normal photosynthesis and causing death. Modified crops produce mutant Y unaffected by X and continue photosynthesising and surviving. Weed removal reduces competition for water, minerals, light and space, potentially increasing crop yield.

X does not increase yield by acting as fertiliser. The different responses of modified crops and weeds allow weed control and reduced resource competition.

b(i); original allocation: 2 mark(s)

The recipient's immune system may recognise transplanted tissue as foreign and reject it. Suitable donors and tissues are also scarce, potentially causing long waiting times.

These concern immune compatibility and supply respectively. Do not give two differently worded versions of difficulty finding organs.

b(ii); original allocation: 1 mark(s)

Cloning and harming or sacrificing another person to obtain tissue raises serious ethical concerns about that individual's life and autonomy.

State the ethical issue of using another person as an organ source. No claim about a particular jurisdiction's laws in a particular year is necessary.

b(iii)(1); original allocation: 1 mark(s)

Bone marrow.

The question asks for a body location from which Keith's stem cells can be obtained. Bone marrow is an accepted source in this paper; stem cells are not found only in embryos.

b(iii)(2); original allocation: 3 mark(s)

Culture stem cells to increase their number through division, induce differentiation into suitable nerve cells, then transplant the resulting cells into the damaged site to repair tissue.

Proliferation, differentiation and transplantation each serve a purpose. More stem cells do not automatically constitute functional nervous tissue.

b(iii)(3); original allocation: 2 mark(s)

Differentiation into the required nerve cells and formation of appropriate connections must be controlled reliably. Uncontrolled proliferation and tumour formation must also be prevented.

Identify concrete technical limitations to treatment in this case rather than asserting that it can never work. Research limitations in 2012 do not imply permanent inability to advance.

Examiner's comment (source page(s) 3)

Do not confuse DNA ligase with polymerase. Explain stem-cell treatment through proliferation, differentiation and transplantation in sequence; differentiation cannot be omitted.

Marking reference: HKDSE Biology 2012 Paper 2 Q4, official marking scheme, source page(s) 9.

4. [HKDSE Biology Practice Paper Paper 2 Q4]

← *Hint: Identify the gene or cells changed, then discuss effects and risks.*

(a) Severe combined immune deficiency (SCID) is an inherited disease. One form of SCID is caused by a gene mutation which renders the person unable to produce lymphocytes. Thus, people with this disease are susceptible to infections and, if untreated, rarely live past the age of two. To cure the disease, patients are transplanted with stem cells from the bone marrow of a healthy person. In 2000, it was first demonstrated that the disease could be cured by somatic gene therapy. In this therapy, a virus is used as a vector to incorporate the normal gene into the bone marrow cells of the patient.

(i) Why can transplanting bone marrow stem cells to SCID patients be used for treating the disease? (1 mark)

(ii) What is the basis of using somatic gene therapy for treating SCID? Give **one** advantage of treating SCID patients with somatic gene therapy over transplanting normal bone marrow stem cells to the patients. (2 marks)

(iii) There is another type of gene therapy called 'germ line gene therapy'. State **two** differences in the biological consequences of using germ line gene therapy and using somatic gene therapy for treating SCID. (2 marks)

(iv) In 2002, three out of eleven SCID children who received this somatic gene therapy treatment got leukemia (a type of blood cancer). Some scientists believe that the occurrence of the blood cancer is related to the incorporation of the normal gene into the bone marrow cells using a virus as the vector. Suggest an explanation for this belief. (2 marks)

4. (b) Polymerase chain reaction (PCR) is a technique in modern biotechnology. A cycle of PCR consists of three principal steps, which operate at 95°C, 55°C and 72°C in sequence.

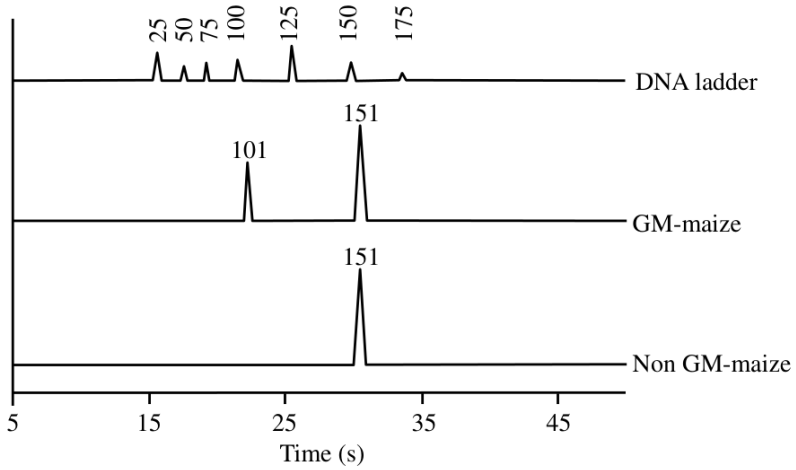
(i) Outline what happens in the three principal steps in a cycle of PCR. (3 marks)

(ii) One application of PCR is Polymerase Chain Reaction–Short Tandem Repeat Analysis (PCR–STR analysis) which can be used in forensics. In a crime scene, a piece of hair suspected to be the criminal's is found. A suspect is arrested one week later.

(1) With reference to the above case, state the significance of PCR in PCR–STR analysis. (1 mark)

(2) Describe how the products of PCR are used in PCR–STR analysis to produce evidence for verifying whether the suspect has committed the crime. (3 marks)

(iii) Another application of PCR is for identifying GM organisms. The following shows the analysis of the DNA of a GM maize and a non-GM maize:
[Note: The number above each peak in the figure indicates the number of base pairs (bp).]



(1) A marker (a polynucleotide chain) with 101 bp is introduced to the maize in producing the GM maize. With reference to the above analysis, suggest the purpose of introducing a marker to the maize in the process of producing the GM maize. (2 marks)

(2) Illustrate with an example how GM plants can help promote people's health. (2 marks)

(3) What are the possible impacts on the ecosystem of growing GM plants that produce a toxin to kill insects? Describe **two** possible impacts. (2 marks)

✓ **Answer** Answers, reasoning and corresponding marks are given below in the order of the original subparts.

Explanation **a(i); original allocation: 1 mark(s)**

Healthy bone-marrow stem cells can differentiate into normal lymphocytes, restoring some immune function and improving resistance to infection.

Their key properties are proliferation and differentiation; they are not injected as antibodies.

a(ii); original allocation: 2 mark(s)

Introduce a functional gene into the patient's somatic cells so its expression compensates for the defective gene. Using the patient's own cells reduces difficulties with finding a compatible marrow donor and immune rejection.

Gene therapy can restore function without necessarily deleting the original defective DNA from every cell.

a(iii); original allocation: 2 mark(s)

Germline gene therapy changes gamete-forming cells or early embryos. Changes may pass to offspring and occur in a wider range of cells. Somatic therapy mainly affects treated cells and their mitotic descendants and is generally not inherited through gametes.

Not every germline intervention necessarily changes every cell. The comparison concerns heritability and extent of action.

a(iv); original allocation: 2 mark(s)

Viral-vector insertion may alter other genes, for example activating a proliferation-promoting gene or disrupting normal regulation, causing uncontrolled bone-marrow-cell proliferation and leukaemia.

The mechanism is insertion-site-dependent expression change, not a claim that the therapeutic normal gene is inherently a cancer gene.

b(i); original allocation: 3 mark(s)

95°C: double-stranded DNA separates into single strands.

55°C: primers anneal through complementary pairing on either side of the target.

72°C: thermostable DNA polymerase extends from primer 3′ ends, adding nucleotides to form new strands.

Each step earns 1 mark. Heat disrupts interstrand pairing, not every covalent bond in the DNA backbone.

b(ii)(1); original allocation: 1 mark(s)

Crime-scene hair supplies little DNA. PCR amplifies target STR regions to provide enough material for analysis.

It amplifies target DNA, not records in a suspect database.

b(ii)(2); original allocation: 3 mark(s)

Electrophoresis separates STR amplification products of different lengths, producing a multilocus DNA profile. Compare it with corresponding suspect profiles. Agreement across multiple loci supports that person as a possible source; discrepancies can exclude the corresponding source.

A DNA match is evidence about the origin of biological material, not proof by itself that the person committed the crime. Consider the material, how it arrived and other evidence.

b(iii)(1); original allocation: 2 mark(s)

The 101 bp marker accompanies successfully modified cells. PCR detection of this characteristic peak distinguishes cells receiving foreign DNA from unmodified cells, allowing selection for further culture.

Both have a 151 bp peak, so it cannot distinguish them. Only the added 101 bp peak in modified samples is the requested marker.

b(iii)(2); original allocation: 2 mark(s)

For example, modify rice to produce more β-carotene, which humans convert into vitamin A, helping reduce night-blindness risk in relevant deficiency conditions.

State both the modification and its health effect. “GM food is more nutritious” alone is insufficient.

b(iii)(3); original allocation: 2 mark(s)

An insecticidal trait may affect some non-target insects and alter food webs. Pollen-mediated transfer to compatible relatives may also change competitiveness and community composition.

Assess specific traits and exposure conditions. Do not treat every insecticidal protein as a persistent, lipid-soluble pesticide and assert inevitable biomagnification.

Marking reference: HKDSE Biology Practice Paper Paper 2 Q4, official marking scheme, source page(s) 14.

Chapter 11

Sources of questions, marking references and images

Sources Curriculum and questions

Curriculum basis: the Education Bureau and Hong Kong Examinations and Assessment Authority, Biology Curriculum and Assessment Guide (Secondary 4–6), updated 2015, with reference also to the 2026 HKDSE Biology assessment information. Question text, marking references and candidate-performance information are checked against the original papers and question collections for each year. Sample and practice papers supplement the examination questions and are labelled separately from examination years.

The year, paper section and original question number appear beside each question. Solutions also identify pages in the official marking reference. 'Source page' refers to the page sequence in the cited PDF; printed page numbers may differ between combined and standalone files. Each book has its own question-source index linking original questions to book pages.

The knowledge sections explain concrete examples before introducing terminology, symbols and diagrams. Interpret process arrows and numerical illustrations together with the text. Typical cell or organ diagrams are not complete records of every specimen. Original question figures are retained with their questions.

Education Bureau: Biology Curriculum and Assessment Guide

HKEAA: 2026 Biology assessment information

The original structural diagrams of the eye, ear and intestinal villi are by Servier Medical Art, supplied through Bioicons under CC BY 3.0. This edition retains the structural forms and adds English labels, leader lines, function tables and captions. The villus diagram's original text has been replaced with corresponding labels.

- Eye cross-section: Servier Medical Art / Bioicons
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HKDSE Biology 2024 Paper 1B Q3: Wikimedia Commons: Mitochondria, mammalian lung - TEM.jpg
HKDSE Biology 2024 Paper 1B Q7: original paper image: Pixlr; seaweed images: Foras na Mara Marine Institute, Explorers Wild About Wildlife on the Seashore: Seaweed Information Sheets
HKDSE Biology 2024 Paper 1B Q9: Microscopy of Nature: Stomata
HKDSE Biology 2024 Paper 2 Q2: The Conservancy Association: The Ecology of Long Valley