

Microbiology, Immunity and Forensics revision notes

Microbiology, Immunity and Forensics is topic 6 of Pearson Edexcel International Advanced Level Biology (YBI11) and is examined in Unit 4; its core practicals 13 and 14 can be examined in Unit 4 and in Unit 6 (Practical Skills in Biology II). It covers culturing and measuring microorganisms, how bacteria and viruses cause disease, the immune response, antibiotics and infection control, and the DNA techniques and evidence used in forensic science. It is A Level only content.

For Pearson Edexcel International AS Biology XBI11 and International A Level Biology YBI11. **A Level only** is IA2 content (Units 4 to 6), not needed for the International AS.

1 Culturing and measuring microorganisms **Spec 6.1–6.4** **A Level only**

Culturing microorganisms using aseptic technique **Spec 6.1**

Microorganisms are grown (**cultured**) in a **culture medium** that supplies everything they need: a carbon source (such as glucose), a nitrogen source (such as amino acids or ammonium ions), mineral ions, water and sometimes growth factors such as vitamins. The medium is either a solid **nutrient agar** gel poured into Petri dishes, or a liquid **nutrient broth** in a flask or tube.

The aim of **aseptic technique** is to stop two things: unwanted microorganisms from the air, skin and bench entering the culture (contamination), and the cultured organisms escaping into the environment or infecting the worker.

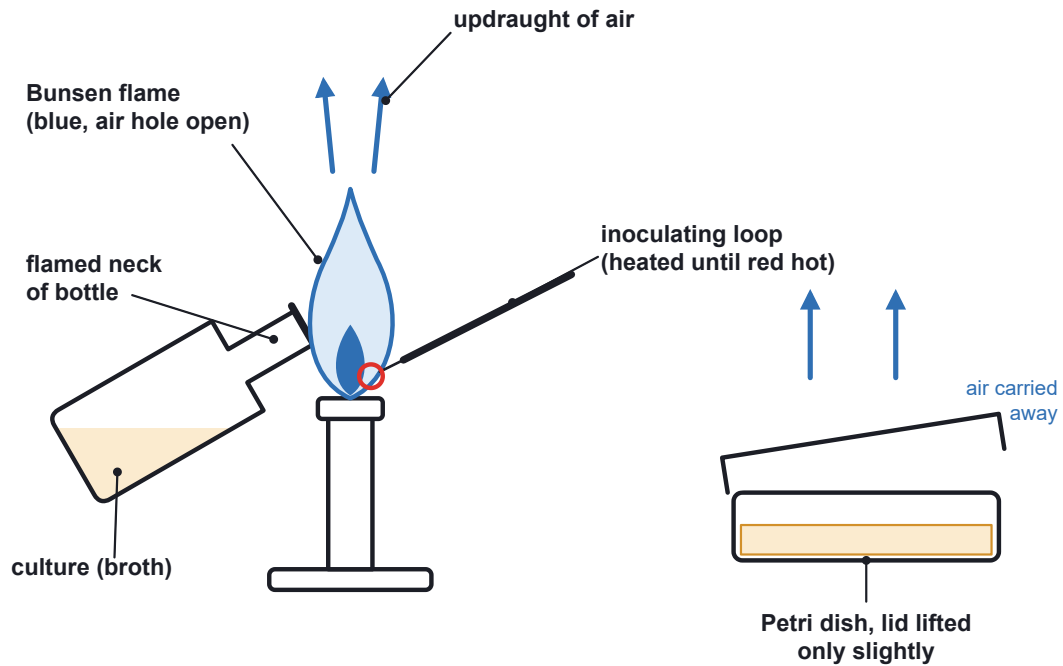
Principles and techniques

Aseptic technique: what is done and why	
Technique	Reason
Sterilise media, glassware and pipette tips in an autoclave (steam at 121 °C under pressure for at least 15 minutes) before use	Kills all microorganisms, including heat-resistant spores, so only the organism being studied grows
Wipe the bench with disinfectant and wash hands before and after	Removes microorganisms that could contaminate the culture or infect the worker
Pass the neck of a bottle or tube and the inoculating loop through a Bunsen flame; heat the loop until red hot and let it cool before use	Kills microorganisms on the surfaces; the flame creates an updraught of air that carries airborne microorganisms away from the opening; a cool loop does not kill the culture being transferred
Keep lids on, lifting the Petri dish lid only slightly and only for as long as needed	Reduces entry of airborne microorganisms
Seal the lid with two or four short strips of adhesive tape, not all the way round	Stops the lid being removed accidentally, but still lets oxygen in, so anaerobic pathogens are not selected for
Incubate plates upside down (inverted)	Condensation collects in the lid and cannot drip on to the agar and spread the colonies
Incubate at 25 °C in schools and colleges (not above 30 °C); commercial and clinical work often uses 37 °C	Slower growth of human pathogens, which grow best at body temperature, is safer
Autoclave or disinfect all used cultures and equipment before disposal	Prevents release of microorganisms

Inoculating a plate

1. Sterilise the inoculating loop in a flame until red hot, then let it cool for a few seconds near the flame.
2. Remove the bottle cap with the little finger of the hand holding the loop, flame the neck of the bottle, and take a loopful of culture.
3. Flame the neck again, replace the cap, then lift the Petri dish lid slightly and streak the loop gently across the agar surface (or spread a measured volume with a sterile spreader to give a lawn).
4. Replace the lid, tape it, label the base with a marker pen (name, date, organism) and incubate inverted.
5. Re-sterilise the loop in the flame.

Exam tip: Always give the reason with the technique. Marks go for 'flame the neck of the bottle to kill microorganisms and create an updraught', not just 'flame the bottle'.



Aseptic technique: flame the loop and the bottle neck, keep the dish lid lifted only slightly, and work in the **updraught** of the flame.

Measuring the growth of microorganisms **Spec 6.2**

Growth of a population of microorganisms is an increase in the number of cells (or in their total mass). Four methods are named in the specification.

Cell counts

A sample of the culture is placed on a **haemocytometer** (a slide with a grid etched on it that holds a known small volume) and the cells in a number of squares are counted under a microscope. The mean count per square is scaled up to give cells per cm^3 . A **total cell count** includes dead cells as well as living cells. A stain such as methylene blue, which living yeast cells decolorise but dead cells do not, can be used to count only living cells.

Dilution plating (viable count)

A **serial dilution** of the culture is made, for example by adding 1 cm^3 of culture to 9 cm^3 of sterile water (a 10^{-1} dilution) and repeating this step each time, giving 10^{-2} , 10^{-3} and so on. A measured volume (often 0.1 cm^3) of the more dilute tubes is spread on agar plates, incubated, and the colonies counted. Each colony is assumed to have grown from one **colony-forming unit** (cfu), a single living cell or a small clump. Only plates with about 30 to 300 colonies are used, because fewer is unreliable and more cannot be counted accurately.

cfu per cm^3 of original culture = (number of colonies $\div$ volume plated in cm^3) $\times$ dilution factor

Dilution plating

0.1 cm³ of a 10⁻⁴ dilution of a bacterial culture was spread on an agar plate. After incubation 56 colonies grew. Calculate the number of colony-forming units per cm³ of the original culture.

1. Colonies per cm³ of the diluted sample = $56 \div 0.1 = 560$ cfu per cm³.
2. The dilution factor is 10⁴ (10⁻⁴ dilution).
3. Original culture = $560 \times 10^4 = 5.6 \times 10^6$ cfu per cm³.

Answer: 5.6×10^6 cfu per cm³

Maths skill: Dilution factor and sample volume are the usual traps. Divide by the volume you plated, then multiply by the dilution factor, and write the answer in standard form with units.

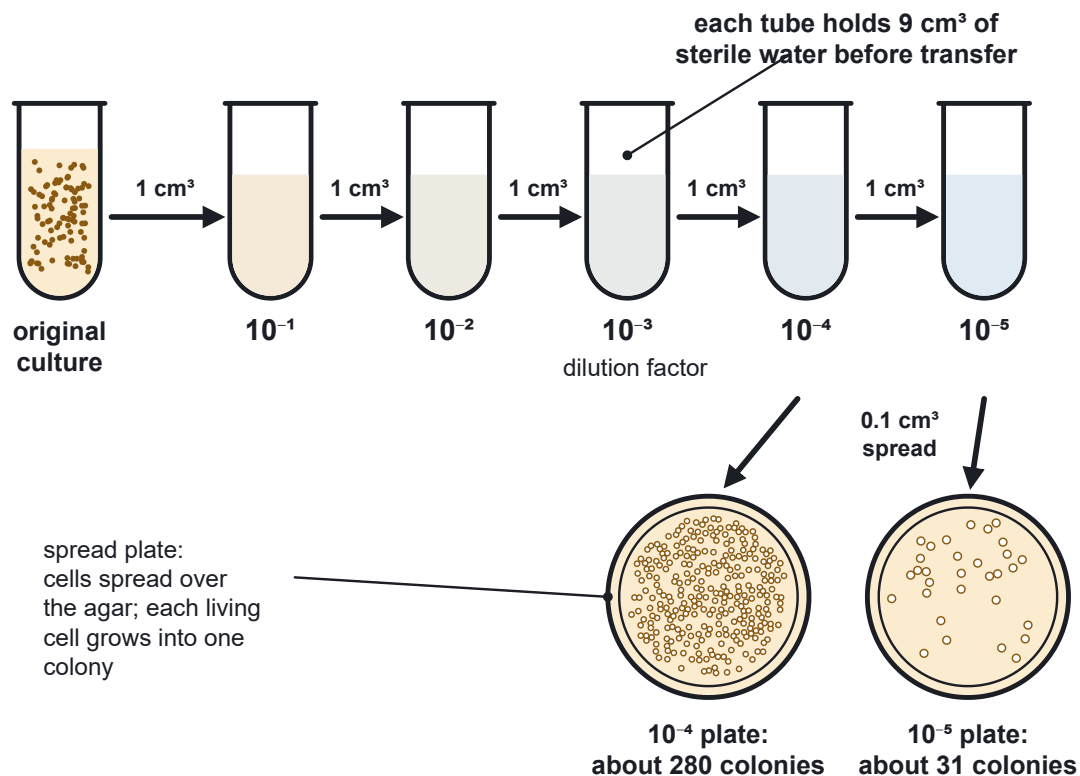
Mass

A known volume of culture is centrifuged, the supernatant discarded, and the cells dried in an oven at about 80 to 100 °C until the mass is constant. **Dry mass** is a better measure of growth than wet mass, because wet mass includes a variable amount of water. Mass is used when cells are too small or too many to count, or for filamentous fungi. It cannot tell living from dead cells, and the sample is destroyed.

Optical methods: turbidity

As cells multiply, a liquid culture becomes cloudier (more **turbid**). A **colorimeter** (or spectrophotometer) shines light through the culture and records the absorbance, or the percentage transmission. More cells scatter more light, so absorbance rises as the population grows. It is quick, does not destroy the sample and allows repeated readings from the same culture. A **calibration curve** (absorbance plotted against cell number found by another method) turns readings into cell numbers. Turbidity counts dead cells too, and is unreliable at very low or very high cell densities.

Comparing the methods				
Method	Measures	Living and dead cells	Main advantage	Main limitation
Cell count (haemocytometer)	Number of cells per cm ³	Both, unless a viability stain is used	Direct count of cells	Slow; small sample; hard to count at high density
Dilution plating	Number of colony-forming units	Living only	Counts only viable cells	Slow (incubation); clumps counted as one
Dry mass	Mass of cells per cm ³	Both	Works for cells too small or many to count	Destroys sample; time-consuming
Turbidity	Absorbance of the culture	Both	Quick; repeated readings; sample kept	Needs calibration; unreliable at extremes

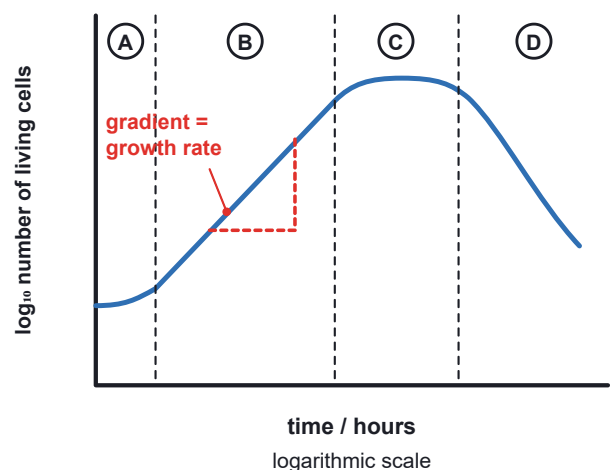
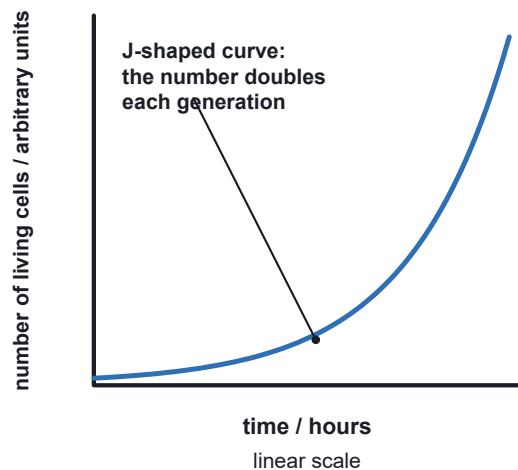


Serial dilution and spread plating: each transfer of 1 cm³ into 9 cm³ of sterile water gives a ten-fold dilution.

The bacterial growth curve and growth rate constants Spec 6.3

When bacteria are inoculated into a fixed volume of broth (a closed culture, with no nutrients added or waste removed) and the number of living cells is plotted against time, the curve has four phases. Cell number is usually plotted as log₁₀ so that the very large range fits on the graph and the exponential phase appears as a straight line.

Phases of the growth curve		
Phase	What happens to cell number	Why
Lag phase	Little or no increase	Cells are adjusting to the new medium: they take up nutrients, synthesise enzymes and ribosomes, and grow in size; few cells divide yet
Exponential (log) phase	Doubles every generation: fastest rate of increase	Nutrients and space are plentiful and there is little waste; cells divide by binary fission at a constant rate, so the number of cells doubles in each generation time
Stationary phase	Constant: births equal deaths	Nutrients become limiting, toxic waste (for example acids that lower the pH) accumulates and oxygen may run short, so division slows until the rate of division equals the rate of death
Death (decline) phase	Falls	Nutrients are exhausted and toxic waste is high, so more cells die than divide



- (A) Lag phase: cells adjust to the medium (make enzymes, grow); few divide yet.
- (B) Exponential phase: cells divide by binary fission, so the number doubles each generation.
- (C) Stationary phase: births = deaths (nutrients limiting, toxic waste building up).
- (D) Death phase: deaths > births (nutrients exhausted).

The **growth curve** of a closed culture: on the log graph the exponential phase is a straight line whose gradient is the growth rate.

Exponential growth and growth rate constants

In the exponential phase each cell divides once per **generation time** (the time for the population to double). After n generations a population starting with N_0 cells has $N_n = N_0 \times 2^n$ cells. Rearranging with logarithms gives the number of generations from the start and end numbers.

$$\text{number of generations, } n = (\log_{10} N_t - \log_{10} N_0) \div 0.301$$

$$\text{exponential growth rate constant, } k = n \div t \text{ (generations per hour) } \text{ generation time} = 1 \div k$$

The constant 0.301 is $\log_{10} 2$. The growth rate constant k is the number of generations per unit time during the exponential phase; it is the gradient of the straight line on a graph of $\log_2$ (cell number) against time, or the gradient of $\log_{10}$ cell number against time divided by 0.301.

Growth rate constant

A broth culture contained 2.0×10^3 cells per cm^3 at the start of the exponential phase and 1.28×10^5 cells per cm^3 6 hours later. Calculate the exponential growth rate constant.

- $\log_{10} (1.28 \times 10^5) = 5.107$ and $\log_{10} (2.0 \times 10^3) = 3.301$, so the difference is 1.806.
- Number of generations, $n = 1.806 \div 0.301 = 6.0$.
- $k = n \div t = 6.0 \div 6 \text{ h} = 1.0$ generation per hour (generation time 1 hour).

Answer: $k = 1.0$ generation per hour

Maths skill: Logarithms on a calculator and reading a log scale are IAL-only skills (A.0.5, A.2.5). If a question does not give the formula, show the doubling idea: $1.28 \times 10^5 \div 2.0 \times 10^3 = 64 = 2^6$, so 6 generations.

Exam tip: Do not say that cells 'stop dividing' in the stationary phase. The correct idea is that the rate of division equals the rate of death, so the number of living cells is constant.

Core practical 13: rate of growth in liquid culture Spec 6.4

Core practical: Core practical 13: Investigate the rate of growth of microorganisms in a liquid culture, taking into account the safe and ethical use of organisms.

A typical investigation uses a harmless organism, such as baker's yeast (*Saccharomyces cerevisiae*) or a non-pathogenic strain of *Escherichia coli*, in nutrient broth, and asks how the rate of growth is affected by an independent variable such as temperature, pH or glucose concentration.

Method

1. Prepare sterile broth and set up identical flasks or tubes, each with the same volume of broth and the same starting cell density. Use aseptic technique throughout.
2. Set the independent variable (for example water baths at 20, 25, 30, 35 and 40 °C for yeast, or broth at different pH).
3. Inoculate each flask with the same volume of culture, mix gently, and take a time-zero reading.
4. At regular intervals (for example every 30 or 60 minutes for several hours) swirl to resuspend and remove a sample with a sterile pipette. Measure absorbance in a colorimeter using sterile broth as the blank, or do a haemocytometer count or dilution plating.
5. Repeat each condition at least three times and calculate mean readings.
6. Plot cell number or absorbance (or $\log_{10}$ of it) against time and compare gradients of the exponential phase, or calculate the growth rate constant k for each condition.

Variables and controls	
Type	Examples
Independent variable	Temperature, pH, glucose concentration or antibiotic concentration
Dependent variable	Cell number or absorbance over time (rate of growth = change in cell number $\div$ time, or k)
Controlled variables	Volume and type of broth; starting cell density and volume of inoculum; strain of organism; aeration or shaking; light; time between readings; same colorimeter wavelength and cuvettes

Processing results

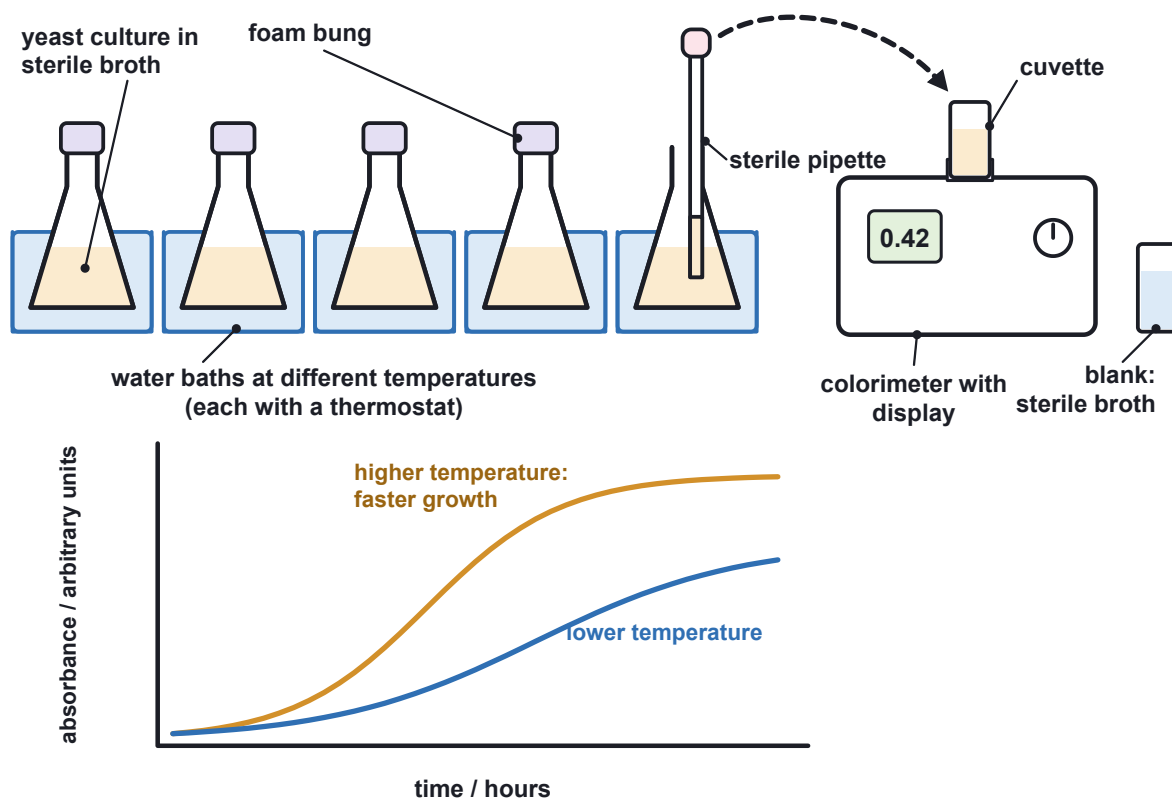
Rate of growth in the exponential phase is the gradient of a line on the graph. For a curved graph, draw a **tangent** to find the gradient at a given time. A growth rate constant can be found from the number of generations per hour. Compare the means for each condition and say whether differences are likely to be real, using error bars or range.

Safe and ethical use of organisms

- Use only non-pathogenic strains, such as laboratory strains of yeast or *E. coli* K12, and never culture samples from the environment or from people, which could contain pathogens.
- Carry out a risk assessment; wear a lab coat and gloves, tie back hair, cover cuts, wash hands, and disinfect the bench before and after.
- Use aseptic technique, incubate at 25 °C or lower for bacteria in school, and tape the lids on any plates.
- Autoclave or disinfect all cultures and equipment before disposal; do not tip live cultures down the sink.

- Ethical use: microorganisms are living organisms, so use only as many as are needed, plan to avoid repeating work unnecessarily, and kill (autoclave) and dispose of cultures responsibly once finished.

Exam tip: In a planning question give a named strain that is safe, a reason for each control, and what you will do with the cultures afterwards. These are easy marks that are often missed.



Core practical 13: growth of a culture at different temperatures measured as absorbance in a colorimeter zeroed with a sterile broth blank.

Quick check

1. Why are Petri dishes incubated upside down?
2. What does a viable count measure that a total cell count does not?
3. Name the four phases of the bacterial growth curve in order.
4. What is the formula for the number of generations from the start and end numbers of cells?
5. Why is a colorimeter blank of sterile broth used in core practical 13?

2 Bacteria, viruses and infection Spec 6.5-6.7 A Level only

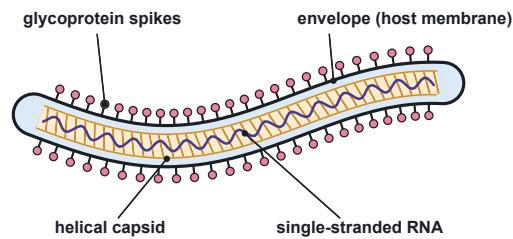
Structure of bacteria and viruses Spec 6.5(i)

Bacteria are living prokaryotic cells, about 0.5 to 5 μm long. **Viruses** are far smaller (most are about 20 to 300 nm across; Ebola filaments are about 80 nm wide but often over 1 μm long) and are not cells: they have no cytoplasm, no ribosomes and no metabolism of their own, and they can reproduce only inside a host cell.

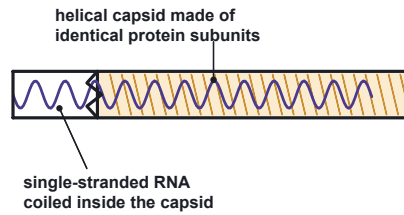
Bacteria compared with viruses		
Feature	Bacterium	Virus
Cell structure	Prokaryotic cell: cell-surface membrane, cell wall of peptidoglycan, cytoplasm	Acellular: no membrane or cytoplasm of its own
Genetic material	Circular DNA (nucleoid), often plasmids too	DNA or RNA (single or double stranded), never both, in small amounts
Protein coat	None (cell wall is a different structure)	Capsid made of protein subunits (capsomeres) around the nucleic acid
Envelope	None	Present in some viruses: lipid bilayer from the host membrane with viral glycoproteins
Ribosomes	70S ribosomes	None; uses host ribosomes
Reproduction	Binary fission; can reproduce outside a host	Only inside a host cell, using its enzymes, ribosomes and ATP
Susceptible to antibiotics	Yes	No

Four named viruses

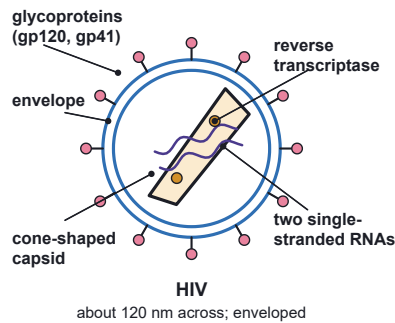
Structure of the viruses named in the specification				
Virus	Nucleic acid	Capsid	Envelope	Notes
Ebola virus	Single-stranded RNA	Helical capsid forming a long filament	Yes, with glycoproteins	Infects human cells; filamentous particle
Tobacco mosaic virus (TMV)	Single-stranded RNA	Helical (rod-shaped) capsid of identical protein subunits	No	Infects plant cells, causing mosaic discolouration of leaves
HIV	Two copies of single-stranded RNA, plus reverse transcriptase	Cone-shaped capsid	Yes, with glycoproteins (gp120, gp41) that bind to host cells	Infects helper T cells
Lambda phage (λ phage)	Double-stranded DNA	Icosahedral head with a tail and tail fibres	No	A bacteriophage: infects bacteria (<i>Escherichia coli</i>)



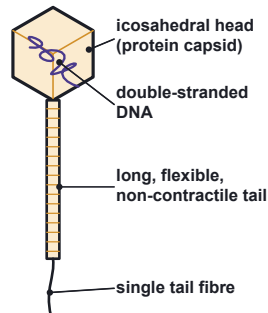
Ebola virus
filament about 80 nm wide, up to 1 μ m long; enveloped



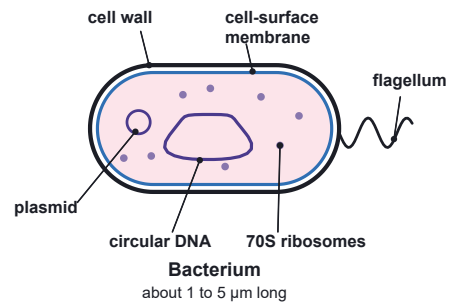
Tobacco mosaic virus (TMV)
rigid rod about 300 nm long; no envelope



HIV
about 120 nm across; enveloped



Lambda (λ) phage
bacteriophage; about 200 nm long; no envelope



Bacterium
about 1 to 5 μ m long

Not drawn to scale: sizes are approximate.

The viruses named in the specification compared with a bacterium (not drawn to scale).

Common mistake: Viruses are not cells and are not made of 'a nucleus and cytoplasm'. Say 'nucleic acid in a capsid, with or without an envelope'. Never say viruses are killed by antibiotics.

The terms lytic and latency Spec 6.5(ii)

Lytic cycle	A viral life cycle in which the virus uses the host cell to make many copies of itself at once; the host cell bursts (lyses) and releases the new particles.
Latency	A state in which viral nucleic acid persists in the host cell without producing new virus particles, and no symptoms need occur; the virus can later become active.

The lytic cycle (for example lambda phage)

1. Attachment: tail fibres bind to specific receptors on the bacterial surface.
2. Injection: the phage DNA enters the bacterium; the empty capsid stays outside.
3. Replication and synthesis: the host's enzymes and ribosomes copy the viral DNA and make viral capsid proteins.
4. Assembly: new capsids form and are filled with DNA.
5. Lysis: viral lysozyme breaks down the cell wall; the host bursts and releases hundreds of phage particles.

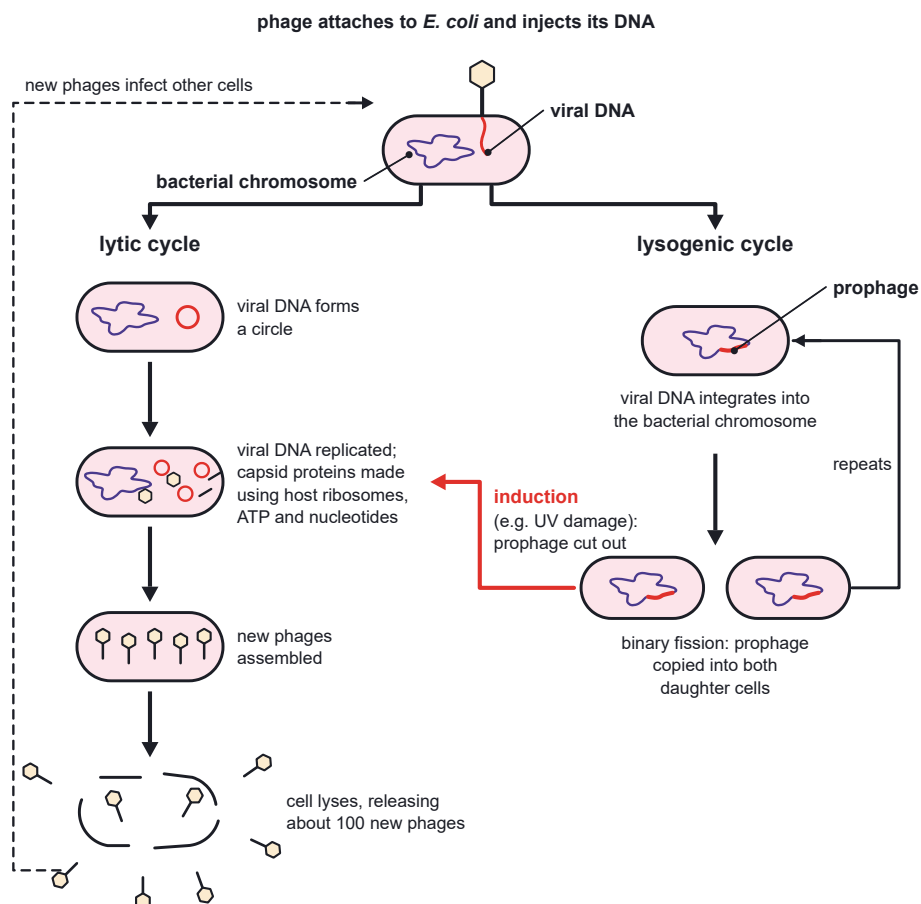
Latency (the lysogenic state of lambda phage; HIV)

In latency the viral DNA is inserted into the host chromosome. In a bacterium it is called a **prophage** and in a human cell infected by HIV, a **provirus** (made by reverse transcriptase from the RNA genome). It is copied each time the host DNA replicates and passed to daughter cells, and the host cell is not

destroyed. A trigger such as stress, ultraviolet light or activation of the infected cell switches the virus into the lytic cycle.

Lytic cycle compared with latency		
Feature	Lytic cycle	Latency
Viral nucleic acid	Replicated separately from the host DNA; many copies made	Integrated into host DNA (or held silent)
New virus particles	Made at once in large numbers	None made
Host cell	Destroyed (lysed)	Survives and divides
Symptoms	Usually during the lytic phase	Often none; the host can be a hidden carrier

Exam tip: Latent HIV in a provirus is protected from the immune system and antiviral drugs because no viral proteins are being made. That is why people with HIV need life-long treatment.



The lytic cycle and latency of **lambda phage**: in latency the viral DNA is a **prophage** inside the host chromosome.

How *Mycobacterium tuberculosis* and HIV cause disease Spec 6.6

Tuberculosis (TB)

TB is caused by the bacterium *Mycobacterium tuberculosis*. It is spread in droplets coughed out by an infected person and breathed in. The bacteria reach the alveoli and are engulfed by **macrophages**. The bacterium has a thick, waxy cell wall and prevents the lysosomes fusing with the phagosome, so it is not digested and multiplies slowly inside the macrophage.

1. Bacteria in droplets are inhaled and reach the alveoli of the lung.

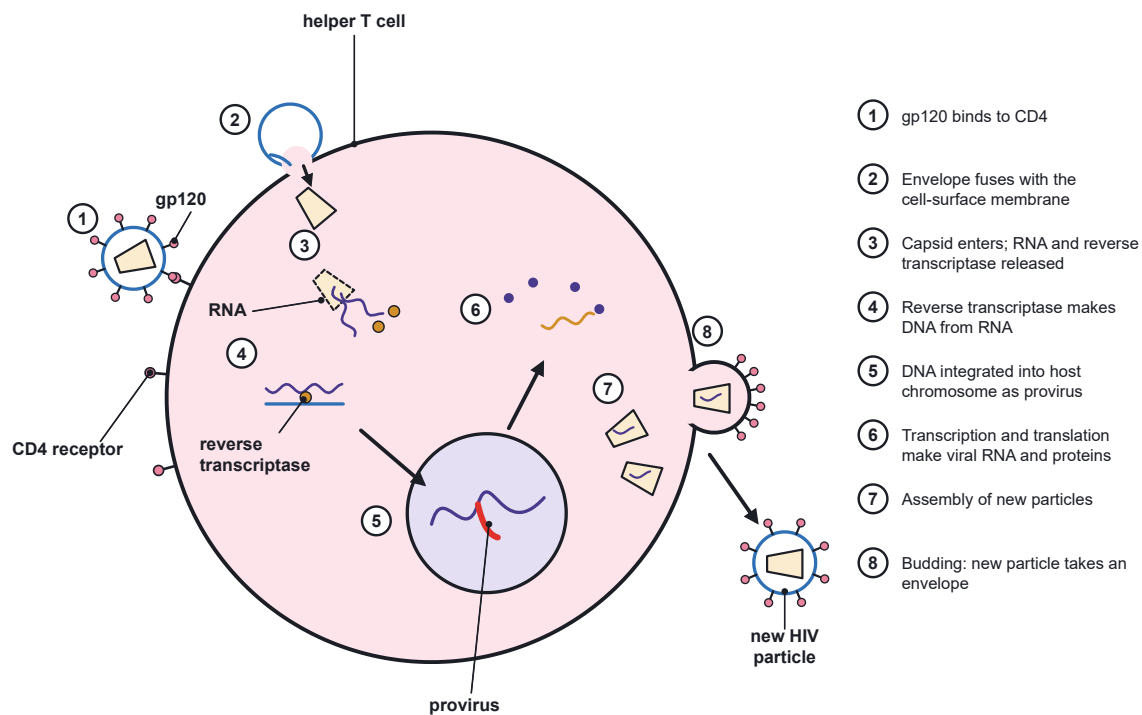
2. Macrophages engulf them but cannot destroy them; the bacteria multiply inside the macrophages.
3. The immune system responds: macrophages and T cells gather round the infected cells and form a ball of cells and dead tissue called a **tubercle**, which walls off the bacteria. Most people have no symptoms at this stage (latent TB).
4. If the immune system is weakened (for example by HIV, malnutrition or age), the bacteria multiply, the tubercle breaks down and spreads to other parts of the lung. Lung tissue is destroyed, leaving cavities.
5. Symptoms include a persistent cough with blood-stained sputum, weight loss, fever and night sweats. Without treatment, lung function fails and the infection can spread through the blood, causing death.

HIV and AIDS

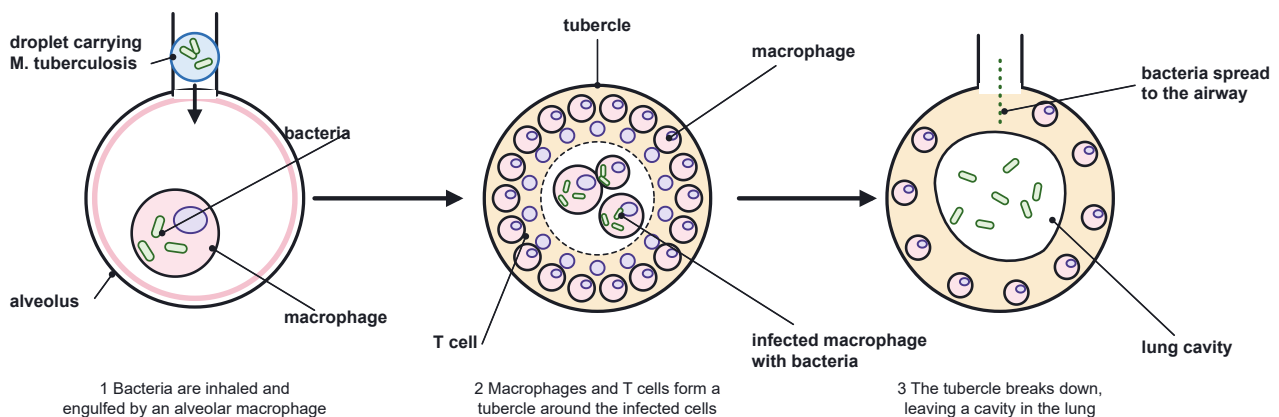
The human immunodeficiency virus (HIV) is spread by exchange of body fluids (sexual contact, blood, mother to child). It infects **helper T cells** (and some macrophages), which carry the CD4 receptor.

1. A glycoprotein on the HIV envelope (gp120) binds to the CD4 receptor on the helper T cell.
2. The viral envelope fuses with the cell-surface membrane and the capsid enters the cytoplasm.
3. The capsid breaks down, releasing RNA and the enzyme reverse transcriptase. This makes a DNA copy of the RNA, and the DNA is inserted into the host chromosome by the enzyme integrase as a provirus (latency).
4. When the cell is activated, the host's enzymes transcribe the proviral DNA to make viral RNA and proteins, which are assembled into new virus particles.
5. New particles bud off from the cell-surface membrane, taking a piece as their envelope. Large numbers of virus particles are released and the T cell dies.

Helper T cells are essential for activating B cells and killer T cells. As HIV destroys them, the number of helper T cells falls and the person develops **AIDS** (acquired immune deficiency syndrome): they lose the ability to fight infections and some cancers. Death usually results from opportunistic infections such as TB, pneumonia or a cancer, rather than from HIV directly. HIV makes people far more likely to develop active TB, and TB is a leading cause of death in people with HIV.



HIV life cycle in a helper T cell, from binding to CD4 to budding of new particles.



TB in the lung: bacteria in macrophages are walled off in a **tubercle**, which can break down to leave a cavity.

Exam tip: A 'describe how HIV causes death' answer needs the chain: infects helper T cells, destroys them, fewer B and killer T cells activated, immune system fails, death from opportunistic infection.

Routes of entry and protective barriers Spec 6.7

Major routes of entry

Routes pathogens take into the body		
Route	How	Examples
Gas exchange system	Breathing in droplets or spores	TB, influenza, measles
Digestive system	Eating or drinking contaminated food or water	Cholera, <i>Salmonella</i> food poisoning
Breaks in the skin	Cuts, wounds, bites from vectors, injections, needles	Tetanus, malaria (mosquito bite), HIV and hepatitis B (needles)
Mucous membranes of the genital tract and eye	Direct contact with infected fluids or surfaces	HIV, gonorrhoea, chlamydia, conjunctivitis
Across the placenta or in breast milk	Mother to fetus or baby	HIV, rubella

Barriers to infection

Non-specific barriers	
Barrier	How it protects
Skin	A tough layer of keratinised dead cells forms a physical barrier. Sebum from sebaceous glands contains fatty acids, making the surface acidic and hostile to many microorganisms. Cuts are sealed quickly by blood clotting.
Stomach acid	Hydrochloric acid keeps the stomach at about pH 1 to 2, killing most microorganisms taken in with food and drink. Pepsin works at this pH.
Gut flora	Harmless bacteria in the gut compete with pathogens for nutrients and space, and produce substances (such as lactic acid) that inhibit pathogens. Antibiotics can reduce gut flora and allow pathogens to grow.
Skin flora	Resident bacteria on the skin compete with pathogens for nutrients and space and produce acids and antimicrobial substances. Normal flora can themselves cause infection if they enter the body through broken skin.

Other barriers are mucus, which traps microorganisms, and cilia on the lining of the airways, which sweep mucus up and out of the gas exchange system. Tears and saliva contain lysozyme (see the next subtopic).

Common mistake: 'Gut flora fight pathogens' is too vague. Say they **compete** for nutrients and space and produce inhibitory substances, so pathogens cannot establish themselves.

Quick check

1. What is the difference between a capsid and an envelope?
2. Why do viruses only reproduce inside host cells?
3. What is a provirus?
4. Where does *Mycobacterium tuberculosis* survive in the body?
5. Name two ways that gut flora protect against infection.

3 The immune response Spec 6.8–6.12 A Level only

Non-specific responses to infection Spec 6.8

Inflammation

When tissue is damaged or infected, mast cells and damaged cells release **histamine** and other signalling molecules (cytokines).

1. Histamine causes vasodilation of arterioles, increasing blood flow to the area, so the tissue becomes red and warm.
2. Capillary walls become more permeable, so more fluid leaves the blood as tissue fluid, causing swelling, which may press on nerve endings and cause pain.
3. Chemical signals attract phagocytes (neutrophils and monocytes that become macrophages), which leave the capillaries and engulf pathogens.

Lysozyme

Lysozyme is an enzyme found in tears, saliva, mucus and in phagocytes. It hydrolyses the peptidoglycan in the cell walls of bacteria. The weakened wall can no longer resist osmotic pressure, so water enters by osmosis and the bacterium bursts (lysis). Lysozyme does not act on viruses.

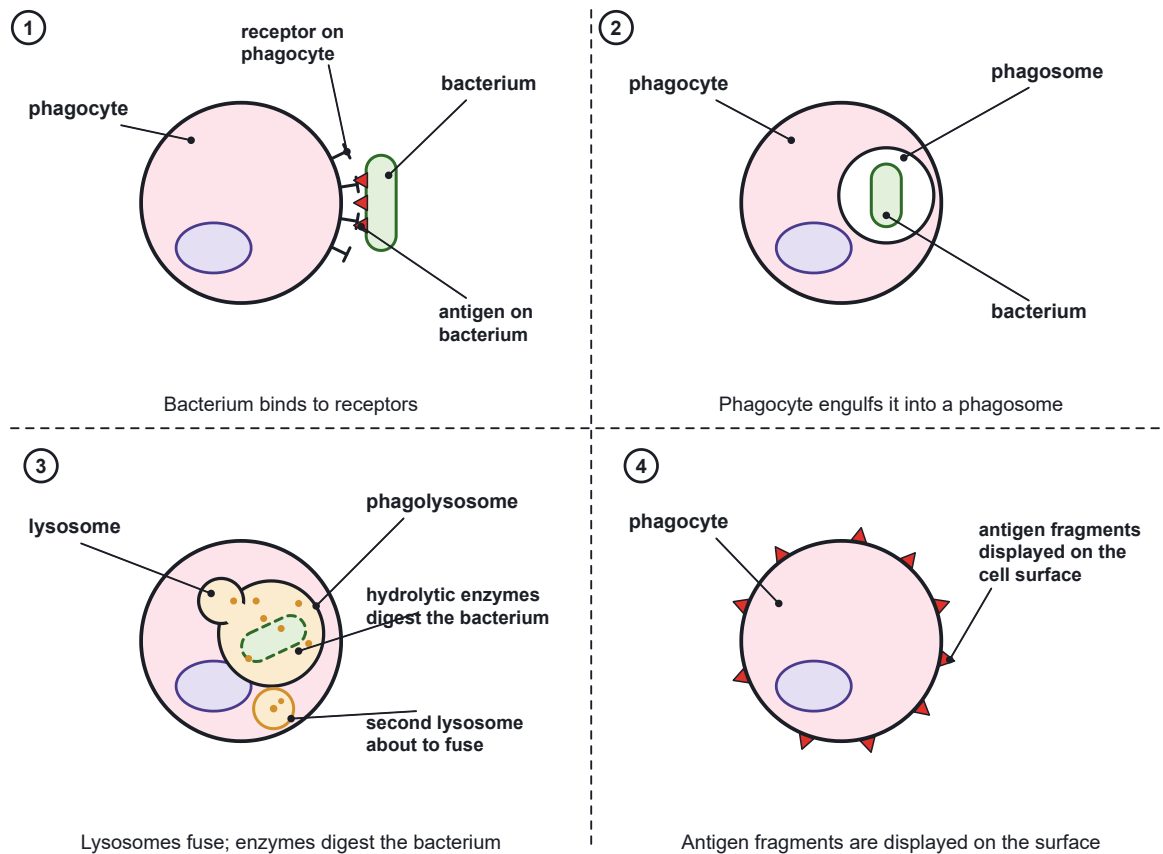
Interferon

Interferons are cytokines (signalling proteins) released by cells infected with a virus. They diffuse to neighbouring uninfected cells and bind to receptors on the cell-surface membrane. This switches on genes that make enzymes that break down viral mRNA and inhibit protein synthesis. Viral proteins cannot be made, so the virus cannot replicate in these cells and its spread is slowed.

Phagocytosis

1. A phagocyte (neutrophil or macrophage) recognises a pathogen, often because it is coated with antibodies or has foreign surface molecules, and the pathogen binds to receptors on the phagocyte.
2. The phagocyte engulfs the pathogen by endocytosis, enclosing it in a vesicle called a phagosome.
3. Lysosomes fuse with the phagosome and release lysozyme and other hydrolytic enzymes, which digest the pathogen.
4. Soluble products are absorbed into the cytoplasm. A macrophage then displays pieces of the pathogen's antigens on its surface (antigen presentation) to start the specific response.

Exam tip: 'Non-specific' means the response is the same whatever the pathogen. Do not say it is 'not immune': it is the first part of the innate immune response.



Phagocytosis and antigen presentation by a phagocyte.

Antigens, antibodies and antigen-presenting cells Spec 6.9

Antigen	A molecule (usually a protein or glycoprotein) on the surface of a pathogen, a foreign cell or a toxin, that is recognised as foreign by the immune system and stimulates an immune response.
Antibody	A protein (immunoglobulin) made by a plasma cell that binds specifically to one antigen.

Antibody structure

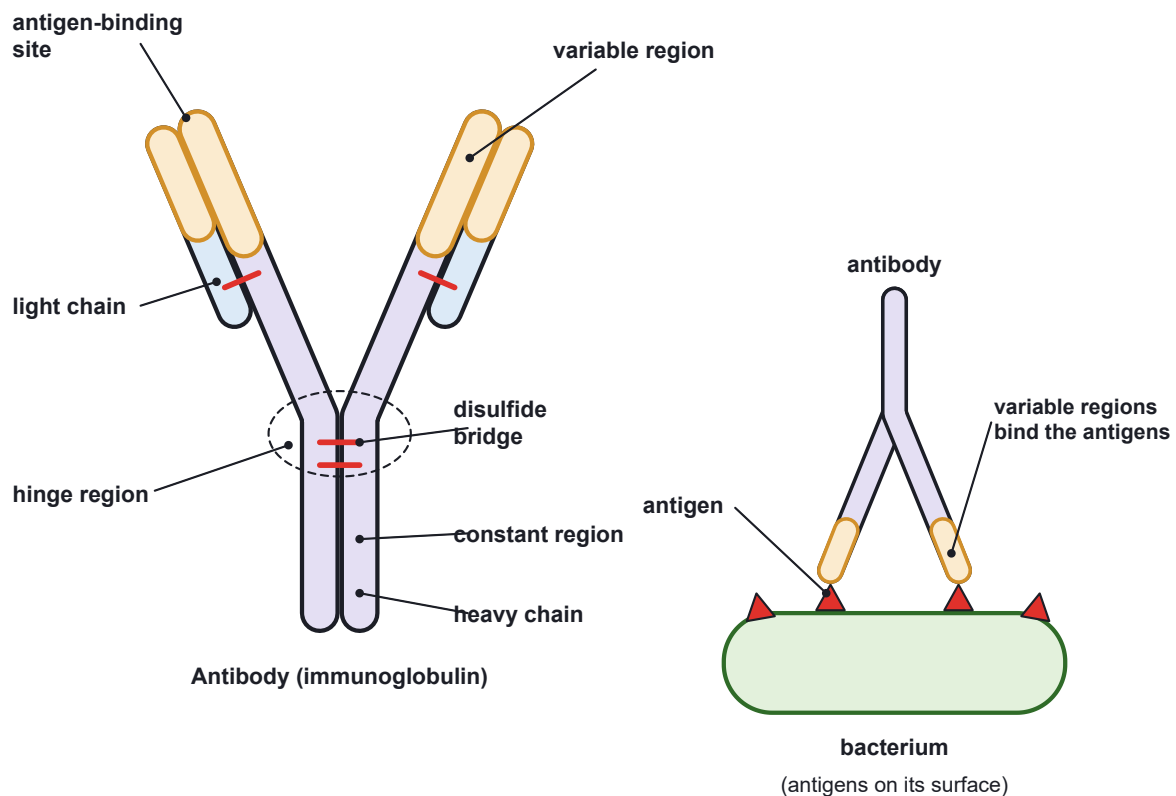
An **antibody** is a Y-shaped protein with four polypeptide chains: two identical heavy chains and two identical light chains, held together by disulfide bridges. Each arm has a **variable region** whose shape is complementary to one antigen and forms the antigen-binding site. The rest is the constant region, which is the same for all antibodies of one class, and a flexible hinge region lets the arms move. The tertiary structure of the variable region is different for each antibody, which is why each antibody binds only one antigen.

How antibodies help to destroy pathogens

- Agglutination: each antibody has two binding sites, so antibodies link pathogens together into clumps, which are easier for phagocytes to engulf.
- Opsonisation (marking): antibodies bound to the surface of a pathogen make phagocytosis easier, because phagocytes have receptors for the constant region.
- Neutralising toxins and blocking attachment: antibodies bind to toxins or to the proteins pathogens use to attach to or enter host cells.

The cells involved

Cells in the specific response	
Cell	Role
Macrophage	Engulfs pathogen, digests it, and displays pieces of its antigens on its surface so that T cells can recognise them; also a phagocyte in the non-specific response
Antigen-presenting cell (APC)	Any cell that displays foreign antigen on its surface to T cells (macrophages, dendritic cells, and body cells infected by a virus). Presents the antigen to T cells with a complementary receptor
Plasma cell	A differentiated B cell (B effector cell) packed with rough endoplasmic reticulum, which secretes large numbers of one type of antibody



An **antibody**: the **variable regions** at the tips bind a specific antigen.

Common mistake: Antibodies are made by plasma cells, not by 'B memory cells' or by macrophages, and they are proteins, not cells.

B cells and T cells in the immune response Spec 6.10

The specific immune response depends on lymphocytes, white blood cells made in the bone marrow. Each lymphocyte has receptors for one antigen. **B cells** mature in the bone marrow and **T cells** mature in the thymus.

Sequence of the response to a new pathogen

1. A macrophage or other antigen-presenting cell engulfs the pathogen and displays its antigens on its surface.
2. A T helper cell with a receptor complementary to this antigen binds to the antigen-presenting cell and is activated. It divides by mitosis (clonal expansion) to make a clone of T helper cells.

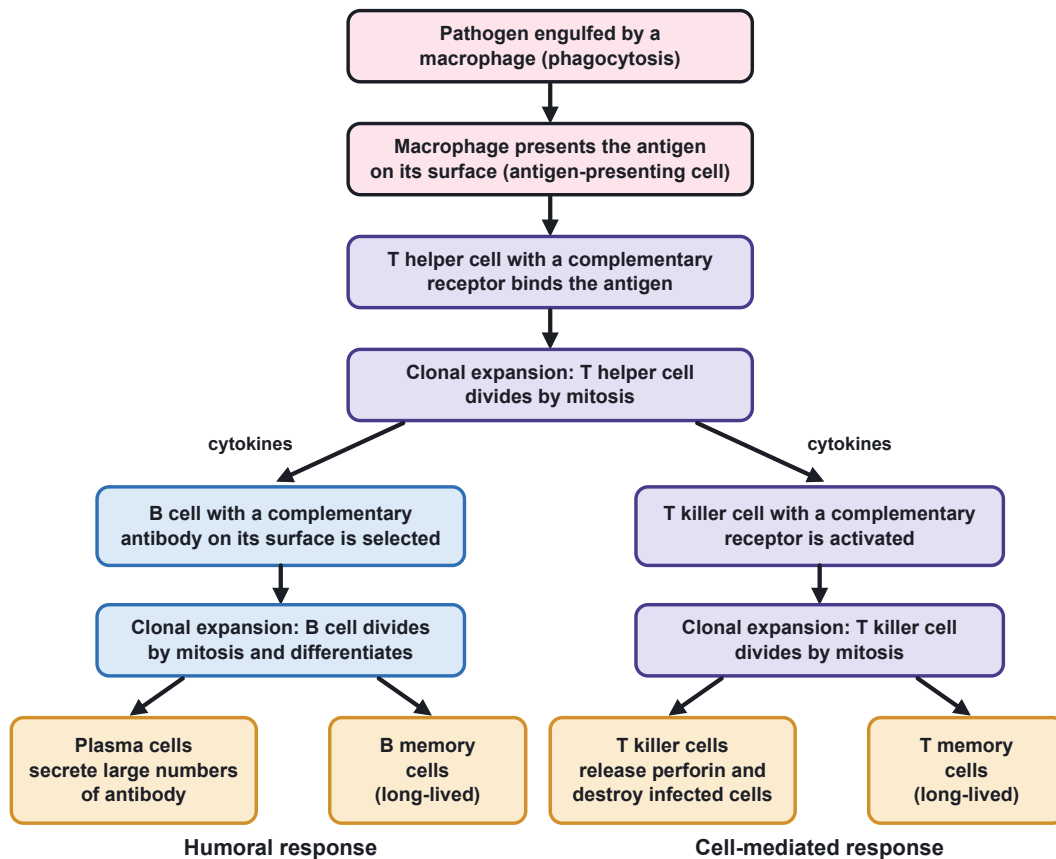
3. T helper cells release cytokines. These stimulate B cells whose antibodies on the surface are complementary to the antigen (clonal selection) to divide by mitosis, and they also activate T killer cells.
4. The B cells differentiate into plasma cells (B effector cells), which secrete large quantities of antibody into the blood, and into B memory cells.
5. Activated T killer cells find body cells displaying the antigen (for example virus-infected cells) and kill them.
6. T and B memory cells remain in the blood after the infection has been cleared.

Roles of B cells and T cells		
Cell	Type	Role
B cell	B effector (plasma cell)	Makes and secretes antibodies for the humoral response; short-lived
B cell	B memory cell	Long-lived; remains in the blood and, on re-exposure to the same antigen, divides rapidly to form plasma cells and more memory cells
T cell	T helper cell	Binds antigen presented by APCs and releases cytokines that activate B cells, T killer cells and macrophages
T cell	T killer (cytotoxic) cell	Kills infected body cells and cancer cells. Releases perforin to make pores in the target cell membrane and enzymes that cause apoptosis (cell-mediated response)
T cell	T memory cell	Long-lived; gives a faster response of killer and helper T cells when the antigen is met again

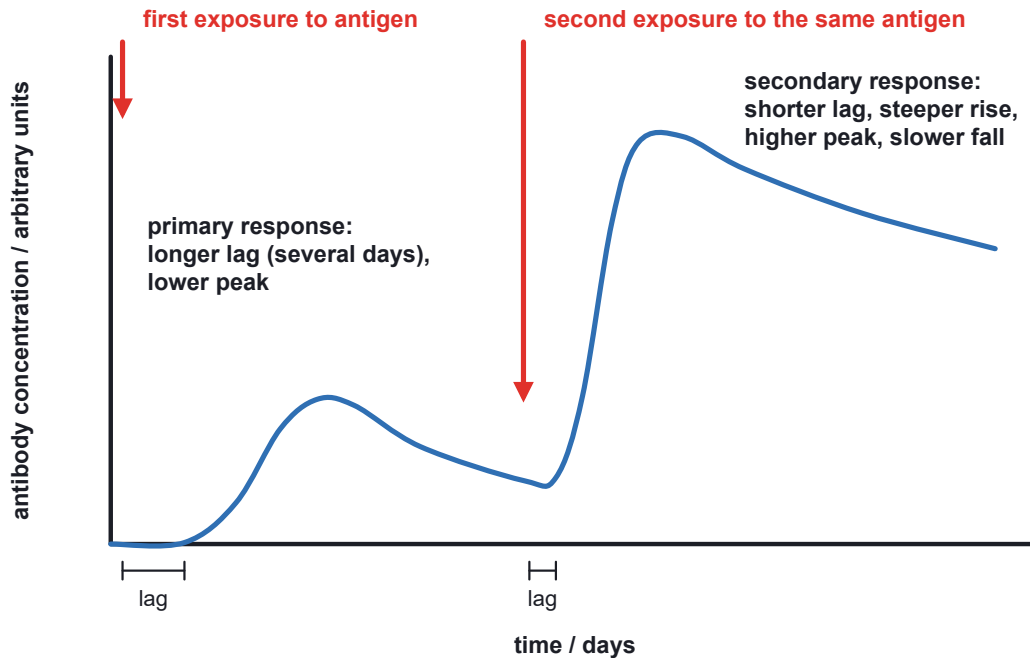
The antibody response by B cells is called the **humoral response** (antibodies are in the body fluids). The response by T cells, which directly kill infected cells, is the **cell-mediated response**.

Primary and secondary responses

The first time a pathogen is met (the **primary response**), only a few B and T cells have receptors for its antigens, so it takes several days for enough plasma cells to make antibody, and the person may become ill. On a second exposure (the **secondary response**), B and T memory cells are activated immediately and divide to produce a large number of plasma cells much faster. Antibody concentration rises faster, reaches a higher level and lasts longer, so the pathogen is destroyed before symptoms appear.



Clonal selection and the humoral and cell-mediated responses.



Primary and **secondary** immune responses: memory cells give a faster, larger response.

Exam tip: Quote the cells in order: antigen-presenting cell, T helper, then B cell and T killer. Questions often lose marks by missing the T helper step.

Natural, artificial, active and passive immunity Spec 6.11

Types of immunity		
Type	How it develops	Example
Natural active	Infection by a pathogen: the body makes antibodies and memory cells	Recovering from chickenpox
Natural passive	Antibodies pass from mother to baby: IgG across the placenta, and IgA in colostrum and breast milk	Newborn protected by mother's antibodies
Artificial active	Vaccination: weakened or inactivated pathogen, or its antigens, are given and the body makes antibodies and memory cells	MMR vaccine, tetanus vaccine
Artificial passive	Injection of ready-made antibodies from another person or animal	Antivenom after a snake bite; tetanus immunoglobulin after a deep wound

Active compared with passive immunity		
Feature	Active	Passive
Antibodies made by	The person's own plasma cells	Another person or animal
Memory cells formed	Yes	No
Time to protection	Days to weeks (primary response)	Immediate
Length of protection	Long-lasting (years or life)	Short (weeks to months) as antibodies are broken down

Vaccines contain antigens but do not cause the disease. They stimulate the primary response, so memory cells are in place. If the real pathogen is met later, the secondary response is fast enough to prevent symptoms. Vaccination of a high proportion of the population also protects those who are not vaccinated, because the pathogen has fewer people to spread between (herd immunity).

Common mistake: 'Natural' and 'artificial' describe how the antibodies or antigens get in (infection or mother, versus medicine). 'Active' and 'passive' describe whether the person makes their own antibodies. Do not mix these up.

The evolutionary race between pathogens and hosts Spec 6.12

Pathogens have short generation times and high mutation rates, so they evolve quickly. A pathogen that survives host defences reproduces and passes on the allele. Hosts with a more effective immune response also survive and reproduce, so their offspring are better defended. The pressure from each side selects for change in the other: an **evolutionary race**. Evidence is shown by the many ways pathogens evade the immune system.

Evasion mechanisms shown by pathogens

Mechanism	Example	Why it works
Antigenic variation: changing surface antigens	Influenza virus (mutations and reassortment give new surface proteins); HIV (reverse transcriptase makes many errors); <i>Trypanosoma</i> (switches its surface protein)	Memory cells and antibodies for the old antigen no longer recognise the new type, so there is no secondary response and a new vaccine may be needed
Hiding inside host cells	HIV (inside helper T cells, as a latent provirus); <i>Mycobacterium tuberculosis</i> (inside macrophages); malaria parasite (liver and red blood cells)	Antibodies cannot reach the pathogen inside the cell, and few or none of its antigens are displayed on the host cell surface
Killing or disabling immune cells	HIV destroys helper T cells	Fewer B cells and killer T cells are activated, so the immune response collapses
Preventing digestion by phagocytes	<i>M. tuberculosis</i> stops lysosome fusion; some bacteria have a capsule	Pathogen survives phagocytosis, or is not engulfed at all

These mechanisms are examples of adaptations produced by natural selection. Because the pathogen population keeps producing new variants, the host immune system, vaccines and drugs always have to respond to a moving target.

Exam tip: To support the theory, name the mechanism, give the organism, and link it to the host: 'HIV mutates rapidly, so new antigen variants are not recognised by memory cells'.

Quick check

1. How does lysozyme kill bacteria?
2. How does interferon protect a neighbouring cell?
3. Which cell secretes antibodies?
4. What is the difference between natural passive and artificial passive immunity?
5. Why does antigenic variation help a pathogen survive?

4 Antibiotics and infection control Spec 6.13–6.15 A Level only

Bacteriostatic and bactericidal antibiotics Spec 6.13

Bacteriostatic compared with bactericidal antibiotics		
Feature	Bacteriostatic	Bactericidal
Effect on bacteria	Prevents growth and reproduction; bacteria stay alive	Kills bacteria
Typical mechanism	Inhibits protein synthesis by binding to bacterial (70S) ribosomes, or blocks folic acid synthesis (sulfonamides)	Prevents formation of cross-links in the peptidoglycan cell wall, so the wall is weak and the bacterium bursts by osmosis; or damages the cell membrane; or blocks DNA or RNA synthesis (rifampicin inhibits bacterial RNA polymerase)
Examples	Tetracycline, chloramphenicol	Penicillin, rifampicin
Depends on host immune system?	Yes: the immune system must remove the bacteria once they stop multiplying	Less so: important when the immune system is weak

Antibiotics show **selective toxicity**: they target structures or processes in bacteria that human cells do not have (a peptidoglycan cell wall, 70S ribosomes). This is why they do little harm to human cells, and why they have no effect on **viruses**, which have no cell wall or ribosomes of their own and replicate inside human cells.

Overuse and misuse of antibiotics exerts selection pressure: bacteria with a mutation that gives resistance survive, reproduce and pass the allele on, producing antibiotic-resistant strains such as MRSA.

Common mistake: Do not say 'penicillin kills bacteria by bursting the cell'. Say it stops the cell wall being formed or repaired, so water enters by osmosis and the weakened wall cannot resist, and the bacterium bursts.

Core practical 14: effect of antibiotics on bacteria Spec 6.14

Core practical: Core practical 14: Investigate the effect of different antibiotics on bacteria.

Method

1. Use aseptic technique. Sterilise Petri dishes of nutrient agar; label the base with names of antibiotics, date and your initials.
2. Use a sterile pipette to put a measured volume (for example 0.1 cm³) of a broth culture of a non-pathogenic bacterium, such as *Escherichia coli* K12, on to the agar and spread it evenly over the surface with a sterile spreader to give a lawn.
3. Using sterile forceps (flamed and cooled), place sterile paper discs soaked in different antibiotics at equal spacing on the lawn, and press gently. Include a control disc soaked in sterile water (or no antibiotic).
4. Replace the lid, tape it with two or four short pieces of tape, invert the plate and incubate at 25 °C for 24 to 48 hours (not above 30 °C).
5. Measure the diameter of each clear zone (zone of inhibition) with a ruler, across the centre of the disc, in two directions at right angles, and take the mean. Calculate the area of the zone using πr^2 if needed.
6. Disinfect or autoclave the plates before disposal. Do not open the plates after incubation.

Variables	
Type	Examples
Independent variable	Type of antibiotic (or concentration of one antibiotic)
Dependent variable	Diameter or area of the zone of inhibition
Controlled variables	Volume and type of bacterial culture; agar type and depth; disc size and volume of antibiotic; incubation time and temperature; spacing of discs
Control	A disc without antibiotic (sterile water) shows that the disc and the water do not themselves stop growth

Interpreting results

Around an effective antibiotic the bacteria cannot grow, leaving a clear **zone of inhibition**. A bigger zone means the bacteria are more susceptible to that antibiotic (or the antibiotic diffuses well), so the bigger the zone, the more effective the antibiotic. No zone means the bacteria are resistant. Colonies growing within the zone suggest resistant mutants.

Area of a zone of inhibition

The diameter of a zone of inhibition around a disc was 22 mm. Calculate the area of the zone.

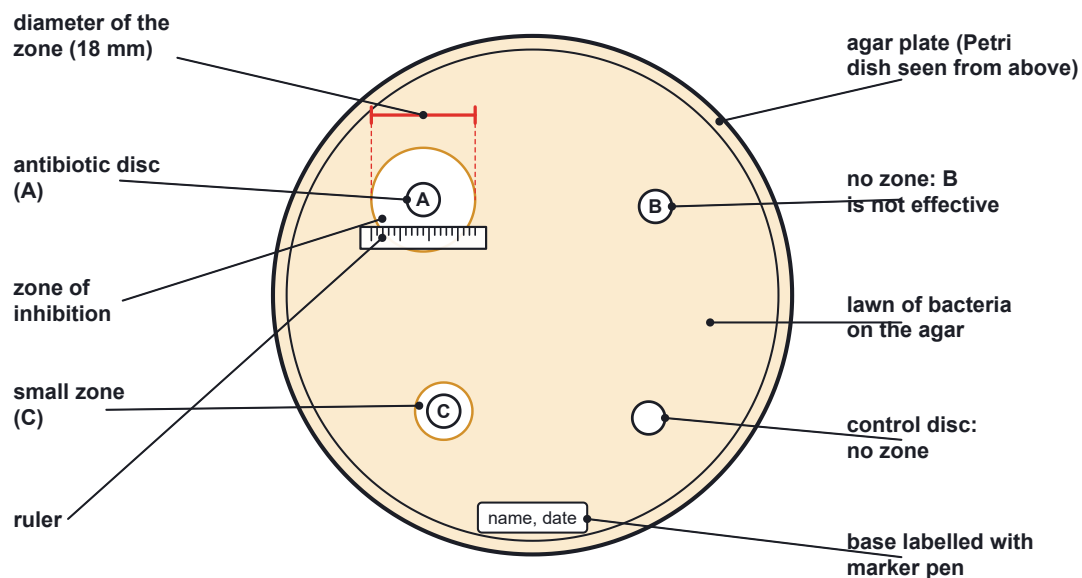
1. Radius = $22 \div 2 = 11$ mm.
2. Area = $\pi r^2 = \pi \times 11^2 = \pi \times 121$.
3. Area = 380 mm² (to 3 significant figures).

Answer: 380 mm²

Maths skill: Compare areas, not diameters, if the question asks 'how many times larger'. Remember to halve the diameter before squaring.

Safety and limitations

- Use only non-pathogenic bacteria; do not incubate at 37 °C; keep plates taped and closed after incubation; wash hands; disinfect bench; autoclave plates.
- The zone depends on how fast each antibiotic diffuses through agar, not only on how effective it is, which limits the comparison. Zones may overlap if discs are too close.
- Results can be improved with repeats (several plates per antibiotic), a standard concentration, and standard disc size.



Core practical 14: the diameter of the **zone of inhibition** around each antibiotic disc is measured.

Hospital-acquired infections and codes of practice Spec 6.15

A **hospital-acquired infection** (HAI) is an infection that is acquired in hospital, not present when the patient arrived. Common examples are MRSA (meticillin-resistant *Staphylococcus aureus*) and *Clostridioides difficile* (*C. difficile*).

Contributory causes and the codes of practice that follow	
Contributory cause	Code of practice
Many patients have weakened immune systems (elderly, very young, after surgery, on immunosuppressant drugs)	Isolate vulnerable and infected patients; screen patients for MRSA on admission; protective clothing for staff and visitors
Close contact between patients, and staff moving between patients, spreads pathogens by hands and equipment	Hand washing with soap or alcohol gel before and after contact with each patient; bare below the elbow; limited visiting; barrier nursing
Open wounds, surgery, catheters, drips and ventilators give pathogens a route into the body	Sterilise surgical instruments; use aseptic technique; remove invasive devices as soon as possible; cover wounds
Contaminated surfaces and equipment, and dirty air	Regular cleaning and disinfection; single-use items; good ventilation
Overuse and misuse of antibiotics selects for antibiotic-resistant bacteria; killing of gut flora allows <i>C. difficile</i> to grow	Antibiotic stewardship: prescribe only when necessary (never for viral infections); use narrow-spectrum antibiotics where possible; take cultures and test sensitivity first; follow local guidelines; patients take the course exactly as prescribed; keep some antibiotics in reserve

Hospitals also carry out surveillance (monitoring infection rates and the species involved) so that outbreaks are found early and codes of practice are reviewed.

Exam tip: In a six-mark question, give both parts: cause (for example, 'weakened immune systems') and the matching measure (for example, 'isolate patients'). Marks are for the link.

Quick check

- 1 . What is the difference between bacteriostatic and bactericidal?
- 2 . Why do antibiotics not harm human cells?
- 3 . What is a zone of inhibition?
- 4 . Why is a control disc of sterile water used?
- 5 . Give two contributory causes of hospital-acquired infections.

5 Decay, DNA techniques and forensics

Spec 6.16–6.20

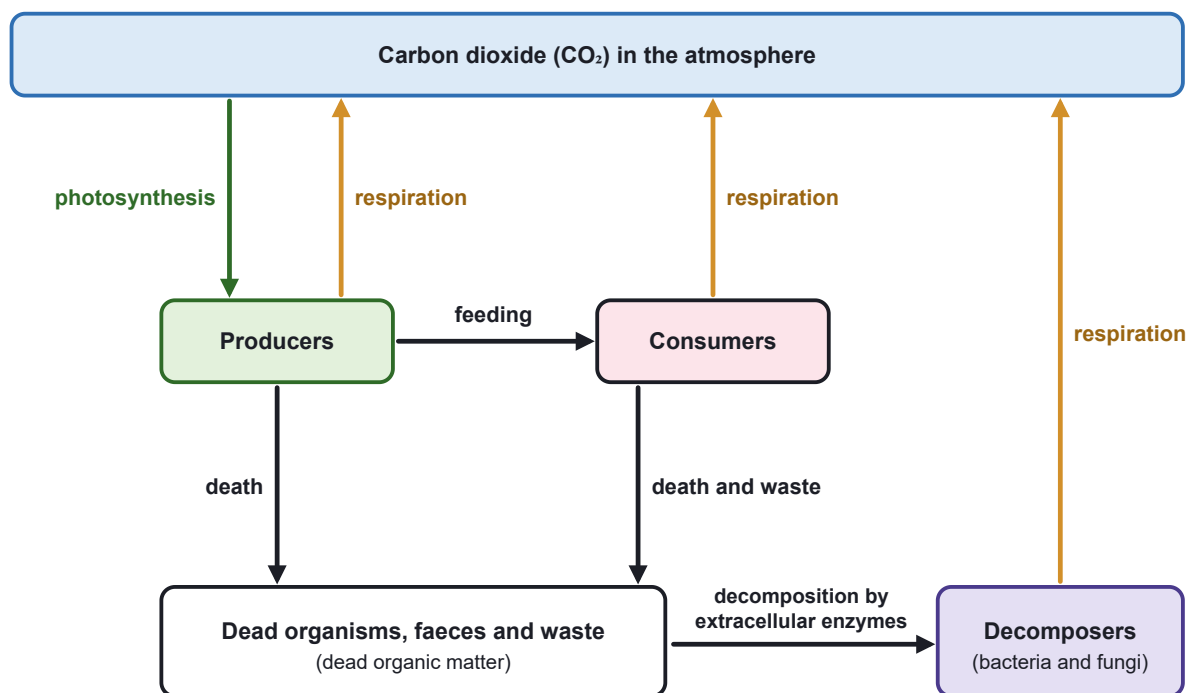
A Level only

Microorganisms, decomposition and recycling carbon Spec 6.16

Decomposers are mostly saprotrophic bacteria and fungi. They feed by **extracellular digestion**: they secrete enzymes (proteases, lipases, carbohydrases such as cellulase) on to dead plants and animals, faeces and other waste. The enzymes hydrolyse large molecules into soluble ones (amino acids, fatty acids, glucose), which the microorganism absorbs. They use some of these for growth and respiration.

1. Producers take carbon dioxide from the atmosphere and fix it into organic compounds by photosynthesis.
2. Carbon passes to consumers when they feed.
3. Producers, consumers and decomposers all release carbon dioxide into the atmosphere by respiration.
4. Dead organisms, faeces and waste are digested by saprotrophs. Carbon in the organic molecules is converted to carbon dioxide when the decomposers respire, and the carbon is available again to producers.

Decomposition is fastest when conditions suit the microorganisms: warm temperatures (enzymes near their optimum), moisture, oxygen for aerobic respiration, and a suitable pH. Cold, dry, waterlogged, anaerobic or acidic conditions slow it, which is why peat and bog bodies persist. Without microorganisms, carbon would remain locked up in dead matter and producers would run short of carbon dioxide.



The **carbon cycle**, showing the role of decomposers.

Exam tip: Say that decomposers secrete extracellular enzymes, absorb the digested products and respire, releasing CO₂. 'They break down dead things' alone gains no marks.

Amplifying DNA by the polymerase chain reaction Spec 6.17

The **polymerase chain reaction (PCR)** makes millions of copies of a particular piece of DNA from a very small sample, such as a hair root at a crime scene. The tube contains:

- the DNA template (the sample)
 - two **primers**: short single-stranded DNA sequences complementary to the start of each strand of the target sequence
 - free DNA nucleotides (dATP, dTTP, dCTP, dGTP)
 - **Taq polymerase**, a DNA polymerase from the bacterium *Thermus aquaticus* that is heat-stable and so is not denatured at 95 °C
 - a buffer solution with magnesium ions
1. Denaturation: heat to about 95 °C. Hydrogen bonds between the complementary base pairs break, separating the two strands.
 2. Annealing: cool to about 55 to 65 °C. Primers bind (anneal) by hydrogen bonds to their complementary sequences at the ends of the target DNA on each strand.
 3. Extension: heat to about 72 °C, the optimum for Taq polymerase. It adds free nucleotides to the 3' end of each primer, building a complementary strand, to make two double-stranded copies.
 4. The cycle is repeated, typically 25 to 35 times in an automated thermal cycler. The amount of DNA doubles each cycle, so after n cycles there are 2^n copies of the target from each original molecule.

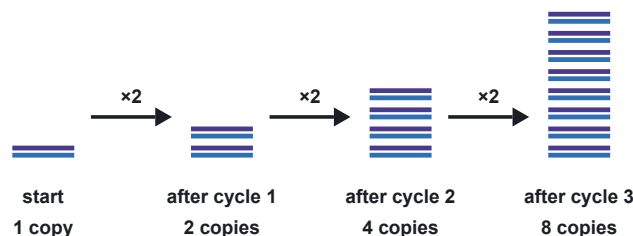
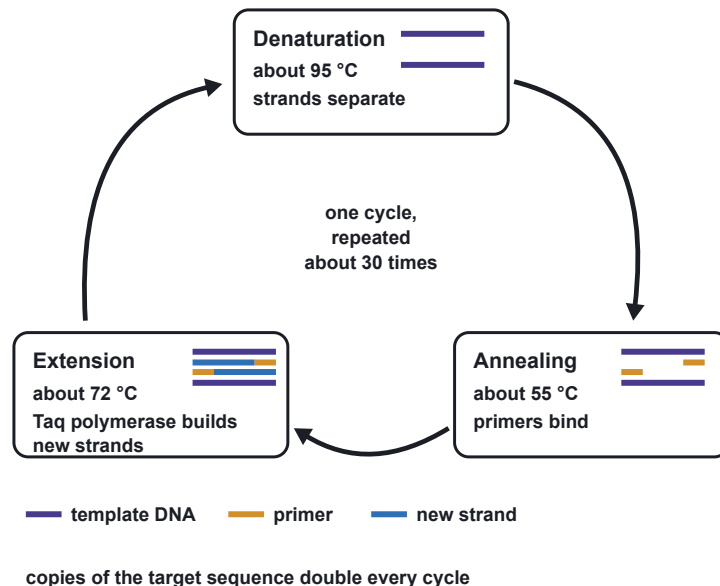
The primers make the technique specific for one sequence, and the use of a heat-stable enzyme means that the polymerase does not have to be added again each cycle. A very small sample can be amplified enough to be analysed. Contamination with other DNA is a risk, so great care is needed.

Amplification

How many copies of a DNA sequence would there be after 25 cycles of PCR starting from one molecule?

1. The number doubles each cycle, so copies = 2^n .
2. $2^{25} = 33\,554\,432$.

Answer: About 3.4×10^7 copies



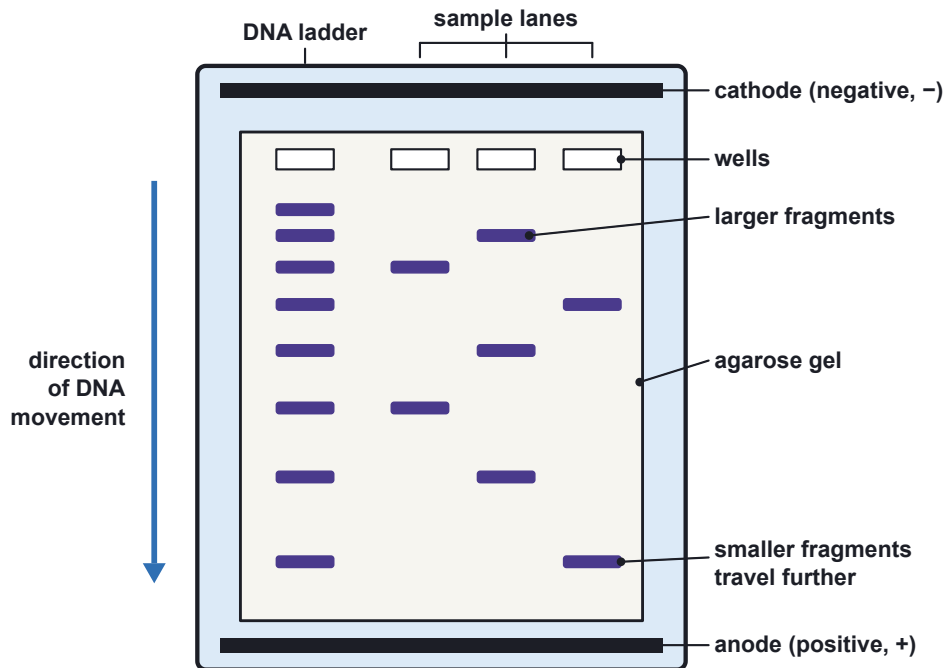
One cycle of **PCR**: the number of copies doubles every cycle.

Separating DNA fragments by gel electrophoresis Spec 6.18

1. DNA is cut into fragments with restriction enzymes (or amplified by PCR).
2. A gel made of agarose (a porous polysaccharide) is covered with a buffer solution that conducts electricity. Samples, mixed with loading dye to make them dense and visible, are put into wells at the negative end. A mixture of fragments of known lengths (a **DNA ladder**) is put in one well.
3. An electric current is applied. DNA is negatively charged because of the phosphate groups, so it moves through the gel towards the positive electrode (anode).
4. The gel acts as a mesh. Shorter fragments move through it more easily, so they travel further in a given time, and longer fragments move more slowly. Fragments of the same length form a band.
5. The DNA is made visible by staining with a fluorescent dye that binds to DNA and is viewed in UV light, or by DNA probes that carry a fluorescent or radioactive label.

The length of a fragment is estimated by comparing how far its band has moved with the bands of the DNA ladder. A graph of $\log_{10}$ fragment length against distance moved is roughly a straight line.

Exam tip: The two key ideas are charge (negative phosphate groups, so moves to the anode) and size (smaller fragments move further through the gel). Fragments are separated by length, not by mass of the whole sample.



Gel electrophoresis: DNA is negatively charged, so it moves towards the **anode**; smaller fragments move further.

DNA profiling Spec 6.19

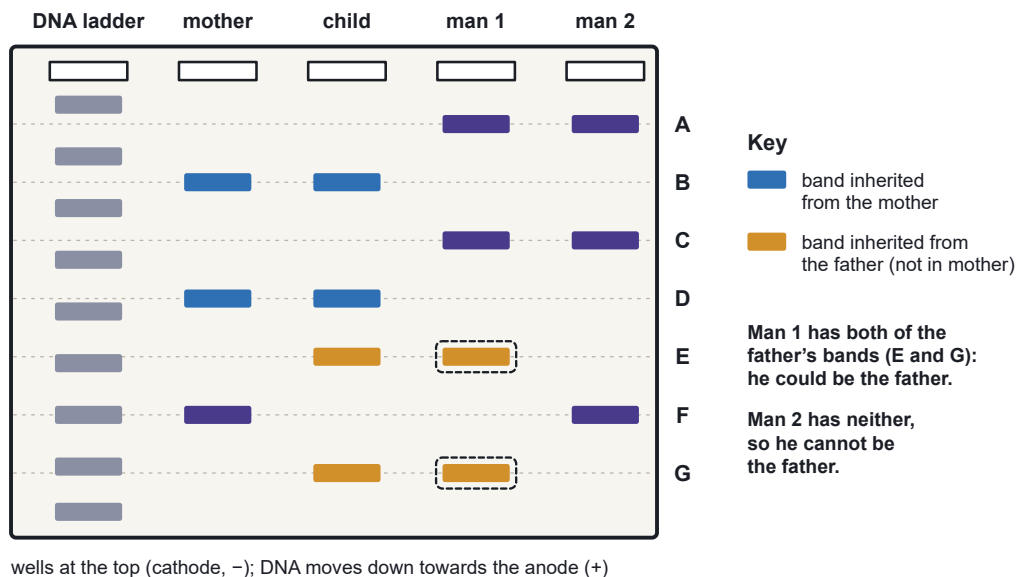
Much of the genome is non-coding DNA, which contains short sequences repeated many times in a row (short tandem repeats, STRs). The number of repeats at each position (locus) differs between individuals, so the length of the fragment differs. Each person inherits one set from each parent, so, apart from identical twins, no two people have the same pattern across several loci.

1. DNA is extracted from a sample (blood, hair root, saliva, tissue or seed).
2. Regions containing STRs are amplified by PCR using primers (often labelled with a fluorescent tag).
3. The fragments are separated by gel electrophoresis and detected, giving a pattern of bands (the DNA profile).
4. Profiles are compared. Bands at the same position in two samples match.

Uses

Uses of DNA profiling	
Use	How profiles are used
Identification: forensic	DNA from a crime scene is compared with DNA from suspects or a database; a match of many bands makes it very unlikely that the DNA is from anyone else
Identification: victims, species and products	Identifies remains; identifies species or individual animals, for example illegal ivory or poached animals; identifies plant varieties or checks the origin of food or timber
Genetic relationships: individuals	Paternity and maternity: every band in a child's profile must be present in the mother or the father. The father's bands that the child has but the mother does not show who the father could be
Genetic relationships: plants and animals	Measures the relatedness of individuals in a breeding programme or wild population, so that inbreeding can be reduced; used to show relationships between species and varieties

Maths skill: Probability: if the chance of a match at one locus is 1 in 10 and loci are independent, the chance of a match at five loci is $(1/10)^5 = 1$ in 100 000. More loci mean a more reliable identification.



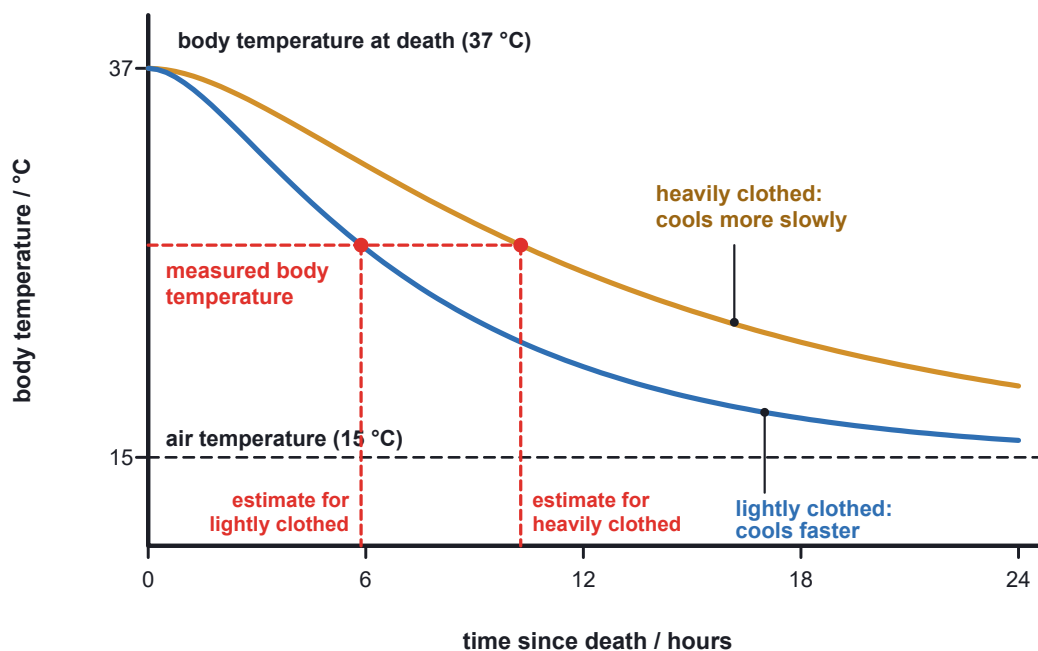
A **paternity** test: every band in the child must come from the mother or the father, so only man 1 could be the father.

Estimating time of death
Spec 6.20

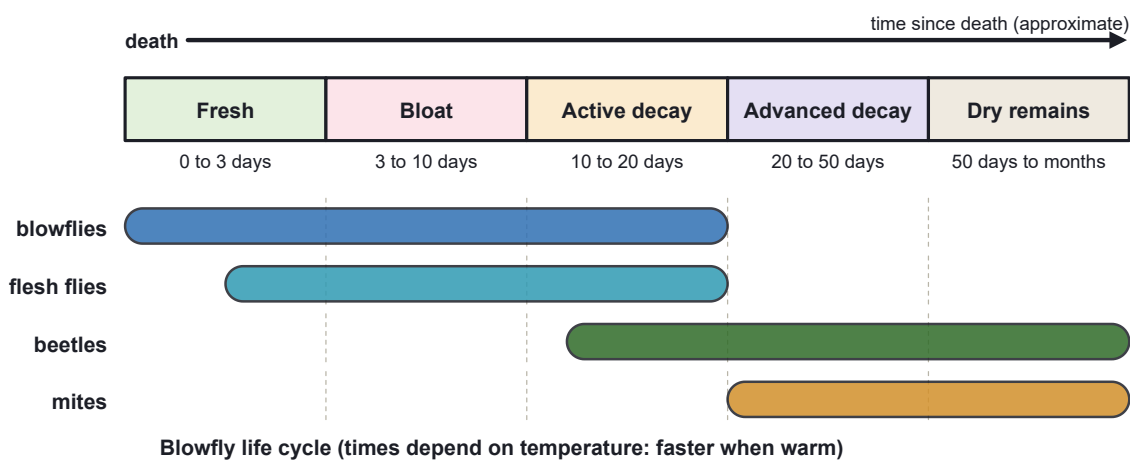
Evidence used to estimate time of death	
Evidence	What is looked at and why it changes with time
Body temperature	After death, metabolism stops and the body cools towards the temperature of its surroundings. Core (rectal) temperature is measured and compared with 37 °C (cooling curve). Speed of cooling depends on air temperature, clothing, body mass and wind or water. Most useful in the first 24 hours or so
Degree of muscle contraction (rigor mortis)	No new ATP is made after death, so myosin heads cannot detach from actin and muscles stay contracted and stiff. It starts in smaller muscles in the face a few hours after death, spreads, and passes off after about 1 to 2 days as muscle proteins are broken down by enzymes. Warm conditions make it start and end sooner
Extent of decomposition	Bacteria in the gut, and enzymes from dead cells (autolysis), digest tissues. In sequence the body shows discolouration, bloating due to gases, softening and liquefaction of tissues and finally dry remains and bones. Slower in cold, dry or burial conditions
Stage of succession	Organisms colonise a body in a sequence as its condition changes: blowflies arrive within minutes to hours, then flesh flies, then beetles that feed on drier tissue, later mites and species that feed on hair and bone. The community present shows the stage of decay
Forensic entomology	Blowflies lay eggs on a body, which hatch into larvae (maggots), grow through stages (instars), pupate and emerge as adults. Species and size of the oldest larvae, or the stage of pupae, give the time since the eggs were laid, allowing for temperature (growth is faster when warm). Eggs are laid only after death, so the age of the oldest larvae gives a minimum time since death

Each method is only an estimate, and the earlier the body is found, the smaller the error. Conditions such as air temperature, burial, water, clothing and whether the body is indoors alter all of the changes. A forensic scientist therefore uses evidence from more than one method, records the environmental conditions at the scene and takes samples of insects at once.

Exam tip: Always say what changes and why: 'rigor mortis occurs because ATP is no longer produced, so myosin heads cannot detach from actin'. For succession, say that different species arrive as the condition of the body changes.



Body cooling curves: a measured temperature gives an estimate of time since death, which depends on clothing.



Times are approximate: decomposition is faster in warm, moist conditions and slower in cold, dry ones.

Decomposition and succession: different insects colonise a body as its condition changes (times approximate).

Quick check

- 1 . How do saprotrophs digest dead matter?
- 2 . Why is Taq polymerase used in PCR?
- 3 . Why do shorter DNA fragments travel further in gel electrophoresis?
- 4 . What is an STR?
- 5 . Why does rigor mortis happen?

Quick check answers

Culturing and measuring microorganisms

1. So condensation collects in the lid and does not drip on to the agar and spread colonies.
2. Only living cells that can divide to form colonies.
3. Lag, exponential (log), stationary, death (decline).
4. $n = (\log_{10} N_t - \log_{10} N_0) \div 0.301$.
5. To set zero absorbance for the broth alone, so readings show only the cloudiness caused by the cells.

Bacteria, viruses and infection

1. The capsid is the protein coat around the viral nucleic acid. The envelope is an outer lipid layer, taken from the host cell membrane, present in some viruses.
2. They have no ribosomes or metabolism, so they use the host's enzymes, ribosomes and ATP.
3. A DNA copy of the HIV genome made by reverse transcriptase and integrated into the host cell's chromosome.
4. Inside macrophages in the lungs, which cannot digest it.
5. They compete with pathogens for nutrients and space, and they produce substances that inhibit pathogens.

The immune response

1. It hydrolyses peptidoglycan in the cell wall, so the bacterium bursts by osmosis.
2. It switches on genes for enzymes that break down viral mRNA and block protein synthesis, so the virus cannot replicate.
3. The plasma cell (B effector cell).
4. Natural passive: antibodies pass from mother to baby. Artificial passive: ready-made antibodies are injected.
5. Memory cells and antibodies for the old antigen do not recognise the new antigen, so there is no fast secondary response.

Antibiotics and infection control

1. Bacteriostatic antibiotics stop bacteria growing and dividing. Bactericidal antibiotics kill them.
2. They show selective toxicity: they target structures that bacteria have and human cells lack, such as the peptidoglycan wall and 70S ribosomes.
3. The clear area around an antibiotic disc where bacteria cannot grow.
4. To show that the disc and water do not themselves stop bacterial growth.
5. Weakened patients; poor hygiene or contact between patients; use of invasive devices; overuse of antibiotics.

Decay, DNA techniques and forensics

1. They secrete extracellular enzymes on to it and absorb the soluble products.
2. It is heat-stable, so it is not denatured at about 95 °C and works at 72 °C.
3. They move more easily through the mesh of the gel.
4. A short tandem repeat: a short sequence of non-coding DNA repeated many times, with a different number of repeats in different people.
5. ATP is no longer made, so myosin heads cannot detach from actin and muscles stay contracted.