Edexcel International A Level (IAL) Biology

Your notes

Microbiology

Contents

Your notes

Culturing Microorganisms

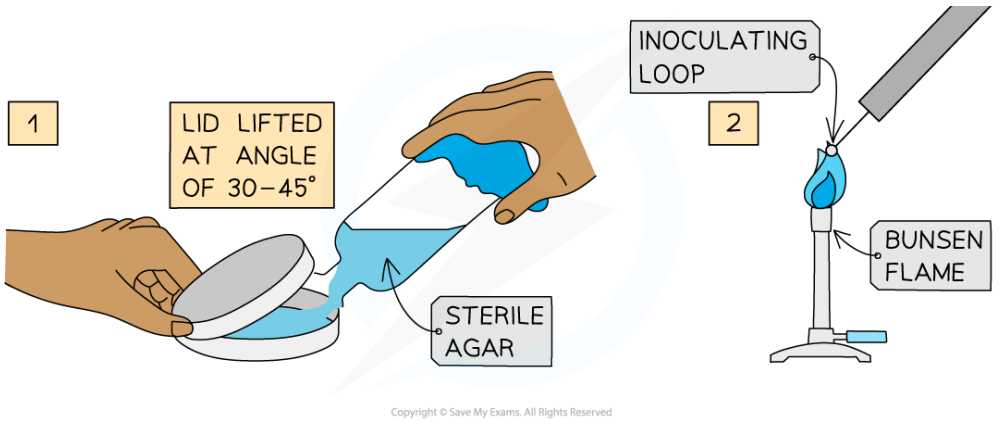
- Most microorganisms are only visible using a **microscope**
- For microbe investigations it is therefore necessary to **culture microorganisms**
  - This **grows enough** microorganisms **to make measurements** during investigations
  - E.g. bacteria reproduce by cloning themselves, so when they are grown on agar gel they form a **colony** of identical individuals that is visible to the naked eye
- Microorganisms must be provided with everything they **need to grow** such as
  - Nutrients
  - Oxygen
    - Note that anaerobic microorganisms would require the absence of oxygen
  - Optimum pH
  - Favourable temperature
- Microorganisms should be cultured with great care
  - There is always the risk that a mutation could lead to the formation of pathogenic **strains**
  - Pathogenic bacteria from the environment could **contaminate** the bacterial culture being investigated
- Remember to
  - Follow health and safety precautions
  - Ensure that all equipment are **sterilised** before culturing the bacteria
    - Sterilising involves killing microorganisms, e.g. by heating to a high temperature or the use of antimicrobial chemicals
  - Keep the culture in the laboratory
  - Seal cultures in a plastic bag and sterilise at high temperature and pressure before disposal

Culturing steps

- **Obtain a supply** of the type of microorganism to be cultured
- Provide them with the **correct type of nutrients** to facilitate growth
  - A **nutrient growth medium** (plural media) containing carbon, nitrogen, and minerals is typically used

- The medium could be in the form of a **liquid culture** or a **solid nutrient agar,** a type of gel extracted from seaweed

- Ensure that the nutrient medium is kept under **sterile conditions** until use
- By adjusting the type of nutrients in the medium, conditions will be created for the optimal growth of a certain type of microorganism; this is known as a **selective medium**
  - Selective media can be used to **identify mutant strains** of microorganisms or those that are **resistant to antibiotics**
  - They are also useful for **identifying genetically modified microorganisms**
- Microorganisms are introduced to a growth medium using **inoculation** with a sterilised inoculation loop
- Inoculation can be used to **transfer microorganisms between media**, e.g.
  - From agar gel into a liquid culture flask
  - From a liquid culture flask onto agar gel
- The new medium should be sealed or covered to **avoid contamination** from microorganisms in the air; if growing aerobic microorganisms any seal or cover should not be airtight
  - Flasks can be sealed with a sterile cotton wool stopper
  - Petri dishes can be covered with a lid
- **Label** the medium clearly and **incubate** at around 20 °C to prevent the growth of pathogenic microorganisms
  - Microorganisms that are pathogenic to humans will grow best at around 37 °C
  - In a hospital or research laboratory a higher temperature might be used to obtain faster results



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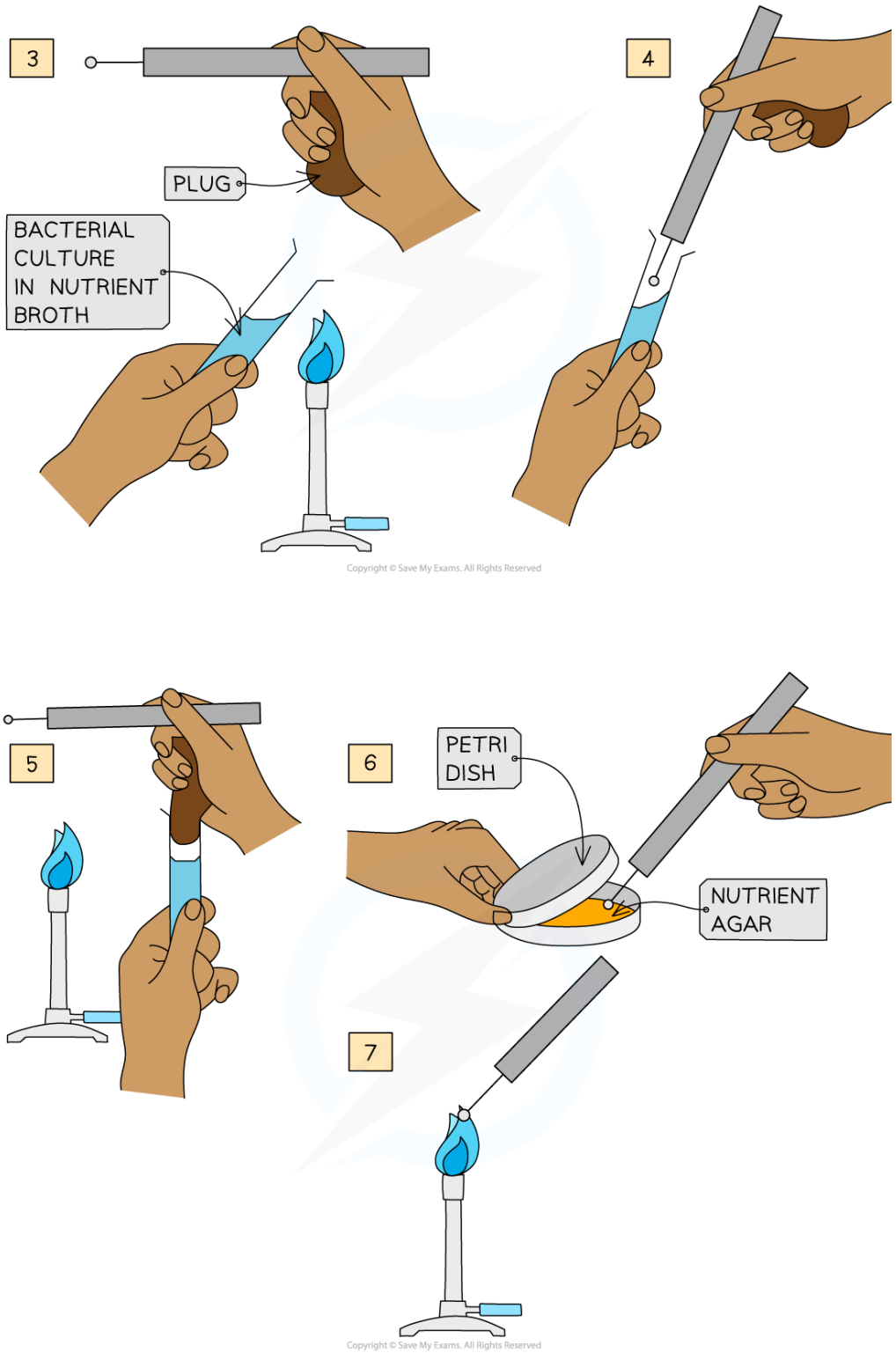

A metal inoculating loop can be used to transfer microorganisms from a liquid broth medium onto an agar gel medium. A Bunsen burned enables sterilisation of the loop between uses.

Growing a single type of microorganism

Your notes

- In order to grow a single type of microorganism, or a **pure culture**, the specific microorganism must be **isolated**
- This can be done by using knowledge about the needs of the microorganism to be cultured or those of microorganisms that may contaminate the culture
- Examples include
  - Growing the culture under either **aerobic** or **anaerobic conditions** to reduce the variety of microorganisms in the culture
  - Using a **selective medium** that is tailored to the specific requirements of the desired microorganism
  - **Indicator media** can provide a colour change to distinguish desired colonies from the rest
- Being able to isolate pathogenic microorganisms is useful in the diagnosis and treatment of diseases

Your notes

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Measuring the Growth of Microorganisms

- Bacteria and most other microorganisms are **too small** to count with the naked eye
- There are a **variety of methods** that can be used to **count** microorganisms and it is important to be able to make an appropriate choice when conducting investigations
- These methods include
  - Cell counts
  - Dilution plating
  - Measuring area and mass
  - Optical methods

Cell counts

- A **microscope** and **haemocytometer** can be used to count **single-celled microorganisms**
  - A haemocytometer is a microscope slide with a rectangular chamber that is marked with grid lines
  - The chamber can hold a standard volume of 0.1 mm^3
  - Haemocytometers were originally used to count blood cells
    - Haemo = blood
- Counting cells using a haemocytometer involves the following
  - A nutrient broth is diluted with an equal volume of **trypan blue**
    - This is a dye that will **stain dead cells blue**
    - It enables the investigator to only count the **living cells**
  - The chamber of the haemocytometer is filled with the stained nutrient broth
  - The number of living cells in the **four corner squares** of the grid are counted
    - Each corner square consists of **16 smaller squares**
    - Consistency needs to be used when deciding whether to count cells that are on the lines that border the corner squares
      - E.g. Counting cells on the top and right-hand borders and ignoring cells on the bottom and left-hand borders
  - The **mean number of cells** from the four corner squares can be calculated
- The haemocytometer is **calibrated** to allow the calculation of the number of cells in a known volume of broth

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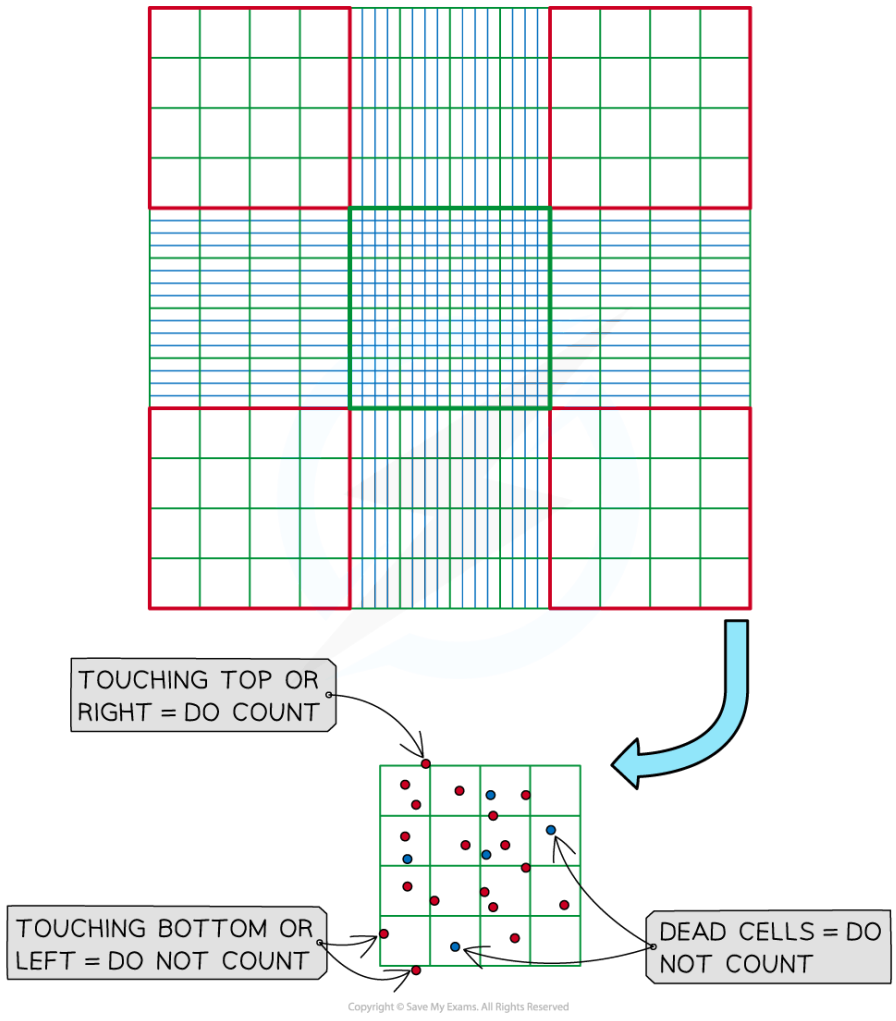
- Because the haemocytometer chamber can hold exactly 0.1 mm^3 of liquid, it is possible to estimate of the cell count in 1 ml of nutrient broth using the following calculation

No. of cells per ml nutrient broth = mean cell count x dilution factor x 10^4

- Multiplying by the dilution factor enables calculation of the bacterial cell count in the original broth rather than the diluted broth
  - The multiplication by 10^4 enables calculation of the bacterial cell count in 1 ml rather than in 0.1 mm^3
    - 1 ml = 1 cm^3
    - 1 cm^3 = 0.1 mm^3 x 10 000
    - 10 000 = 1 x **10^4**
- E.g. in a scenario where a nutrient broth is diluted by a factor of 100 and the four corner grid squares contain 20, 14, 19, and 16 cells
  - This gives a mean cell count of 17.25
  - No. cells per ml nutrient broth = 17.25 x 100 x 10^4 = 17 250 000



The living cells in the corner squares of a haemocytometer grid can be counted to determine the number of microorganisms in a standard volume of nutrient broth

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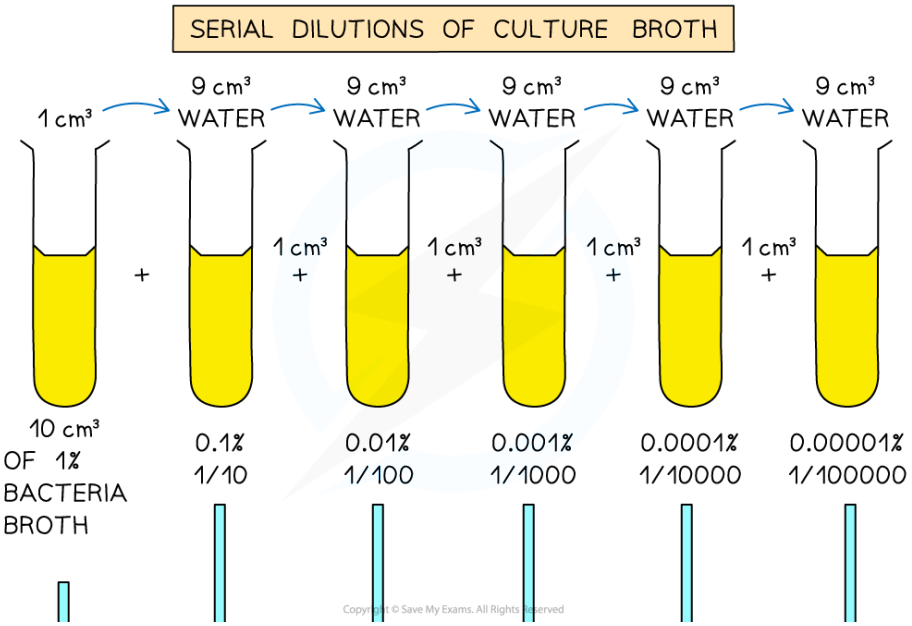
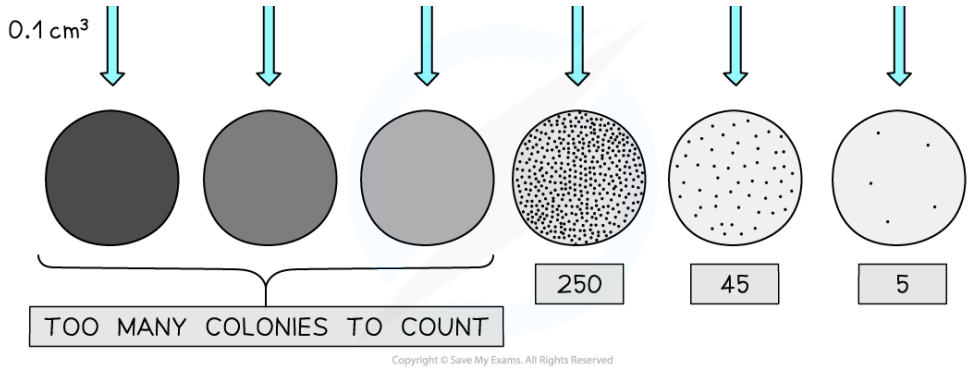
Dilution plating

- This method can be used to determine the **total viable cell count** in a nutrient broth
  - The nutrient broth is transferred to agar where the bacteria use nutrients in the agar gel to reproduce
  - A single cell that lands on agar reproduces by cloning itself, resulting in a **mass of identical cells known as a colony**
  - **Each microbial colony** that grows on agar gel originated with one viable microorganism, so can be counted as **one viable cell**
- Individual microbial colonies can be difficult to identify on an agar plate as they tend to form one large mass
- This problem can be overcome by **diluting the original cultures** before transferring the samples to agar; this reduces the number of cells in the original sample so that individual colonies are visible on an agar plate
  - This is why the technique is known as **dilution plating**
- To calculate the total viable cell count, the **number of colonies** are **multiplied by the dilution factor**
- A **mean** can be determined if more than one plate is used





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Dilution plating can provide a way to determine the total viable cell count of a microbial culture

Area and mass of fungi

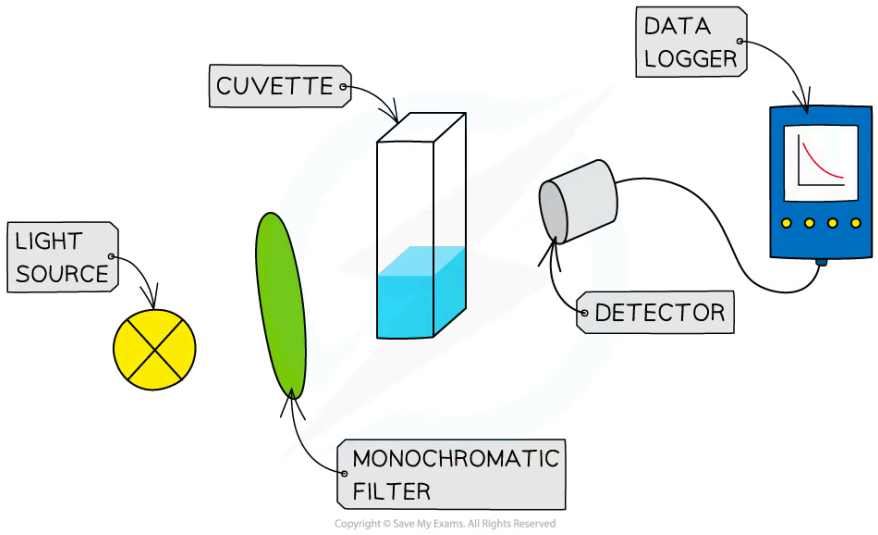
- Fungi do not always live as single-celled organisms, but can form a mass of elongated cells known as a **fungal mycelium** (plural mycelia); this means that the methods described above can be unsuitable for measuring the growth of some fungi
- Measuring the **diameter of individual areas of the mycelium** can be used to determine the growth of fungi
  - Petri dishes of agar are inoculated with fungal spores and incubated at a suitable temperature
  - The resulting areas of fungal mycelia are then measured
- This can be used to **compare growth rates** in different conditions, e.g. at different temperatures
  - The **larger** the mean diameter, the **greater the growth** of the fungi
- **Testing the dry mass** of fungi is another effective way to measure fungal growth
  - A liquid nutrient broth is inoculated with fungal spores
  - Samples of the nutrient broth are removed at set time intervals
  - The fungal mycelia are removed by filtering or centrifugation
  - The material is dried in an oven overnight and its mass measured
- The **higher the mass**, the **more fungal growth** has occurred

Optical methods

- **Turbidimetry** is a specialised form of colorimetry that can be used as an **alternative method** to measure the number of cells in a sample
  - **Turbidity** is a measure of **how cloudy** a solution is
    - More turbid = more cloudy
    - Less turbid = less cloudy

- Colorimetry uses a machine called a **colorimeter** to shine a beam of light at a sample and measure the amount of light that is either transmitted through or absorbed by the sample

- The **higher the number of cells** the **more turbid** the solution becomes
- More turbid solutions will **absorb more light** and **allow less light through**; this can be measured by a colorimeter
- This provides an **indirect measure** of the number of microorganisms present
- A **calibration curve** can be constructed by measuring the turbidity of a series of control cultures while also counting the cells in each culture using a **haemocytometer**; the results are plotted in a graph of **turbidity against cell count**
- This curve can then be used to **estimate** the cell count of unknown samples by measuring their turbidity and then reading their cell count from the graph

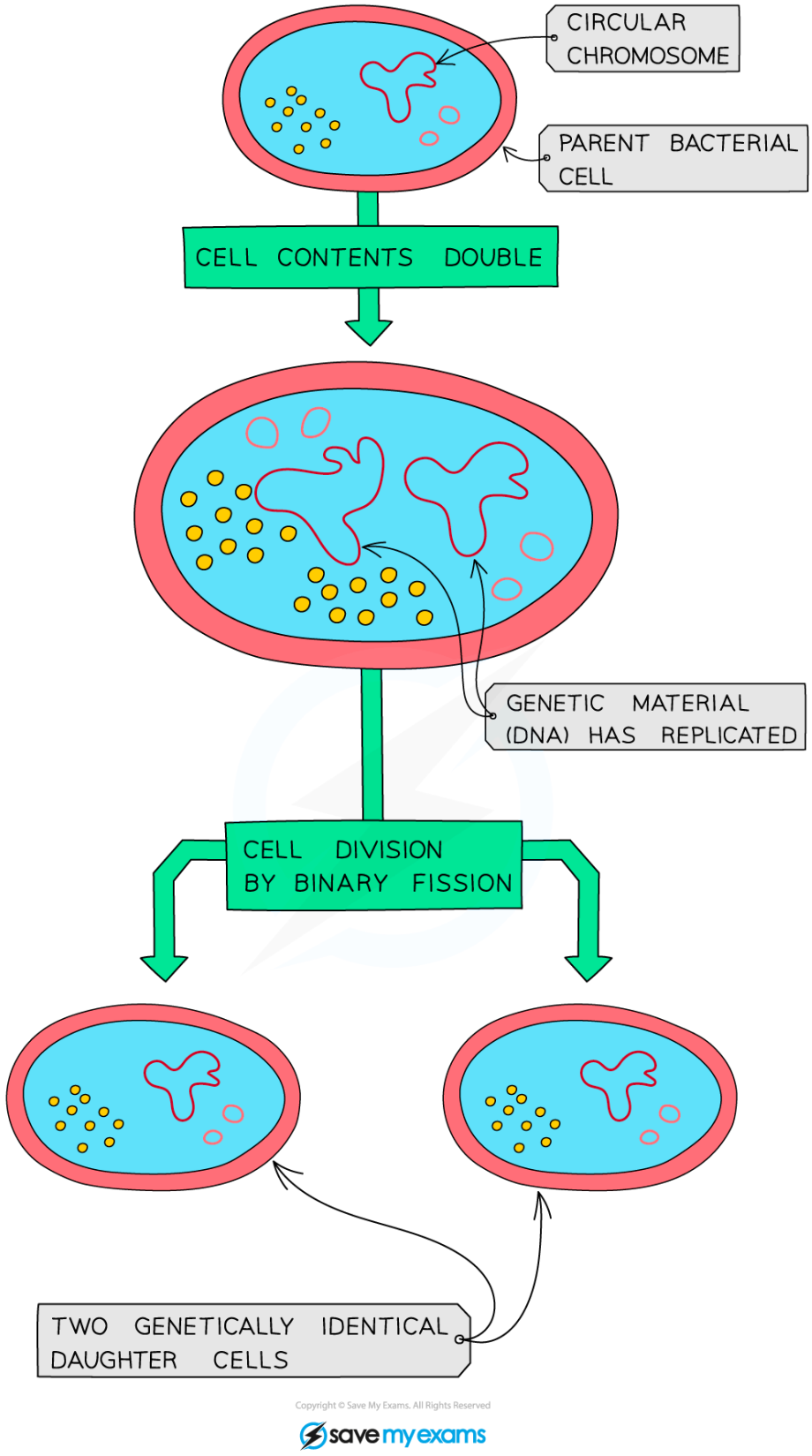


A colorimeter can be used to determine the turbidity of a solution containing microorganisms. The solution containing bacteria is placed into a container called a cuvette and the amount of light that can pass through, or is absorbed by, the solution can be measured.

Your notes

The Bacterial Growth Curve

- Bacteria divide using the process of **binary fission** during which one cell will divide into two identical cells
- The process is as follows
  - The single, circular DNA molecule undergoes DNA replication
  - Any plasmids present undergo DNA replication
  - The parent cell divides into two cells, with the cytoplasm roughly halved between the two daughter cells
  - The two daughter cells each contain a single copy of the circular DNA molecule and a variable number of plasmids



The process of binary fission produces two identical daughter cells

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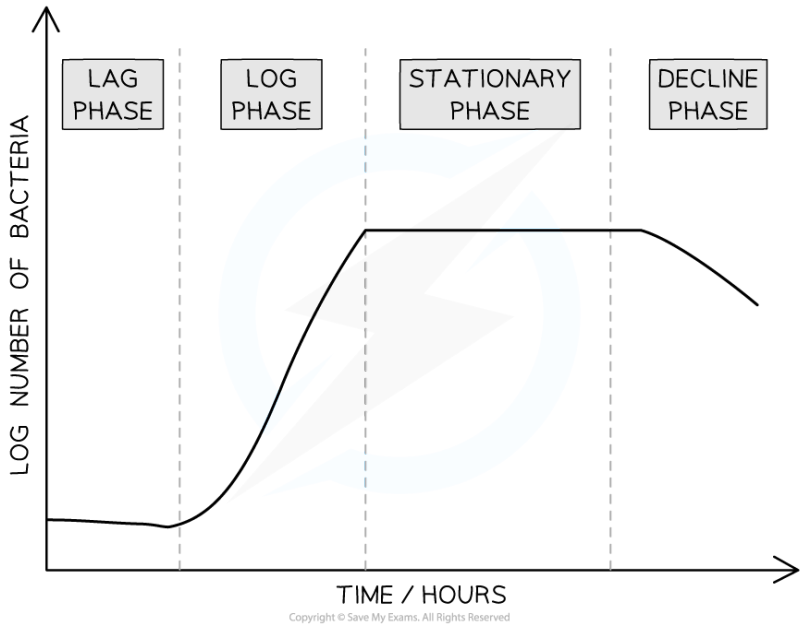
Bacterial growth curve

Your notes

- The growth of a bacterial population follows a specific pattern over time; this is known as a **growth curve**
- There are 4 phases in the population growth curve of a microorganism population
  - **Lag phase**
    - The population size **increases slowly** as the microorganism population adjusts to its new environment and gradually starts to reproduce
  - **Exponential phase**
    - With high availability of nutrients and plenty of space, the population moves into **exponential growth**; this means that the population **doubles with each division**
    - This phase is also known as the **log phase**
  - **Stationary phase**
    - The population reaches its maximum as it is **limited by its environment**, e.g. a lack of resources and toxic waste products.
    - During this phase the number of microorganisms dying equals the number being produced by binary fission and the **growth curve levels off**
  - **Death phase**
    - Due to lack of nutrients and a build up of toxic waste build up, death rate exceeds rate of reproduction and the population **starts to decline**
    - This phase is also known as the **decline phase**

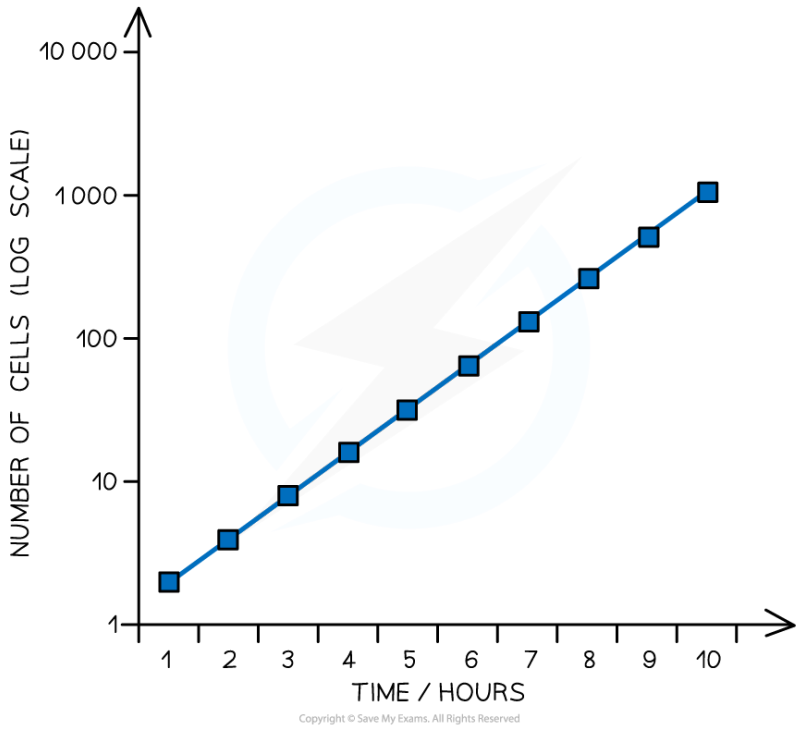


There are four phases in the standard growth curve of a microorganism

Using logarithms in growth curves

Your notes

- During the **exponential growth phase** bacterial colonies can grow at rapid rates with very large numbers of bacteria produced within hours
- Dealing with experimental data relating to **large numbers** of bacteria can be difficult when using traditional linear scales
  - There can be a wide range of numbers reaching from single figures into millions
  - This makes it hard to work out a suitable scale for the axes of graphs
- **Logarithmic** scales can be very useful when investigating bacteria or other microorganisms
  - The numbers in a logarithmic scale represents logarithms, or powers, of a base number
  - If using a $\log_{10}$ scale, in which the base number is 10, the numbers on the y-axis represent a **power of 10**, e.g. **1**=10^1 (10), **2**=10^2 (100), **3**=10^3 (1000) etc.
- Logarithmic scales allow for a **wide range of values** to be displayed on a single graph
- For example, if yeast cells were grown in culture over several hours the number of cells would increase very rapidly from the original number of cells present
- The results from such an experiment are shown in the graph below using a log scale
  - The number of yeast cells present at each time interval was converted to a logarithm before being plotted on the graph
    - This can be done using a log function on a calculator
  - The log scale is easily identifiable as there are **not equal intervals** between the numbers on the y-axis
  - The **wide range of cell numbers fit** easily onto the same scale



When a $\log_{10}$ scale is used, the scale increases by a factor of 10 each time; this allows large increases in numbers to be shown on a single graph

Examiner Tips and Tricks

You won't be expected to convert values into logarithms or create a log scale graph in the exam. Instead you might be asked to interpret results that use logarithmic scales or explain the benefit of using one! Remember that graphs with a logarithmic scale have **uneven intervals** between values on one or more axes.

Exponential growth rate constants

- To calculate the number of bacteria in a population the following formula can be used

$$N_t = N_0 \times 2^{kt}$$

- *Where*
  - N_t = the number of organisms at time *t*
  - N_0 = the number of organisms at time *0*
  - k = the exponential growth rate constant
  - t = the time for which the colony has been growing
- To use this equation the exponential growth rate constant k must be calculated

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- This refers to the **number of times the population doubles in a given time period**

- The following formula can be used to calculate the exponential growth rate constant

$$k = \frac{\log_{10} N_t - \log_{10} N_0}{\log_{10} 2 \text{ x } t}$$

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Worked Example

A bacterial colony started with 2 individuals and after 3 hours of growing there were 926 bacteria in the colony.

1. Calculate the exponential growth rate constant of this colony

2. Calculate the number of bacteria in the colony after 5 hours

Answer:

Step 1: Calculate the exponential growth rate constant

$$k = \frac{\log_{10} N_t - \log_{10} N_0}{\log_{10} 2 \text{ x } t}$$

$$k = \frac{\log_{10}(926) - \log_{10}(2)}{\log_{10} 2 \text{ x } 3}$$

$$k = \frac{2.67}{0.30 \text{ x } 3}$$

$$k = 3$$

Step 2: Calculate the number of bacteria after 5 hours

$$N_t = N_0 \text{ x } 2^{kt}$$

$$N_t = 2 \text{ x } 2^{(3)(5)}$$

$$N_t = 2 \text{ x } 2^{15}$$

$$N_t = 65\ 536$$

Your notes

Rate of Growth of Microorganisms

- There are several ways to investigate the growth of microorganisms
- In this example the **change in turbidity of a yeast solution** will be used as a measure of the growth of microorganisms in a liquid culture over time
  - Turbidity = cloudiness of a solution

Apparatus

- Cloths and antimicrobial solution
- Conical flask
- Glucose solution
- Dried baker's yeast
- Bunsen burner
- Cotton wool
- Magnetic stirrer
- Aluminium foil
- Colorimeter
- Dropper pipette
- Cuvettes
- Optional
  - Microscope
  - Microscope slides with cover slips and graph paper photocopied on to acetate OR a haemocytometer

Method

1. Use antimicrobial solution to sterilise the work area
2. Place 250 cm^3 glucose solution into a conical flask
   - This solution will be the **liquid culture medium**
3. Inoculate the glucose solution with 1.25 g dried yeast using aseptic techniques
   - Work **next to a Bunsen flame** to ensure that microorganisms in the air are drawn away from the nutrient broth
4. Seal the flask using a cotton wool stopper immediately after inoculation is complete to prevent contamination

5. Swirl to mix the yeast with the glucose solution and place on a magnetic stirrer
6. While stirring continuously, loosely cover the stopper with aluminium foil and incubate the flask at 20 °C
7. Fill a cuvette with plain glucose solution and use this as a blank to calibrate the colorimeter
    - This ensures that the colorimeter is **reset to zero** before each measurement is taken
8. Transfer about 3 cm^3 of the yeast suspension into a cuvette using a dropping pipette
9. Use the colorimeter to measure the absorbance and record this in a suitable table against time
10. Repeat steps 7–9 at intervals over a 12 hour period
    - E.g. this could involve taking samples every 30 minutes for the first two hours and then every 2–3 hours after this
11. Plot a graph of absorbance against time

- The temperature or concentration of the glucose solution can be changed and the experiment repeated to investigate the effect these changes would have on the growth of the yeast

Optional extension to practical

- Turbidity measurements are useful as the **turbidity of the medium is mainly a measure of the number of living cells** in suspension; however, the measurement can be affected by the presence of **dead cells** and **other suspended particles**, so other methods of cell counting can be used to back up the turbidity findings, e.g.
    - Use a **haemocytometer** to estimate the number of yeast cells in the glucose solution
    - Calculate the area of the microscope's field of view using graph paper copied on to acetate, and **use the number of cells in the field of view** to estimate the number of cells in the glucose solution
- If using the area of the microscope's field of view the following steps can be used to estimate the number of cells in the growth medium
    - Count the number of squares of acetate graph paper visible when using the x4 objective lens and use this to calculate the area of the field of view under a x40 objective lens; this will be 0.01 of the area of the x4 objective lens
    - Stain the yeast suspension with methylene blue and place one drop of the suspension on a microscope slide
    - View under the x40 objective lens and count the number of yeast cells in the field of view
    - Calculate the volume of this drop by measuring the volume of 10 drops and dividing by 10

Your notes

- To calculate the volume under the field of view when using the x40 objective lens, use the following formula

volume = (area of field of view of x40 objective lens ÷ area of cover slip) x volume of one drop

- To calculate the number of yeast cells in 1 mm^3 of medium the following formula can be used

number of cells per mm^3 = average cell count in field of view ÷ volume of field of view

- Note that if many cells overlap when viewed under x40 objective lens a serial dilution of the yeast suspension will be required

Safety

- **Eye protection** should be worn
- Care should be taken around Bunsen burners
- **Aseptic techniques** should be used when transferring microorganisms
- Cultures should be **incubated at a safe temperature** for school laboratory work; 20 °C and not 37 °C
- Work spaces and hands should be **thoroughly disinfected** at the end of the experiment
- Cultures should be **safely destroyed** at the end of the experiment

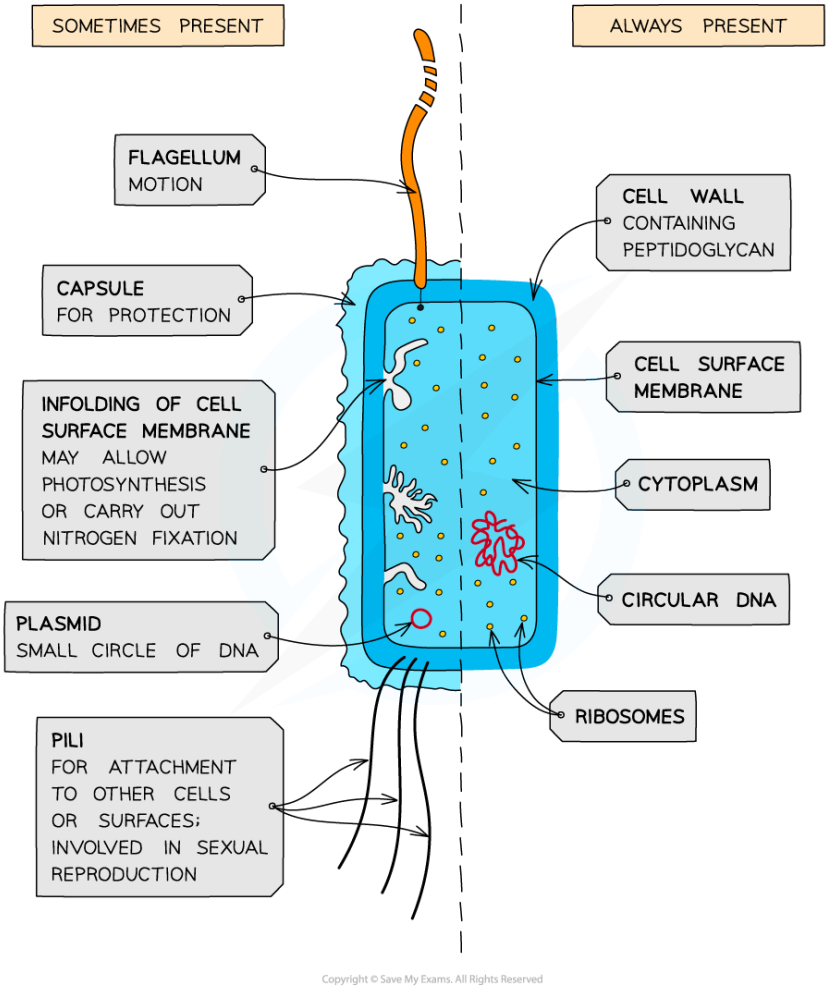
Your notes

Your notes

Comparison of Bacterial & Viral Structure

Bacteria

- Bacteria are single-celled **prokaryotes**
- Prokaryotic cells are much **smaller** than eukaryotic cells
- They also differ from eukaryotic cells in having
  - A **cytoplasm** that **lacks membrane-bound organelles**
  - **Ribosomes** that are smaller (70 S) than those found in eukaryotic cells (80 S)
  - **No nucleus,** instead having a **single circular bacterial chromosome** that is free in the cytoplasm and is **not associated with proteins**
  - A cell wall that contains the glycoprotein **murein**
    - Murein is sometimes known as **peptidoglycan**
- In addition, many prokaryotic cells also have the following structures
  - Loops of DNA known as **plasmids**
  - **Capsules**
    - This is sometimes called the **slime** capsule
    - It helps to **protect bacteria** from drying out and from attack by cells of the immune system of the host organism
  - Flagella (singular flagellum)
    - Long, tail-like structures that rotate, enabling the prokaryote to **move**
    - Some prokaryotes have **more than one**
  - Pili (singular pilus)
    - Thread-like structures on the surface of some bacteria that enable the bacteria to **attach** to other cells or surfaces
      - Involved in **gene transfer** during sexual reproduction
  - A cell membrane that contains folds known as **mesosomes;** these infolded regions can be the site of respiration
- Some bacteria are disease-causing, or **pathogenic**, but not all bacteria cause harm to other organisms



Prokaryotic cells have a peptidoglycan cell wall, no membrane-bound organelles, a circular chromosome, and 70S ribosomes

Viruses

- **Viruses** are **non-cellular infectious particles**
- They are relatively simple in structure, and much smaller than prokaryotic cells
- Structurally they have
  - **A nucleic acid core**
    - Their genomes are either DNA or RNA, and can be single or double-stranded
  - **A protein coat** called a '**capsid**' made of repeating units known as **capsomeres**
- They do not possess a plasma membrane, cytoplasm, or ribosomes
- Some viruses have an outer layer called an **envelope** formed from the **membrane-phospholipids** of the cell they were made in

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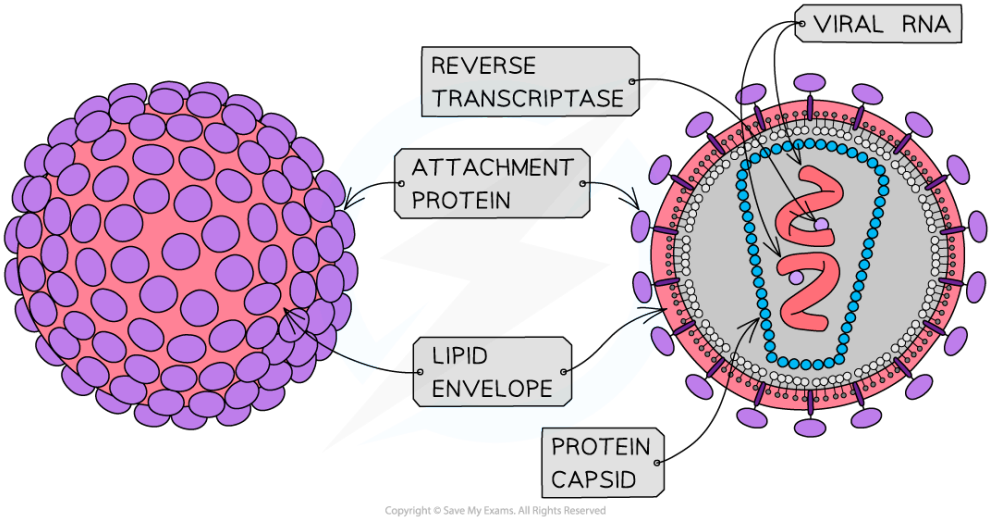
- The fact that lipid envelopes are formed from the membrane of a viral host cell means that very few plant viruses have lipid envelopes

- Some contain **proteins** inside the capsid which perform a variety of functions
  - E.g. HIV contains the enzyme **reverse transcriptase** which converts its RNA into DNA once it has infected a cell
- Viruses also contain **attachment proteins,** also known as **virus attachment particles,** that stick out from the capsid or envelope
  - These enable the virus to attach itself to a host cell
- Viruses can only reproduce by **infecting living cells** and using the **protein-building machinery** of their host cells to produce new viral particles
- Viruses are classified on the basis of the **genetic material they contain** and how they replicate
  - They can be classified into the following categories
    - DNA viruses
    - RNA viruses
    - Retroviruses



HIV contains RNA as its genetic material. It is surrounded by a protein capsid, as well as having an outer lipid envelope and attachment proteins

DNA viruses

- They contain **DNA** as genetic material
- Viral DNA acts as a **direct template** for producing new viral DNA and mRNA for the synthesis of viral proteins
- Examples: smallpox, adenoviruses, and bacteriophages

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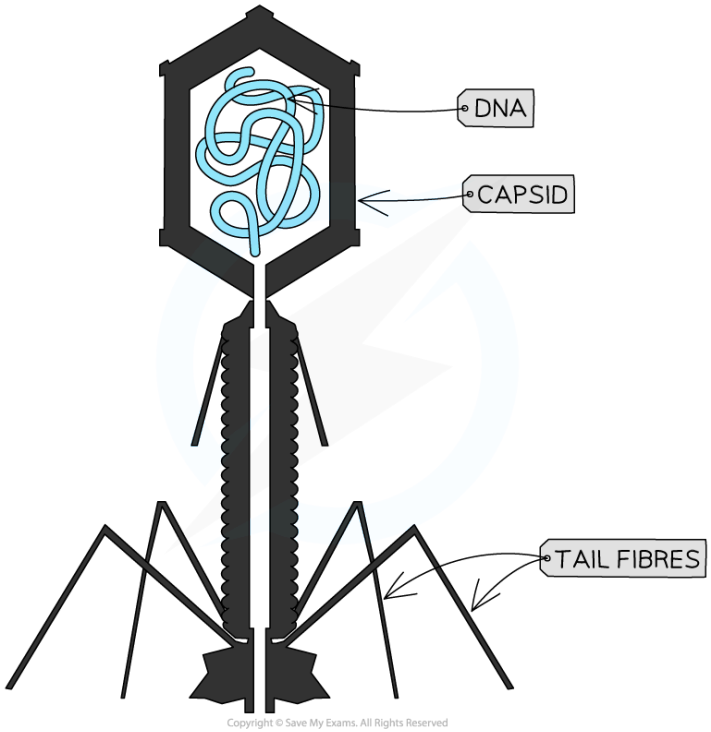
- Bacteriophages are viruses that infect bacteria, such as the **λ (lambda) phage**



Bacteriophage viruses, such as the λ phage, are examples of DNA viruses

RNA viruses

- They contain **RNA** as genetic material
  - Most have a **single strand** of RNA
  - They **do not** produce DNA at all
- Mutations are **more likely** to occur in RNA viruses than DNA viruses
- Examples: **tobacco mosaic virus (TMV)**, **ebola virus**

Retroviruses

- **Special type** of RNA virus that **does produce DNA**
- They contain a **single strand of RNA** surrounded by a protein capsid and lipid envelope
- Viral RNA controls the production of an enzyme called **reverse transcriptase**
- This enzyme catalyses production of **viral DNA** from the **single strand of RNA**
- The new viral DNA is incorporated into the **host DNA** using **integrase** enzymes where it acts as a **template** to produce viral proteins and RNA
- Example: **HIV** (Human Immunodeficiency Virus)

Lytic & Latency

Your notes

- Viruses can only reproduce **within a host cell** as they lack the cellular machinery to do so on their own
- They can enter a host cell in a variety of different ways
  - Bacteriophages **inject** their genetic material into bacteria
  - Some animal viruses enter the cell via endocytosis by fusing their viral envelope with the host cell surface membrane
  - Plant viruses will often use a **vector** such as an insect to breach the cell wall
- Once inside the host cell one of the following pathways can occur
  - Lysogenic
  - Lytic

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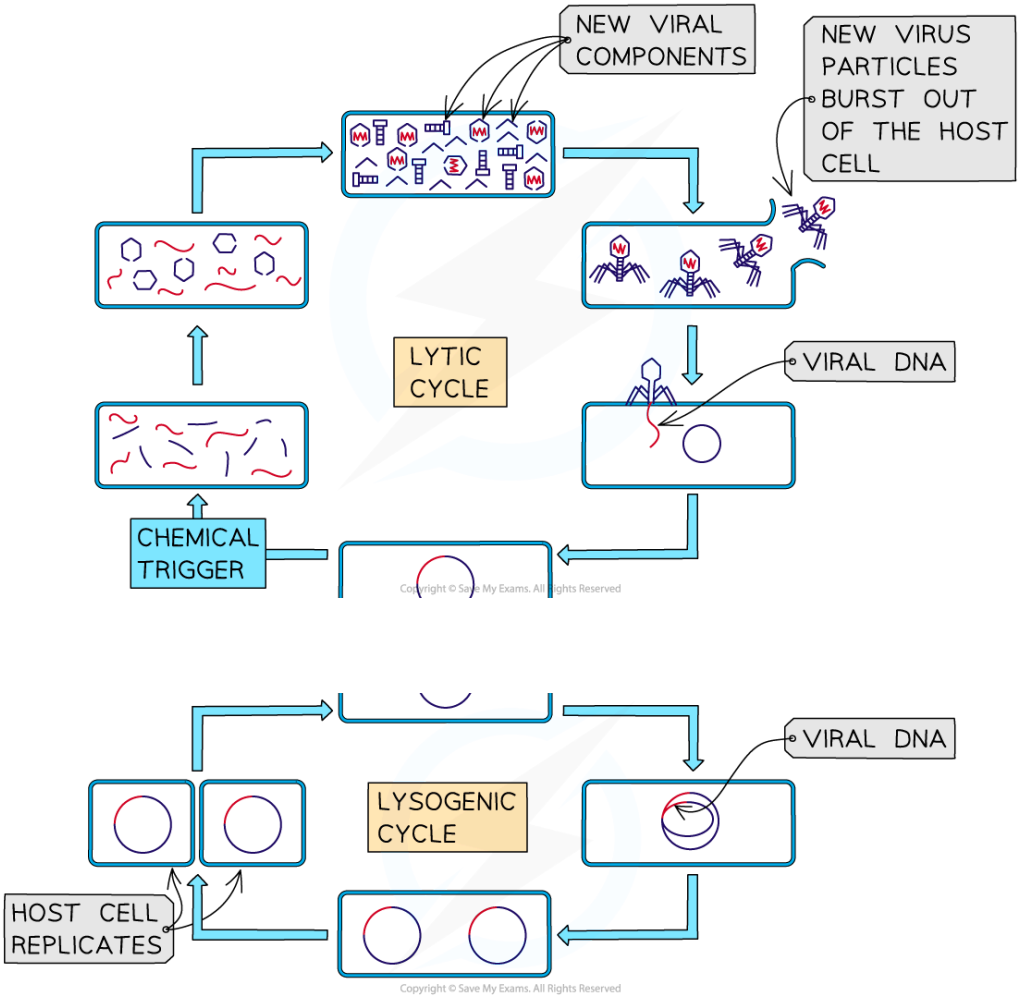
Lysogenic pathway

- Some viruses will **not immediately** cause disease once they infect a host cell
- Viral DNA known as a **provirus** is inserted into the host DNA, but a viral gene coding for a **repressor protein** prevents the viral DNA from being transcribed and translated
  - Every time the host DNA copies itself, the **inserted viral DNA will also be copied**
- This is called **latency** and the time during which it occurs is known as a **period of lysogeny**
- Viruses in a lysogenic state may become **activated** and enter the **lytic pathway**
  - Activation may occur as a result of, e.g. host cell damage or low nutrient levels inside a cell

Lytic pathway

- The viral genetic material is **transcribed and translated** to produce new viral components
- These components are assembled into **mature viruses** that accumulates inside the host cell
- Eventually the **host cell bursts** which releases large numbers of viruses, each of which can infect a new host cell
  - Cell bursting is known as cell **lysis**
- This typically results in **disease**



The life cycle of the λ bacteriophage includes a lysogenic and a lytic pathway

Your notes