1.2 Practical Skills: Endorsement Assessment

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CONTENTS

1.2.1 PRACTICAL: ETHICAL USE OF ORGANISMS

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Practical: Ethical Use of Organisms

The safe and ethical use of organisms to measure plant or animal responses or physiological functions

- When using **live animals** in biological experiments, care must be taken to **avoid harming** them. For example:
  - **Aquatic animals** should only be observed for a **short time** before being promptly returned to their main container
  - **Microscopic aquatic animals** (e.g. from pond water samples) should only be observed using **cavity slides** (slides that have a small indentation or 'well' in them that can hold a small amount of water). This ensures the organisms have enough water available to **prevent dehydration or heat damage** from the microscope light
- Biological investigations (especially those investigating **physiological functions and adaptations**) may involve **humans** and so it is important they are **safe.** For example:
  - For pulse rate experiments and other studies into the effects of exercise, the type of exercise and how it will be carried out should be carefully planned in order to **prevent injury**
  - Chemicals such as **caffeine** or **alcohol** should **not** be administered to human participants in a school environment

Example of the safe and ethical use of organsims

- Experiments can be carried out to investigate the effect of abiotic factors on the movement of animals
  - **Choice chambers** and **mazes** are often used in these experiments and **woodlice** and **maggots** are commonly the model animals
- A scientist called J. Cloudsley-Thompson carried out the first experiments on woodlouse behaviour
- One of his experiments focused on the response of woodlouse to humidity
- He used large choice chambers that were divided into two sections

Apparatus

- Choice chambers
- Lids
- Distilled water (fixed volume in each choice chamber)
- Drying agent (fixed volume in each choice chamber)
- Gauze platforms
- Woodlice
- A dark cupboard
- A bright well-lit room

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Method

- Divide each choice chamber into two sections
  - Add a fixed volume of **distilled water** to one side and a fixed volume of **drying agent** to the other
  - This allows for the **humidity to be controlled** in each section
- Insert **gauze platforms** into the choice chambers
  - This keeps the woodlice at a safe distance from the water and drying agent
  - Note that woodlice are able to move between sections
- Divide the woodlice into two even-sized groups
  - Drop the woodlice from group A into **choice chambers kept in the dark**
  - Drop the woodlice from group B into **choice chambers kept in the light**
- The woodlice can be dropped gently into the chamber using the hole in the lid so that they fall into the centre of the choice chamber
- **Record the position** of the woodlice in each choice chamber after 15 minutes
  - They should fall into the following categories: moving around, stationary on the dry side, stationary in the centre or stationary on the humid side
- Repeat the experiment several times for all conditions

Results and analysis

- A significant percentage of the woodlice (in all groups) **moved to the humid side**
  - This is a beneficial response as it helps to **prevent water loss** from the respiratory surfaces of the woodlice
- The woodlice **responded to humidity more actively when in the light**
  - This is advantageous – if the woodlice are subject to dry conditions during the day when they are more likely to dehydrate then they are even more likely to move towards humid conditions (under a stone, log etc)
- The woodlice tended to **stop moving when in humid conditions**
  - This means they are able to remain within favourable conditions that reduce water loss

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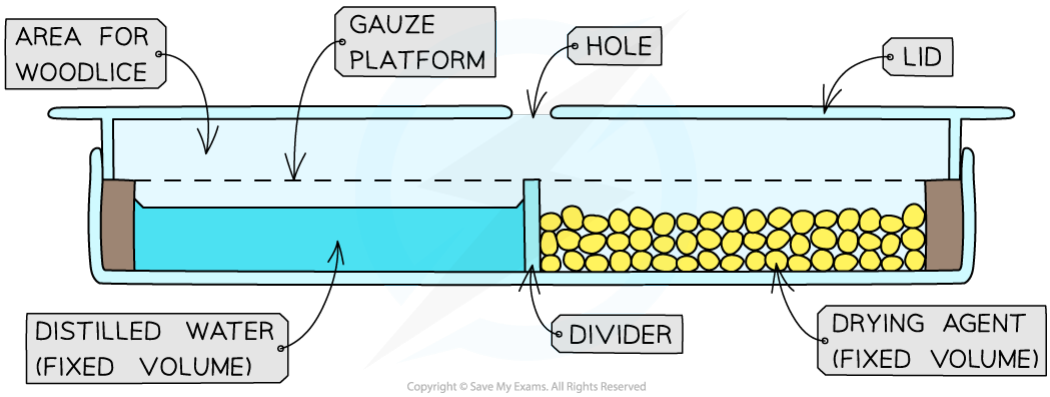

The choice chamber has two sections, one with low humidity and one with higher humidity

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1.2.2 PRACTICAL: ASEPTIC TECHNIQUES

Practical: Aseptic Techniques

- When investigating the effect of antimicrobial substances on microbial growth it is essential that **aseptic (sterile) techniques** are used
- Aseptic techniques ensure the microbes being investigated don't **escape** or become **contaminated** with another unwanted, and possibly pathogenic, microbe
    - This is especially important in preventing the **accidental culture of human pathogens**
- Aseptic techniques include:
    - Washing hands thoroughly to disinfect them
    - No food or drink allowed in the lab
    - Disinfecting work surfaces with disinfectant or alcohol
    - All apparatus, glassware and collecting loops must be fully sterilised before use
    - The culture medium (either a culture broth or an agar plate) must be sterilised
    - Using flamed loops or sterile swabs when transferring cultures to avoid collecting microbes in the atmosphere
    - Flaming culture bottlenecks to prevent contamination
    - Not allowing the growth of microorganisms at body temperature
    - Only removing petri dish lids when necessary
    - Sterilising or disposing of all used equipment promptly once they are no longer needed

Example of a practical using aseptic techniques: testing for bacterial antibiotic resistance using the disc diffusion method

- The disc diffusion method is commonly used to test for **antibiotic resistance** in bacteria
    - It allows for **multiple antibiotics** to be tested at once

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Apparatus

- Sterile agar plates
  - The agar can be made sterile by boiling
- Diluted bacterial broth with a concentration of 1 x 10^8 CFU mm^{-3}
  - Colony-forming unit (CFU): a live bacterial cell that is able to divide and form a colony on the agar plate
- Multiple different antibiotic solutions of a standard concentration
- Paper disks
- Pipettes
- Spreaders
- Bunsen burner
- Gloves
- Goggles
- Incubator

Method

- Pre-soak paper discs in the different antibiotic solutions
  - The different antibiotic solutions need to be the **same concentration** so that the effects of the different antibiotics can be compared
- Spread a sample of the diluted bacterial broth onto the surface of the sterile agar plate
- Lightly press the paper discs onto the surface of the agar
  - Make sure the discs are evenly distributed in the plate
  - They should not be touching the edges of the plate or any other discs
- Keep the agar plate in the incubator overnight
  - The incubator maintains an optimum temperature for bacterial growth
- Remove the agar plate from the incubator and examine the results with the petri dish lid on

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Results

- Overnight the antibiotics will diffuse outwards from each paper disk so that a gradient of antibiotic forms. The antibiotic is most concentrated where the paper disk is located
- If the bacteria being investigated is vulnerable to an antibiotic then a clear area will be visible around the disc
    - There are **no bacteria present in the clear area**
- The clear area will end when the concentration of antibiotic reaches a level that the bacteria are no longer susceptible to
- **More effective antibiotics** require a **lower concentration to kill bacteria** and so they will produce **larger clear zones**
- If a bacteria is **completely resistant** to an antibiotic then there will be **no clear zone** around that particular paper disk

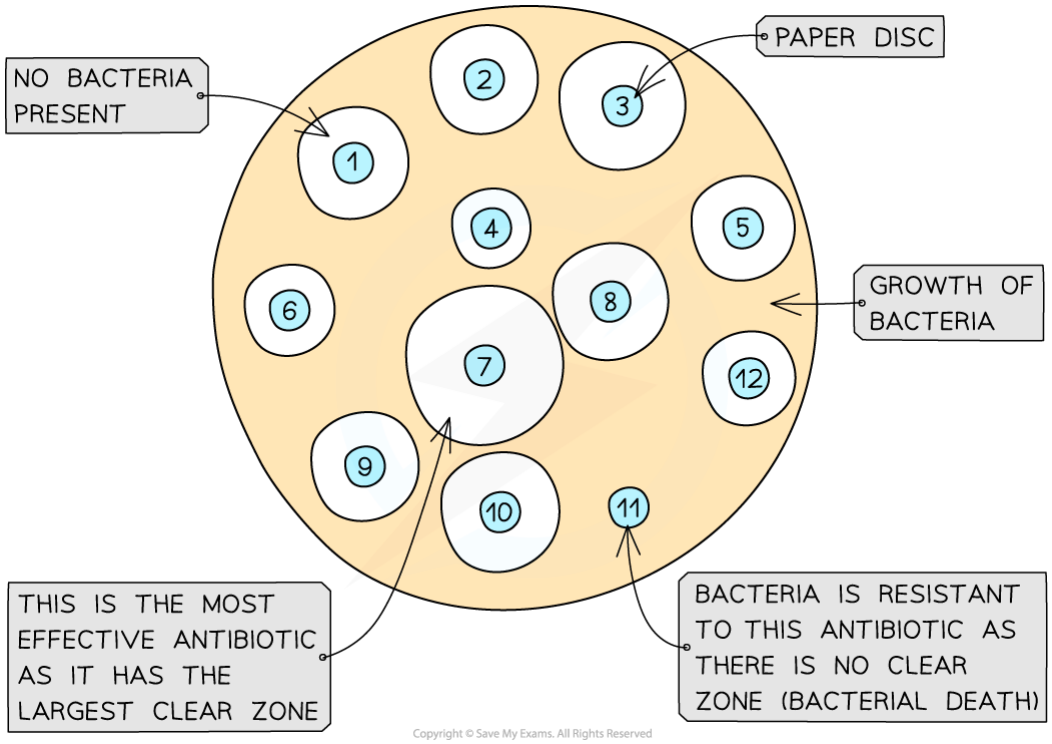

Image showing the bacterial growth on an agar plate following a disc diffusion experiment. The most effective antibiotics produce the largest clear zones while. The antibiotics that the bacteria are resistant to produce no clear zone.

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Exam Tip

It is expected that you will be able to suggest aseptic techniques that should be used for specific experiments. Make sure to learn a few of the ones above so that you can get those marks!

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1.2.3 PRACTICAL: DISSECTION OF GAS EXCHANGE SURFACES IN FISH & INSECTS

Practical: Dissection of Gas Exchange Surfaces in Fish & Insects

The safe use of instruments for the dissection of an animal or plant organ

- Dissections are a **vital part** of scientific research
- They allow for the **internal structures** of **organisms** and **organs** to be examined so that theories can be made about how they **function**
  - For example, dissections can be performed on **fish** to expose and study their **gills** (the gas exchange surface in fish) or on **insects** to expose and study their **spiracles, tracheae and tracheoles** (the gas exchange system in insects)
- There are **ethical concerns** surrounding dissections
  - People worry about how the animals for dissections are **raised** and **killed**
  - It goes against the **religious beliefs** of some individuals
- The biological specimen used for dissection should be from a **reputable source** and should be **disposed of in the correct manner**
- If multiple specimens are being dissected then they should be taken from individual organisms of the same species and roughly the same age
- **Safety is a priority** when conducting dissection experiments with sharp tools like scalpels and scissors
- The method below outlines some precautions that should be taken in the interest of safety

Apparatus

- Scissors
- Scalpel
- Tweezers / Forceps
- Dissection board
- Paper towels
- Biological specimen
- Pins
- Gloves
- Goggles

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Method

- A lab coat, gloves and eye protection should be worn
  - This is done to **avoid contamination** with biological material (which could cause an allergic reaction)
- Place the specimen on the dissecting board
- Use the tools to access the desired structure
  - When using the scalpel **cut away from your body** and keep your **fingers far from the blade** to reduce the chance of cutting yourself
  - Scissors can be used for cutting large sections of tissue (cuts do not need to be precise)
  - A scalpel enables finer, more precise cutting and needs to be sharp to ensure this
- Use pins to move the other sections of the specimen aside to leave the desired structure exposed

Limitations

- It can be hard to see some of the smaller, finer structures within organs
- The specimens do not reflect how the tissue would look in a living organism
- If only a single specimen is dissected then anomalies found within that specimen may be ignored or glossed over
- Dissection instruments (scissors and scalpel) should be sharp to give good clean cuts with as little damage as possible – **blunt instruments are dangerous** (as more force is required to cut) and will not give precise cuts making internal structures harder to distinguish

Exam Tip

You may be asked to suggest a method of dissection for a particular organ. Make sure you **name the specific tools** (e.g. scissors and forceps) that should be used in order to get the marks.

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1.2.4 DRAWING CELLS FROM BLOOD SMEARS

Drawing Cells From Blood Smears

- To record the observations seen under the microscope (or from photomicrographs taken) a labelled biological drawing is often made
- **Biological drawings** are line pictures which show specific features that have been observed when the specimen was viewed
- There are a number of rules/conventions that are followed when making a biological drawing
- The conventions are:
  - The drawing must have a title
  - The **magnification** under which the observations shown by the drawing are made must be recorded
  - A **sharp HB pencil** should be used (and a good eraser!)
  - Drawings should be on plain white paper
  - Lines should be **clear**, **single lines** (no thick shading)
  - **No shading**
  - The drawing should take up as much of the space on the page as possible
  - Well-defined structures should be drawn
  - The drawing should be made with **proper proportions**
  - **Label lines** should not cross or have arrowheads and should **connect directly** to the part of the drawing being labelled
  - Label lines should be kept to one side of the drawing (in parallel to the top of the page) and drawn with a **ruler**
- Drawings of cells are typically made when visualizing cells at a higher magnification power, whereas plan drawings are typically made of tissues viewed under lower magnifications (individual cells are never drawn in a plan diagram)

Blood smears

- A blood smear is when a small amount of blood is spread on a glass microscope slide, stained and covered with a coverslip
- The different blood cells can then be examined using a microscope
  - Red blood cells have no nuclei and a distinct biconcave shape
  - White blood cells have irregular shapes
  - Neutrophils have distinctive **lobed nuclei**
    - They make up roughly 70% of all white blood cells
  - Lymphocytes have very large nuclei that nearly occupy the entire cell

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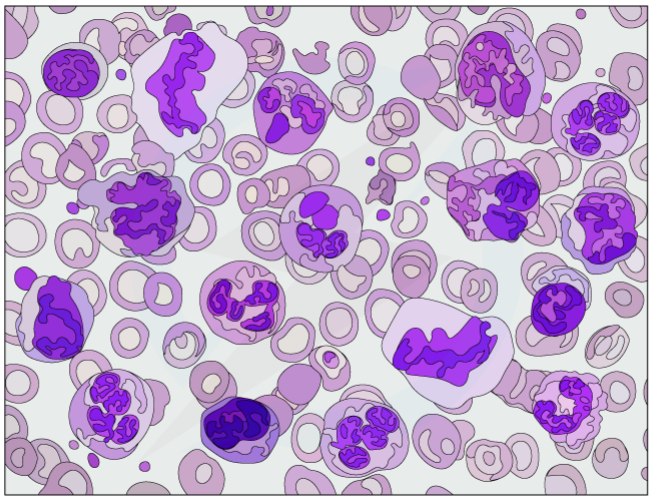
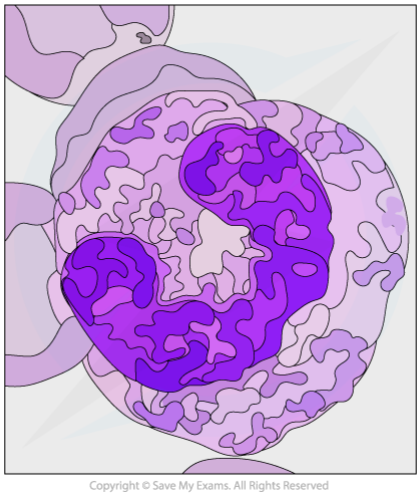


The different blood cells present in a blood smear



The lobed nucleus of a neutrophil

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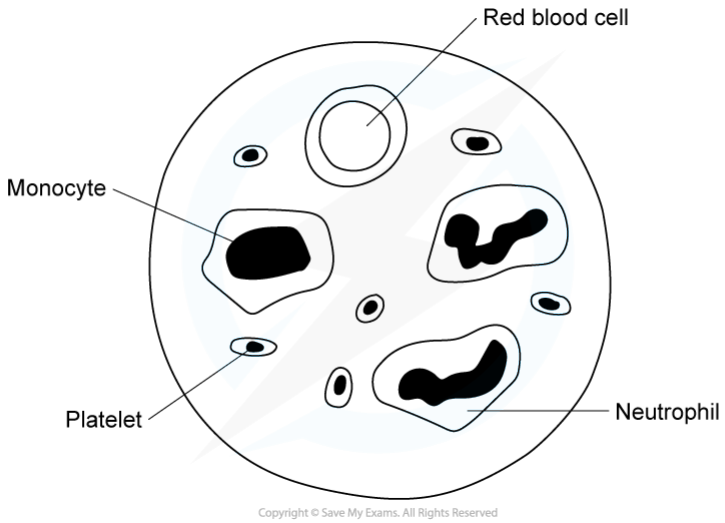

An example drawing of the cells observed (using a microscope) in a blood smear

Exam Tip

When producing a biological drawing, it is vital that you only ever draw what you see and not what you think you see.

To accurately reflect the size and proportions of structures you see under the microscope, you should get used to using the eyepiece graticule.

You should be able to describe and interpret photomicrographs, electron micrographs and drawings of typical animal cells.

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1.2.5 PRACTICAL: INVESTIGATING BIODIVERSITY USING SAMPLING

Practical: Investigating Biodiversity Using Sampling

Use of sampling techniques in fieldwork

- Sampling is a method of investigating the **abundance and distribution of species and populations**
- There are two different types of sampling:
  - **Random**
  - **Non-random**
- In random sampling, the positions of the **sampling points** are completely random or **due to chance**
- In non-random sampling, the positions of the **sampling points are chosen** by the person carrying out the sampling
- There is a range of different sampling equipment which can be used:
  - Quadrats
  - Sweeping nets
  - Pitfall traps
  - Pooters
  - Tullgren funnel

The quadrat method of sampling

Apparatus

- Quadrat
- Random number generator

Method for choosing sample sites

- Mark up a grid on a map or a to-scale drawing of the area being studied and label the grid with coordinates
  - It is important that the area is big enough to get a representative estimate for the specific habitat/ecosystem
- Use a **random number generator** to choose a **set of coordinates**
  - This is done to **avoid sampling bias** which could lead to over or under-estimation (either subconsciously choosing areas that are easier to access or that look like they contain individuals)

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Getting measurements from quadrats

- The contents within quadrats can be counted using different measurements
- Population density, percentage cover and species frequency are all different ways of counting and recording the number of different species and individuals present within a quadrat

Calculating population density (*number of individuals per m^2*):

- Place a quadrat at the generated coordinate
- Count the **number of individuals** in each quadrat
- Use a **running mean** to determine the number of quadrats required to get a representative sample:
  - Calculate the mean number of individuals per quadrat for the first two quadrats found, then the mean of the first three, then the mean of the first four and repeat until there is no further significant change in the mean
- To calculate the **estimated population size** for the whole area or habitat, **divide the whole area by the area of one quadrant**, then multiply this value by the **mean number of individuals per quadrat**

Calculating the abundance of a species using percentage cover (%):

- Usually used for **plants**
- Place a grid quadrat (a square frame split into 100 smaller squares) at this coordinate
  - Each square of a grid quadrat is equivalent to **1% cover**
- Count the number of squares in each quadrat within which the species occupies **over half the square**
  - If **30 squares** contain the species, the percentage cover is **30%**
  - This method is **subjective** and therefore the **same person** should make the estimate for **all samples** to control this variable

Calculating the abundance of a species using frequency (%):

- Place a frame quadrat at multiple coordinates generated
- Count the number of quadrats that contain the species
  - If 3 out of 10 quadrats contain the species the frequency is 30%

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Interpreting results from quadrats

- The results from the quadrats can be used to calculate the predicted frequency and density of a species within an area
- **Species density** indicates how many individuals of that species there are per unit area
  - The number of individuals counted across all **quadrats** is divided by the total area of all the quadrats
  - For example, if 107 bluebells were found across 50 quadrats that are $1m^2$ each the species density would be 107/50 = 2.14 individuals per m^2
- It can sometimes be difficult to count individual plants or organisms. When this is the case **percentage cover** of the species within the quadrat can be estimated instead
  - The quadrat is divided into 100 smaller squares. The number of squares the species is found in is equivalent to its percentage cover in that quadrat
  - For example, if grass is found in 89 out of 100 squares in the quadrat then it has a percentage cover of 89%
- **Species frequency** is the probability that the species will be found within any quadrat in the sample area
  - The number of **quadrats** that the species was present in is divided by the total number of quadrats and then multiplied by 100
  - For example, if bluebells were found in 18 out of 50 quadrats the species frequency would be (18/50) x 100 = 36%

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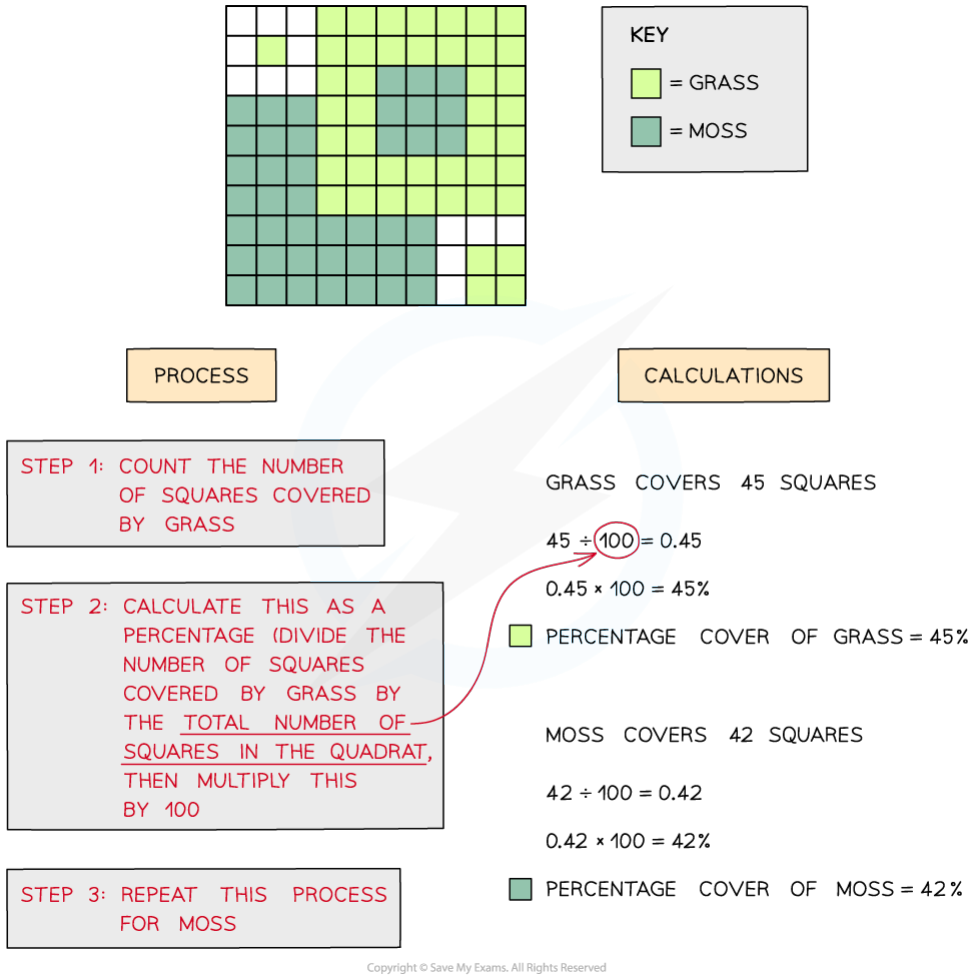

How to estimate percentage cover of one or more species using a quadrat

Limitations of using quadrats

- Quadrats and transects can only be used for **sessile and immobile species** (eg. plants and slow-moving animals)
- Some species can be counted to find their abundance but others that are very small or in high numbers require abundance to be calculated using percentage cover or frequency techniques
    - The frequency technique shows how common a species is but it does not give information on the estimated number of individuals or the size of the population
- **Percentage cover and frequency**, when used together, give a good picture of the **distribution of a species**
    - If a species had a high mean percentage cover but a low frequency it would suggest the species lives in groups in preferred areas of the habitat
    - This can be used to answer questions such as: does the species prefer light or dark and wet or dry areas etc.

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The mark-release-capture method of sampling (estimating population sizes of mobile animals)

- The sampling method described above is only useful for **non-motile** (sessile) organisms
- Different methods are required for estimating the number of individuals in a population of **motile animals**
  - The **mark-release-capture** method is used
- For a single species in the area:
  - The **first large sample is taken**. As many individuals as possible are caught, counted and **marked** in a way that won't affect their survival e.g. if studying a species of beetle, a small amount of brightly coloured non-toxic paint can be applied to their carapace (shell)
  - The marked individuals are **returned to their habitat** and allowed to randomly mix with the rest of the population
  - When a sufficient amount of time has passed **another large sample is captured**
  - The number of marked and unmarked individuals within the sample are **counted**
  - The proportion of marked to unmarked individuals is used to calculate an **estimate of the population size**
  - The formula for the calculation is:

$$\mathbf{N = (n_1 \times n_2) \div m_2}$$

  - Where:
    - **N** = population estimate
    - $\mathbf{n_1}$ = number of marked individuals released
    - $\mathbf{n_2}$ = number of individuals in the second sample (marked and unmarked)
    - $\mathbf{m_2}$ = number of marked individuals in the second sample

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Worked Example

Scientists wanted to investigate the abundance of leafhoppers in a small grassy meadow. They used sweep nets to catch a large sample of leafhoppers from the meadow. Each insect was marked on its underside with non-toxic waterproof paint and then released back into the meadow. The following day another large sample was caught using sweep nets. Use the figures below to estimate the size of the leafhopper population in this meadow.

- No. caught and marked in first sample ($\mathbf{n_1}$) = 236
- No. caught in second sample ($\mathbf{n_2}$) = 244
- No. of marked individuals in the second sample ($\mathbf{m_2}$) = 71

Step One: Write out the equation and substitute in the known values

$$N = (n_1 \times n_2) \div m_2$$

$$N = (236 \times 244) \div 71$$

Step Two: Calculate the population size estimate (N)

$$N = 57{,}584 \div 71$$

$$N = 811$$

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Results from mark-release-capture

- When using the mark-release-capture method, there are a few **assumptions** that have to be made:
  - The marked individuals are given **sufficient time** to **disperse** and **mix** back in fully with the **main population**
  - The marking doesn't affect the **survival rates** of the marked individuals (e.g. doesn't make them more visible and therefore more likely to be predated)
  - The marking remains **visible** throughout the sampling and doesn't rub off
  - The **population** stays the **same size** during the study period (i.e. there are no significant changes in population size due to births, deaths or migrations into or out of the main population)

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1.2.6 PRACTICAL: DATA LOGGERS & COMPUTER MODELLING

Practical: Data loggers & Computer Modelling

Data loggers

- Dataloggers are a tool that allows for the **quick and efficient gathering of data**
- This technology can be used for simple data collection (e.g. breathing rate) or for more complex data collection (e.g. a number of probes monitoring different variables attached to a main computer)
- The information contained within a datalogger can be inputted into a computer and formatted into a **table**
- After this is done the computer is able to calculate the **mean,** perform **statistical tests** and **plot graphs** using the data

Uses of computer modelling

- Computer modelling can be used to study the **theoretical impact** on populations from processes such as:
  - Ecological succession / zonation
  - Infectious disease transmission / epidemiolgy
  - Predator-prey relationships
  - Natural selection
  - Genetic drift
- One of the benefits of these computer programs is that **time** can be **sped up to predict the future outcome** of populations and environments

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Computer programs and natural selection

- **Computer programs** can be used to **model** the effects of **natural selection**
  - They usually start off with a simple **simulated population**, with no particular selection pressures acting upon it
- Natural selection can then be investigated by **changing** various **factors** and observing the **effects** on the simulated population
- Examples of factors that can be changed include:
  - The presence or absence of different biotic and abiotic **selection pressures** (such as predators, disease and food availability)
  - Making new, specifically selected **mutations** appear in the population
  - Changing the **likelihood** that a new mutation will appear in the population
  - Switching which **alleles** are dominant and which are recessive
  - Changing the **environment** the species is in
  - Changing one or more of the **adaptations** of the species in the simulated population
- The effects of these changing factors can be modelled by the program and the **probabilities** of different **outcomes** for the simulated population can be calculated
- One of the benefits of these computer programs is that **time** can be **sped up**
  - This means that natural selection, which for many species would normally occur over very **long time periods** and many many generations, can be modelled and the effects observed in much shorter and more experimentally appropriate timescales

Computer programs and genetic drift

- When a population is significantly **small**, **chance** can affect which **alleles** get passed onto the next generation
- Over time some alleles can be **lost** or **favoured** purely by **chance**
- When there is a gradual change in allele frequencies in a small population due to **chance** and **not** natural selection then **genetic drift** is occurring
- Computer programs similar to those described above can be used to **model** genetic drift

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1.2.7 PRACTICAL: INVESTIGATING THE RATE OF DIFFUSION

Practical: Investigating the Rate of Diffusion

Recording a range of quantitative measurements

- It is important to use **appropriate apparatus** when recording a range of quantitative measurements
- Quantitative measurements include things such as mass, time, volume, temperature, length and pH
  - **Mass** should be recorded using a **digital balance**
  - **Time** should be recorded using a **digital stopwatch**
  - **Volume** should be recorded using a **measuring cylinder**
  - **Temperature** should be recorded using a **digital thermometer** (although water baths have one built-in)
  - **Length** should be recorded using a **ruler**
- The practical below is an example of an experiment that has used the correct apparatus to measure the **dependent variable**

Practical: Investigating the rate of diffusion using agar

- The effect of **surface area to volume ratio** on the **rate of diffusion** can be investigated by **timing** the diffusion of ions through **different sized cubes of agar**
- Coloured agar is cut into cubes of the required dimensions (eg. 0.5cm x 0.5cm x 0.5cm, 1cm x 1cm x 1cm and 2cm x 2cm x 2cm)
  - Purple agar can be created if it is made up with very dilute sodium hydroxide solution and Universal Indicator
  - Alternatively, the agar can be made up with Universal Indicator only
- The cubes are then placed into boiling tubes containing a diffusion solution (such as dilute hydrochloric acid)
  - The acid should have a higher molarity than the sodium hydroxide so that its diffusion can be monitored by a change in colour of the indicator in the agar blocks
- Measurements can be taken of either:
  - The **time taken** for the acid to **completely change** the **colour** of the **indicator** in the agar blocks
    - A **stopwatch** is used
  - The **distance travelled** into the block by the acid (shown by the change in colour of the indicator) in a **given time period** (eg. 5 minutes)
    - A **microscope and a stage micrometer** (essentially a mini ruler) are used
- These times can be converted to rates (1 ÷ time taken)
- A **graph** could be drawn showing how the **rate** of diffusion (rate of colour change) **changes** with the **surface area to volume ratio** of the agar cubes

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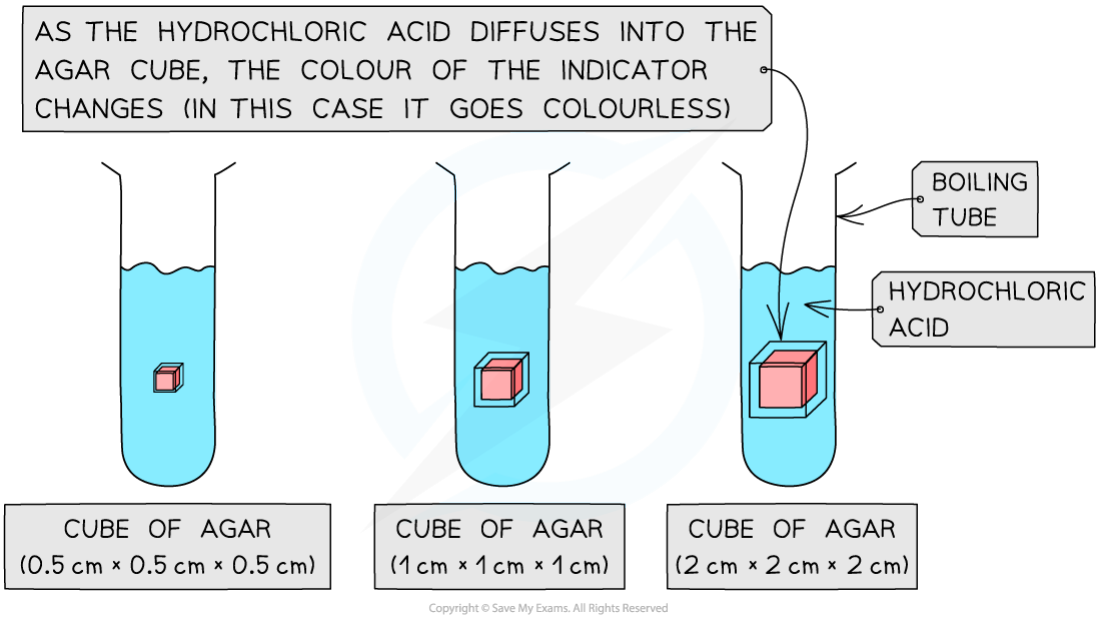

An example of how to set up an experiment to investigate the effect of changing surface area to volume ratio on the rate of diffusion

Exam Tip

When an agar cube (or for example a biological cell or organism) increases in size, the volume increases faster than the surface area, because the volume is cubed whereas the surface area is squared. When an agar cube (or biological cell / organism) has more volume but proportionately less surface area, diffusion takes longer and is less effective. In more precise scientific terms, the **greater the surface area to volume ratio**, the **faster the rate of diffusion**!

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1.2.8 PRACTICAL: INVESTIGATING WATER POTENTIAL

Practical: Investigating Water Potential

Practical 1: Investigating water potential using potato cylinders

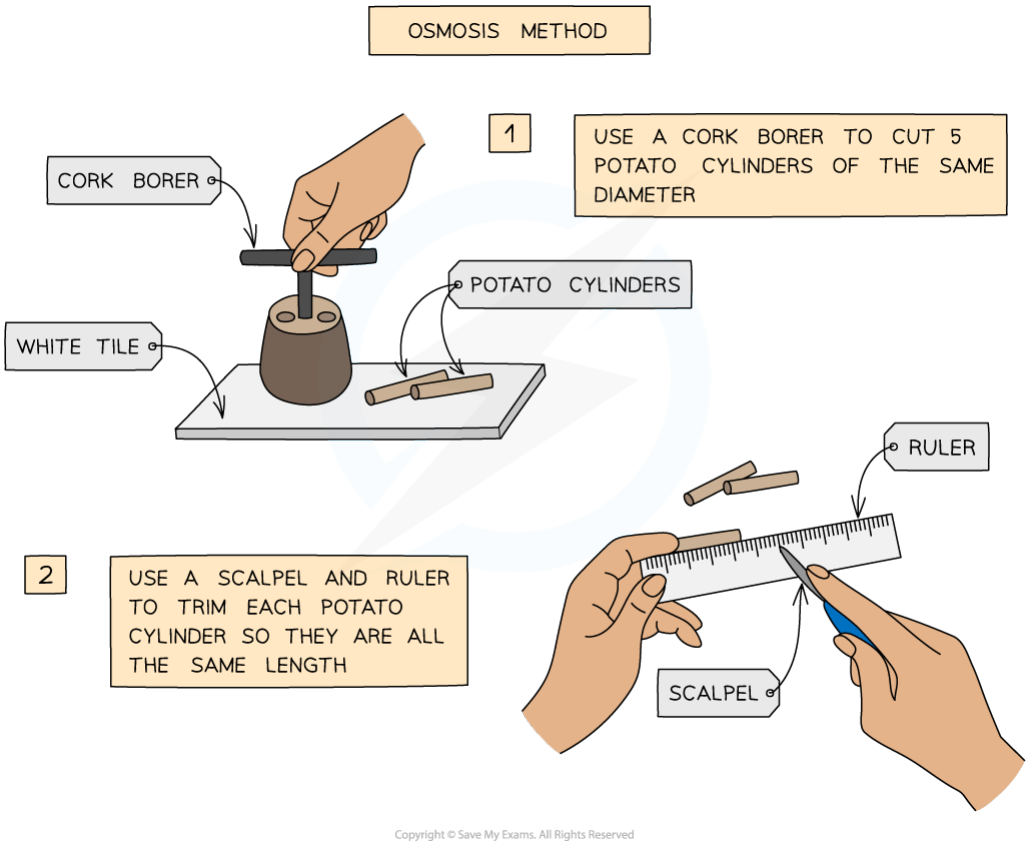
- It is possible to investigate the effects of immersing plant tissue in **solutions of different water potentials** and then **use the results to estimate the water potential of the plant tissue** itself
- The most common osmosis practical of this kind involves cutting **cylinders of potato** and placing them into solutions with a **range of different water potentials (usually sucrose solutions of increasing concentration** – at least 5 different concentrations are usually required)

Method

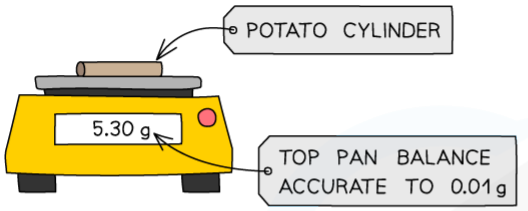
- The required number of potato cylinders are cut (one for each of the solutions you are testing – or more than one per solution if you require repeats)
- They are all cut to the **same length** and, once blotted dry to remove any excess moisture, their **initial mass is measured and recorded** before placing into the solutions
- They are left in the solutions for a set amount of time (eg. 30 minutes), usually in a water bath (set at around 30^{o})
- They are then removed and **dried to remove excess liquid**
- The **final length and mass** of each potato cylinder is then measured and recorded

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇



3 MEASURE THE MASS OF EACH POTATO CYLINDER AND RECORD IN A TABLE OF RESULTS

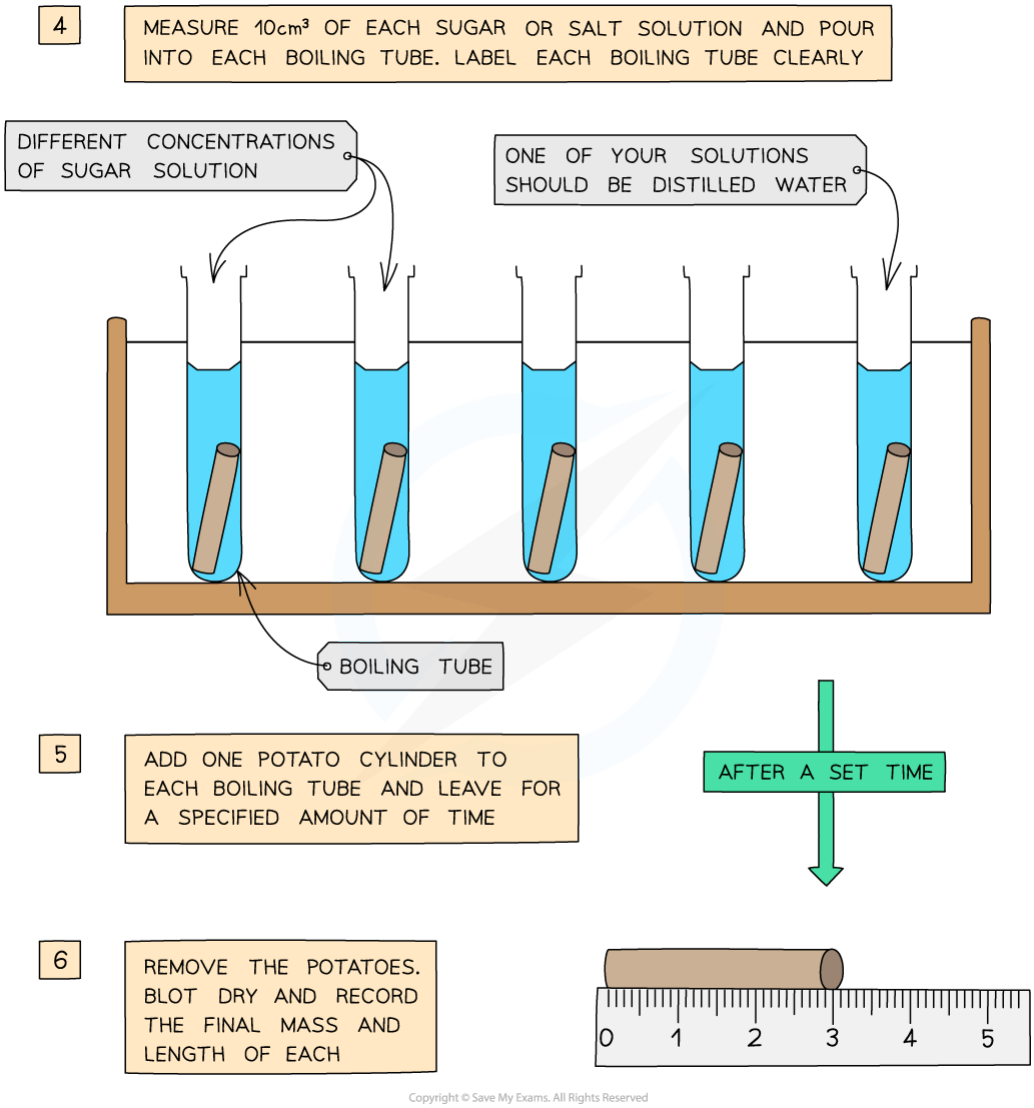


YOU WILL ALSO WANT TO RECORD THE INITIAL LENGTH (mm) IN A SIMILAR TABLE

Concentration of sucrose solution mol/dm³	Initial mass (g)	Final mass (g)	Change in mass (g)	% change in mass
0 (distilled water)	5.30			
0.25	5.32			
0.50	5.29			
0.75	5.31			
1.00	5.29			

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇



You will need to use apparatus appropriately to measure out the volumes of your solutions and record your measurements

Results

- The **percentage change** in mass for each potato cylinder is calculated

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

OSMOSIS ANALYSIS

Concentration of sucrose solution mol/dm³	Initial mass (g)	Final mass (g)	Change in mass (g)	% change in mass
0 (distilled water)	5.30	5.80	+0.50	9.4
0.25	5.32	5.42	+0.10	?
0.50	5.29	5.24	−0.05	−1.0
0.75	5.31	5.11	−0.20	−3.8
1.00	5.29	5.02	−0.27	−5.1

1 CALCULATE THE PERCENTAGE CHANGE IN MASS FOR EACH CYLINDER

$$\frac{(\text{FINAL MASS} - \text{INITIAL MASS})}{\text{INITIAL MASS}} \times 100$$

e.g. FOR 0.25 mol dm³

$$= \frac{(5.42 - 5.32)}{5.32} \times 100$$

$$= 1.9\%$$

To find the percentage change in mass, the change in mass must be divided by the initial mass and then multiplied by 100

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

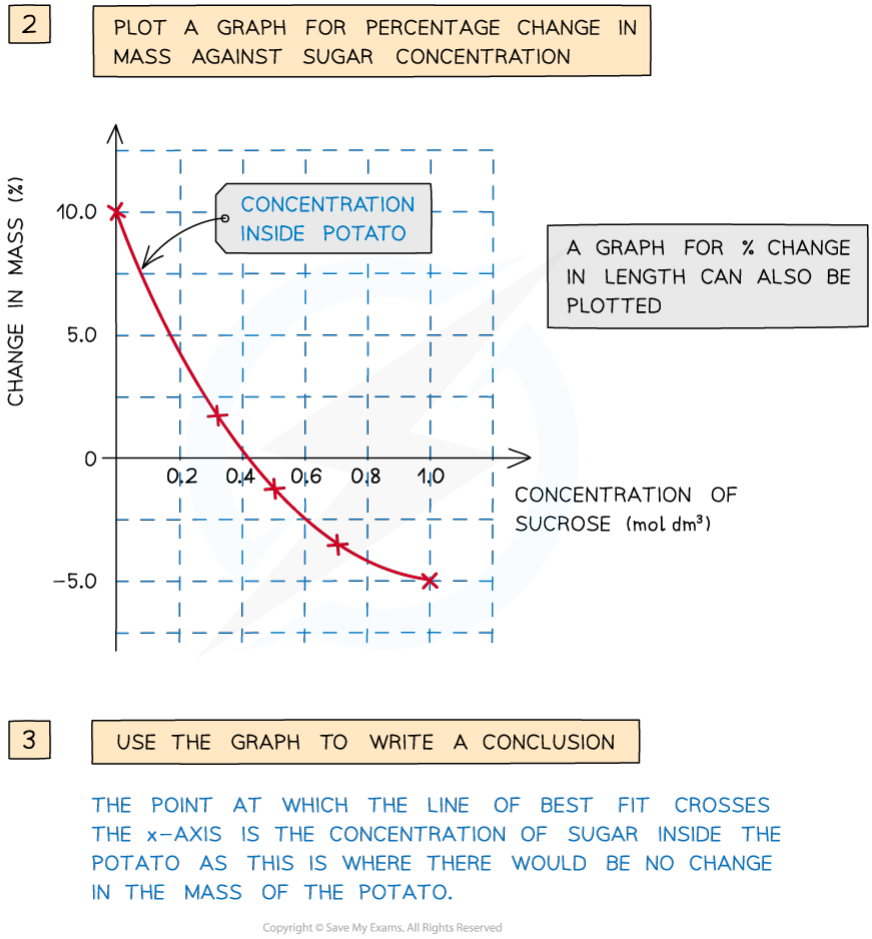
- A **positive** percentage change in mass indicates that the potato has gained water by osmosis (net movement of water from the solution into the potato) meaning the **solution** had a **higher water potential** than the potato
    - The gain of water makes the potato cells **turgid,** as the water exerts turgor pressure (or hydrostatic pressure) on the cell walls – the potatoes will feel hard
- A **negative** percentage change suggests the opposite, that is, the solution had a **lower** water potential than the potato
    - The potato cylinder in the **strongest sucrose concentration** will have **decreased in mass** the most as there is the **greatest concentration gradient** in this tube between the potato cells (higher water potential) and the sucrose solution (lower water potential)
    - More water molecules will move out of the potato cells by **osmosis**, making them **flaccid** and decreasing the mass of the potato cylinder – the potato cylinders will feel floppy
    - If looked at underneath the microscope, cells from this potato cylinder might be **plasmolysed**, meaning the cell membrane has pulled away from the cell wall
- If there is a potato cylinder that has neither increased nor decreased in mass, it means there was **no overall net movement of water** into or out of the potato cells
- The solution that this particular potato cylinder was in had the **same water potential** as the solution found in the cytoplasm of the potato cells, so there was **no concentration gradient** and therefore no net movement of water into or out of the potato cells

Analysis

- The concentration of sucrose inside the potato cylinders can be found if a graph is drawn showing how the percentage change in mass changes with the concentration of sucrose solution
- The point at which the line of best fit **crosses the x-axis** is the concentration of sucrose inside the potato cylinders

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇



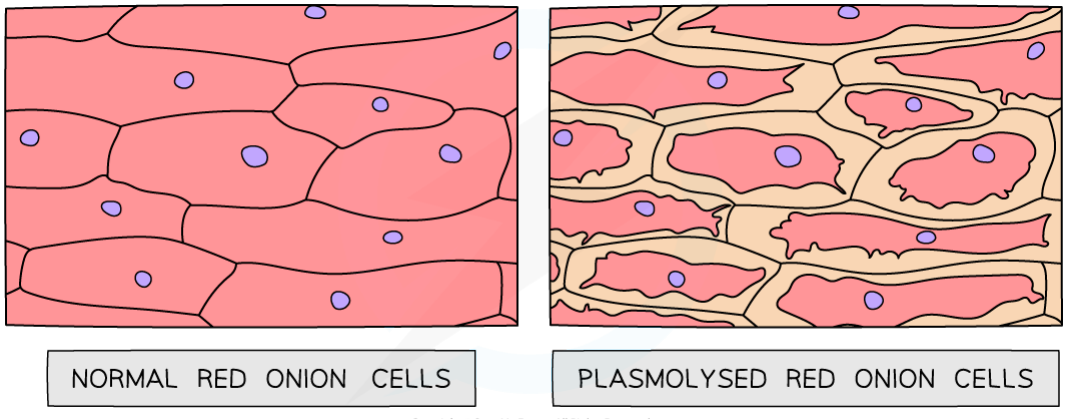
A positive percentage change in mass indicates that the potato has gained water by osmosis (net movement of water from the solution into the potato) meaning the solution had a higher water potential than the potato. A negative percentage change suggests the opposite.

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Practical 2: Investigating water potential using onion cells

- Evidence of osmosis occurring in plant cells can be shown when the cells undergo **plasmolysis**:
  - If a plant cell is placed in a solution with a **lower water potential** than the cell (such as a concentrated sucrose solution), water will **leave** the cell through its partially permeable cell surface membrane by **osmosis**
  - As water leaves the **vacuole** of the plant cell, the volume of the cell **decreases**
  - The protoplast (living part of the cell inside the cell wall) gradually shrinks and no longer exerts pressure on the cell wall
  - As the protoplast continues to shrink, it begins to pull away from the cell wall
  - This process is known as **plasmolysis** – the plant cell is **plasmolysed**
- This process can be observed using **epidermal strips** (sections of the very thin outer layer of tissue in plants)
  - Plants with coloured sap (such as red onion bulbs, rhubarb petioles and red cabbage) make observations easier
- The epidermal strips are placed in a **range of molarities of sucrose solution** or **sodium chloride solutions**, of gradually decreasing water potential
- The strips are then viewed under a light microscope and the **total number** or **percentage** of **onion cells** that have undergone **plasmolysis** can be counted
  - Plasmolysis may take several minutes to occur



Light micrograph of normal red onion cells alongside those that have plasmolysed (artistic impression). The cells on the left are epidermal cells that have been immersed in distilled water, whilst the cells on the right are epidermal cells that have been immersed id 1.0 mol dm⁻³ sucrose solution.

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Exam Tip

Questions involving experiments investigating water potential and osmosis are common and you should be able to use your knowledge of osmosis to explain the results obtained. Don't worry if it is an experiment you haven't done – simply figure out where the higher concentration of water molecules is – this is the solution with the higher water potential – and explain which way the molecules move due to the differences in water potential.

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

1.2.9 PRACTICAL: FACTORS AFFECTING MEMBRANE STRUCTURE & PERMEABILITY

Practical: Factors Affecting Membrane Structure & Permeability

- The **permeability** of **cell membranes** is affected by different **factors** or conditions, such as:
  - **Temperature**
  - **Solvent concentration**
- You can investigate how these different factors affect membrane structure and permeability using **beetroot**
  - Beetroot cells contain a **dark purple-red pigment**
  - The **higher** the permeability of the beetroot cell membrane, the **more** of this pigment leaks **out of the cell**

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Investigating the effect of temperature on membrane permeability

- In this practical, you need to show knowledge and understanding of how to use **appropriate apparatus** to **record a range of quantitative measurements**, including mass, time, volume, temperature, and length. For example:
  - **Mass** should be recorded using a **digital balance**
  - **Time** should be recorded using a **digital stopwatch**
  - **Volume** should be recorded using a **measuring cylinder**
  - **Temperature** should be recorded using a **digital thermometer** (although water baths have one built-in)
  - **Length** should be recorded using a **ruler**
- In addition, you need to show knowledge and understanding of how to use another piece of apparatus, known as a **colorimeter**, that is also appropriate for use in this practical
  - A colorimeter is a machine that passes light through a **coloured liquid sample** and measures how much of that light is absorbed (and therefore gives an indication of how much of the colour is present in the solution)
  - A colour filter is used in the light path to ensure that the correct wavelength of light is used to measure the optical density of the specific pigment in the solution (e.g. the beetroot pigment called betalain)
  - The colorimeter must be **zeroed** before each colorimeter tube (called a **cuvette**) is inserted. This can be done using **distilled water** in a cuvette

Apparatus

- Scalpel
- Cork borer (optional)
- Cutting board
- Ruler
- Digital balance
- Test tubes
- Measuring cylinder
- Water baths
- Digital stopwatch
- Colorimeter

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Method

- Using a scalpel, cut five **equal-sized** cubes of beetroot
  - The pieces must have the same dimensions so that they all have **equal surface areas and volumes**, as these factors could affect the rate at which the pigment leaks out
  - A cork borer can also be used, as long as the cores are cut to the **same length**
  - You should also use a digital balance to check that all pieces have the **same mass**
- **Rinse** the beetroot pieces
  - To **remove** any pigment released during cutting
- Add the beetroot pieces to five different test tubes, each containing the **same volume of water** (e.g. $5cm^3$)
- Put each test tube in a water bath at a different temperature (e.g. 10℃, 20℃, 30℃, 40℃, 50℃) for the **same length of time**
  - The time should be **long enough to allow the pigment to diffuse into the water** (e.g. around 30 minutes)
- Remove the beetroot pieces, leaving just the coloured liquid in the five test tubes
- Use a **colorimeter** to measure how much **light** is **absorbed** as it passes through each of the five samples of coloured liquid
  - The **higher** the **absorbance**, the **more pigment** must have been released, due to a **greater** membrane **permeability**

1.2 Practical Skills: Endorsement Assessment

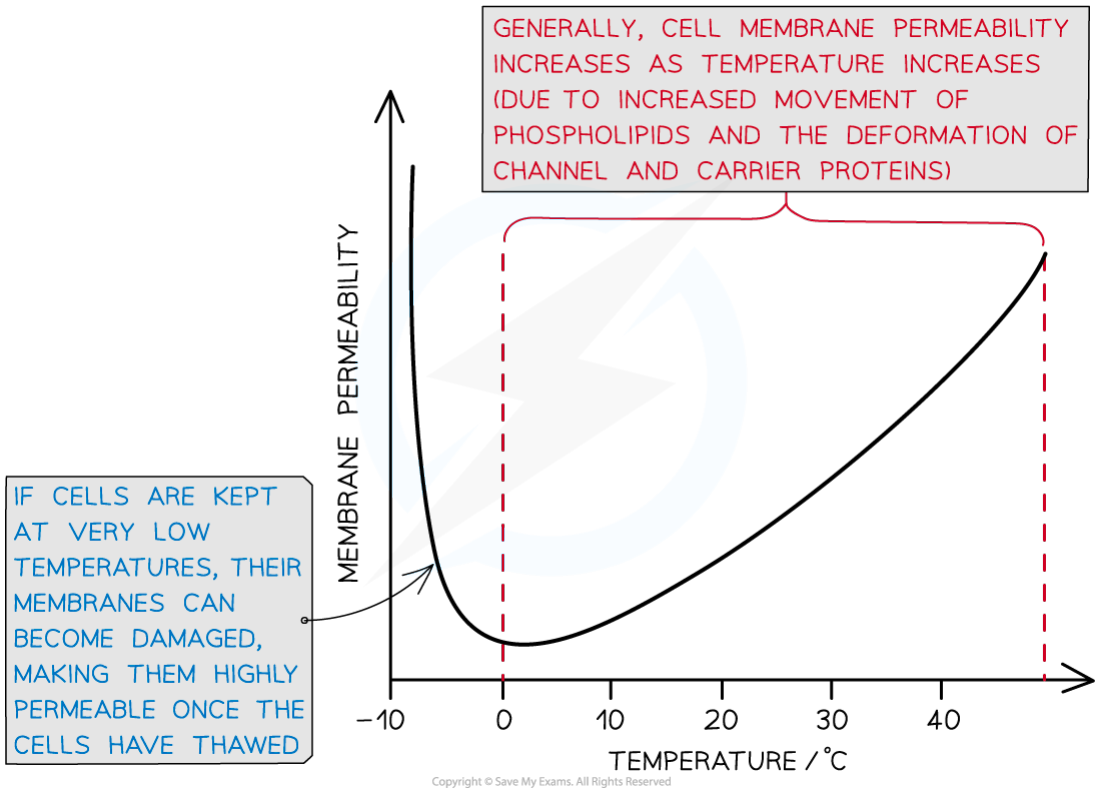
YOUR NOTES ⬇

Results

- The general pattern you would expect to see is that **as temperature increases, membrane permeability also increases**
  - As temperature increases, the phospholipids within the cell membrane **move** more because they have more **energy**
  - Increased movement means the phospholipids are **not** as **tightly packed** together, increasing the permeability of the membrane
  - At high temperatures, the phospholipid bilayer may even start to melt and **breakdown**, further increasing the permeability of the membrane
  - In addition, the volume of water inside the cells **expands**, putting pressure on the membrane, causing channel and carrier proteins to **deform** so they can no longer control what enters and leaves the cell. These factors also increase the permeability of the membrane
  - Temperature also affects the **conformation** (3D shape) **of proteins** as at high temperatures the **intermolecular forces** between amino acids are **broken** which affects the protein's **specificity and function**
- If experimenting with **temperatures below 0℃**, membrane permeability may also be increased (once the cells have thawed again)
  - Increased permeability can be caused by channel or carrier proteins **deforming** at these low temperatures
  - **Ice crystals** that form can also **pierce** the cell membrane, making it highly permeable

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇



Example results showing the effect of temperature on membrane permeability

Limitations

- **Cuvettes** are the small cuboid containers that **hold** the liquid to be measured in a colorimeter
- Cuvettes may differ in **thickness** (very slightly). A thicker (or **scratched**) cuvette will absorb slightly **more light** than a thinner unscratched cuvette
    - Solution: use the **same cuvette** for every reading, or **repeat** the investigation many times and find a **mean**
- The beetroot pieces may not be identical in **size** and shape, meaning some test tubes could contain slightly more beetroot tissue than others
    - Solution: cut the discs as **accurately** as possible using a **scalpel and ruler**, and **repeat** each investigation several times to find a mean
- Some **parts** of beetroot tissue have **more pigment** in their cells than others
    - Solution: conduct several **repeats**, using **different parts** of the beetroot and find a **mean**

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Exam Tip

You could also investigate how solvent concentration affects cell membrane permeability by placing beetroot pieces in test tubes containing increasing concentrations of solvents (such as alcohol or acetone).

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

1.2.10 BIOCHEMICAL TESTS: REDUCING SUGARS & STARCH

Biochemical Tests: Reducing Sugars & Starch

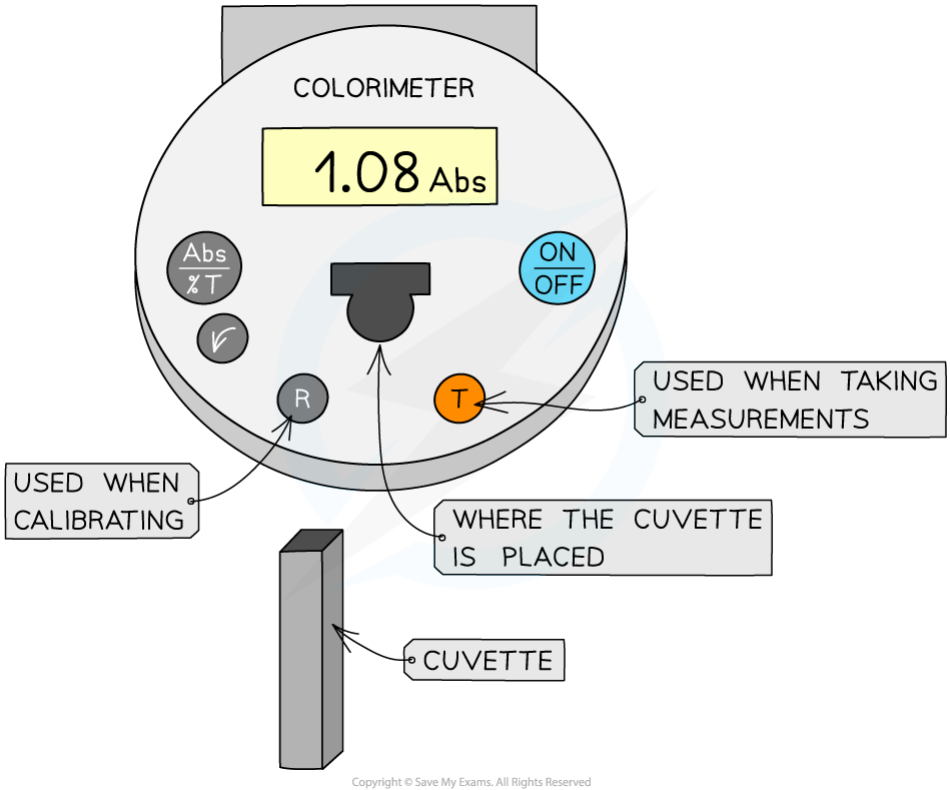
- **Benedict's solution** can be used to carry out a **semi-quantitative** test on a **reducing sugar solution** to determine the **concentration** of reducing sugar present in the sample
  - It is important that an **excess** of Benedict's solution is used so that there is **more than enough copper (II) sulfate** present to react with any sugar present
- The **intensity** of any colour change seen relates to the **concentration** of reducing sugar present in the sample
  - A positive test is indicated along a **spectrum of colour** from **green (low concentration)** to **brick-red (high concentration of reducing sugar present)**
- A semi-quantitative test can be carried out by setting up **standard solutions** with **known concentrations** of a reducing sugar (such as glucose)
- These solutions should be set up using a **serial dilution** of an existing **stock solution**
  - Serial dilutions are created by taking a series of dilutions of a stock solution. The concentration decreases by the **same** quantity between each test tube
  - They can either be 'doubling dilutions' (where the concentration is halved between each test tube) or a desired range (e.g. 0, 2, 4, 6, 8, 10 mmol dm^{-3})
- Each solution is then treated in the same way: add the same volume of Benedict's solution to each sample and heat in a water bath that has been boiled (ideally at the same temperature each time) for a set time (5 minutes or so) to allow colour changes to occur
  - It is important to ensure that an **excess** of Benedict's solution is used
- Any colour change observed for each solution of a known concentration in that time can be attributed to the concentration of reducing sugar present in that solution
- The same procedure is carried out on a sample with an unknown concentration of reducing sugar which is then compared to the **standard solution colours** to estimate the concentration of reducing sugar present
- To avoid issues with human interpretation of colour, a **colorimeter** could be used to measure the absorbance or transmission of light through the sugar solutions of known concentration to establish a range of values that an unknown sample can be compared against a calibration curve

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

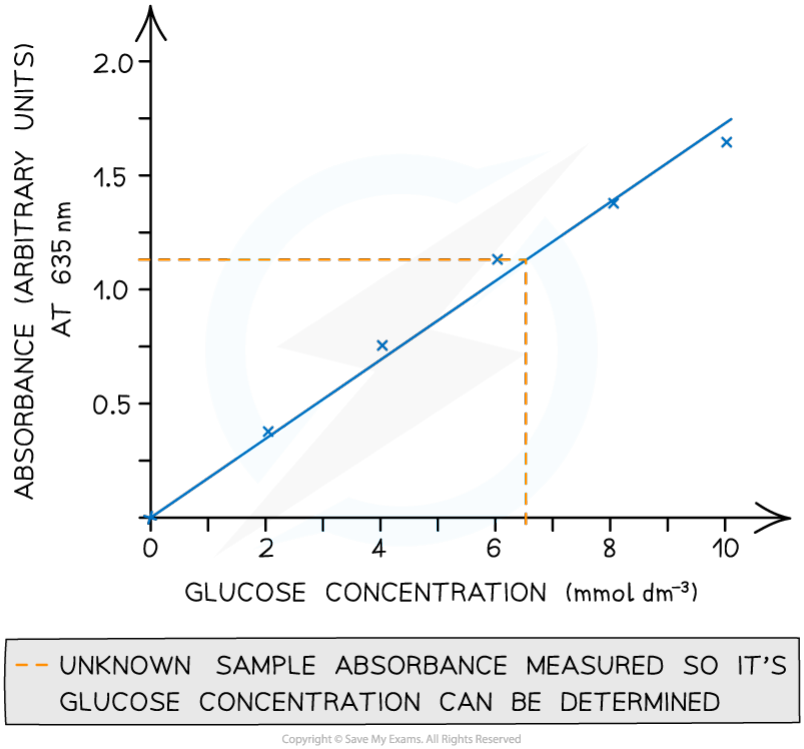
Colorimeters

- A colorimeter is an instrument that beams a specific wavelength (colour) of light through a sample and measures how much of this light is **absorbed** (arbitrary units)
- They provide a **quantitative** measurement
- They contain different wavelengths or colour filters (depends on the model of colorimeter), so that a suitable colour can be shone through the sample and will not get absorbed. This colour will be the contrasting colour (eg. a red sample should have green light shone through)
    - **Remember** that a sample will look red as that wavelength of light is being **reflected** but the other wavelengths will be absorbed
- Colorimeters must be **calibrated** before taking measurements
    - This is completed by placing a blank into the colorimeter and taking a reference, it should read 0 (that is, no light is being absorbed)
    - This step should be repeated periodically whilst taking measurements to ensure that the absorbance is still 0
- The results can then be used to plot a calibration or standard curve
    - Absorbance against the known concentrations can be used
    - Unknown concentrations can then be determined from this graph



1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇



A colorimeter is used to obtain quantitative data that can be plotted to create a calibration curve to be used to find unknown concentrations

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

1.2.11 BIOCHEMICAL TESTS: LIPIDS

Biochemical Tests: Lipids

Identifying biological molecules

- Different **qualitative** reagents can be used to identfy the presence of biological molecules in samples
  - **Ethanol** is used to identify lipids
  - **Biuret reagent** is used to identify proteins
  - **Benedicts solution and iodine** are used to identify carbohydrates
- Qualitative reagents simply determine whether or not a substance is present in a sample
- The quantity or concentration of the substance present is not determined

Practical: the emulsion test for lipids

- The **emulsion test** can be carried out quickly and easily in a lab to determine if a sample contains lipids
- Lipids are **nonpolar** molecules that do not dissolve in water but will dissolve in organic solvents such as ethanol

Apparatus

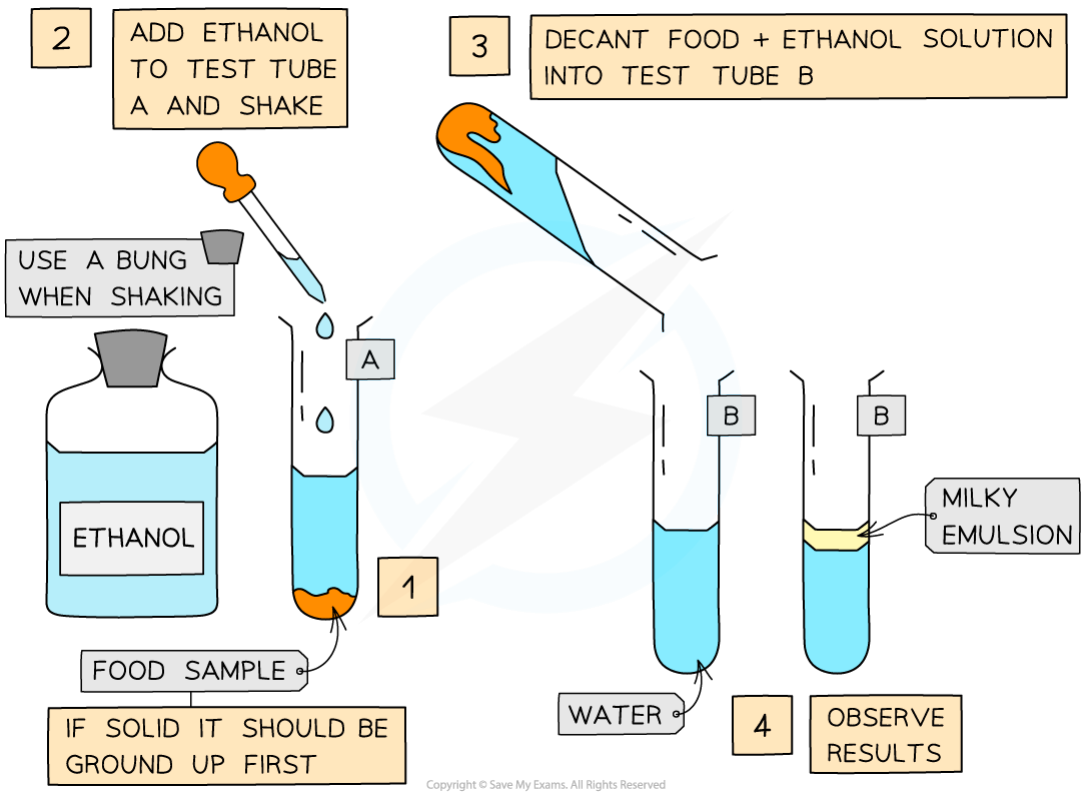
- Test tubes
- Test tube rack
- Ethanol
- Pipettes
- Food sample
- Mortar and pestle (if food sample is solid)
- Water
- Gloves

Method

- Add **ethanol** to the sample to be tested
- **Shake** to mix
- Add the mixture to a test tube of **water**

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇



The Emulsion test for lipids forms a milky colour

Results

- If lipids are present, a **milky emulsion** will form (the solution appears 'cloudy'); the more lipid present, the more obvious the milky colour of the solution
- If no lipid is present, the solution remains clear

Limitations

- This test is qualitative – it does not give a quantitative value as to how much lipid may be present in a sample

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

1.2.12 BIOCHEMICAL TESTS: PROTEINS

Biochemical Tests: Proteins

- The Biuret test can be carried out quickly and easily in a lab to determine if a sample contains proteins
- Biuret 'reagent' contains an **alkali and copper (II) sulfate** which react in the presence of **peptide bonds**

Apparatus

- Test tubes
- Test tube rack
- Food solution
- Control solution (containing no proteins e.g. distilled water)
- Sodium hydroxide
- Copper (II) sulfate solution
- Pipette
- White tile
- Gloves

Method

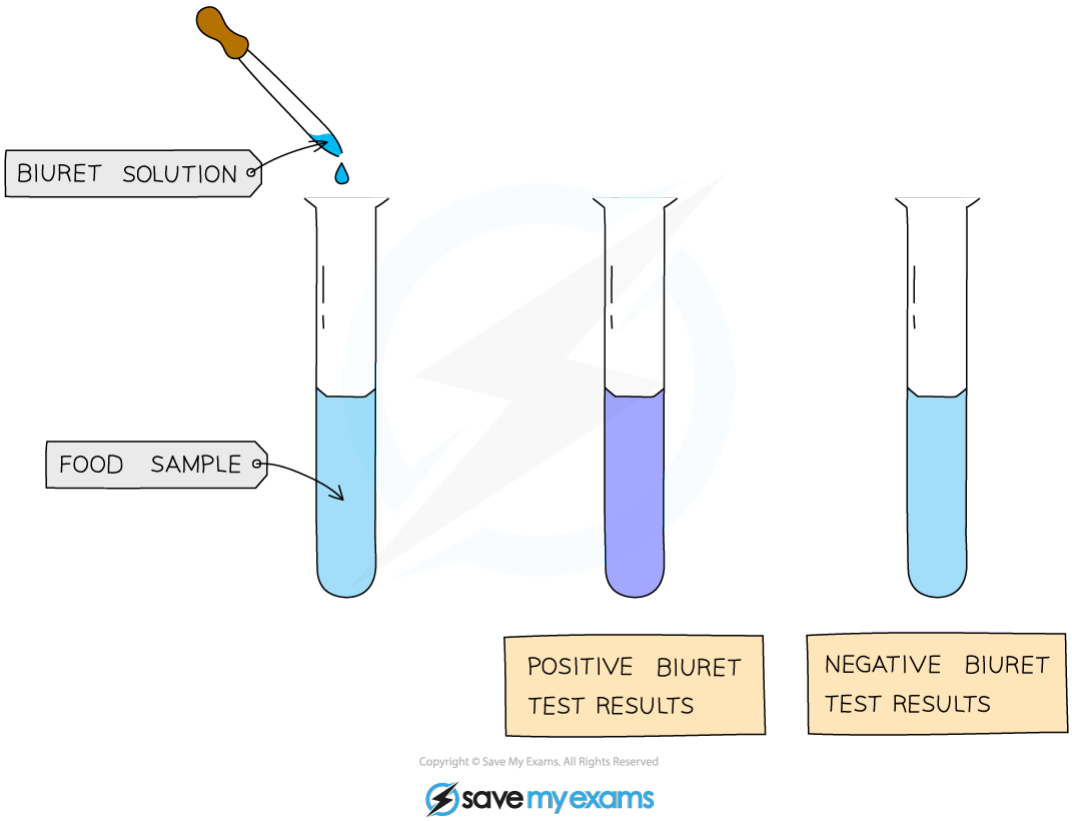
- **Add sodium hydroxide** to the food solution sample to make the solution **alkaline**
- Add a few drops of **copper (II) sulfate solution** (which is blue) to the sample
    - Biuret 'reagent' contains an alkali and copper (II) sulfate
- Repeat steps 1 and 2 using the **control solution**
- **Compare** the colours of the control solution and the food sample solution

Results

- If a colour change is observed from **blue to lilac/mauve**, then protein is present.
    - The colour change can be very **subtle**, it's wise to hold the test tubes up against a **white tile** when making observations
- If no colour change is observed, no protein is present
    - For this test to work, there must be at least two peptide bonds present in any protein molecules, so if the sample contains amino acids or dipeptides, the result will be negative

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇



A positive result from the Biuret test will produce a colour change from blue to mauve/lilac

Limitations

- The Biuret test is qualitative – it does not give a quantitative value as to the **amount** of protein present in a sample
- If the sample contains amino acids or dipeptides, the result will be negative (due to lack of peptide bonds)

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

1.2.13 CHROMATOGRAPHY

Chromatography

- Chromatography is a technique that can be used to **separate a mixture** into its individual components
- Chromatography relies on **differences in the solubility** of the different chemicals (called **'solutes'**) within a mixture
- All chromatography techniques use **two phases:**
  - The **mobile** phase
  - The **stationary** phase
- The components in the mixture **separate** as the mobile phase travels over the stationary phase
- Differences in the **solubility** of each component in the mobile phase affects **how far each component can travel**
- Those components with **higher solubility** will travel **further** than the others
- This is because they spend more time in the mobile phase and are thus carried further up the paper than the less soluble components

Paper chromatography

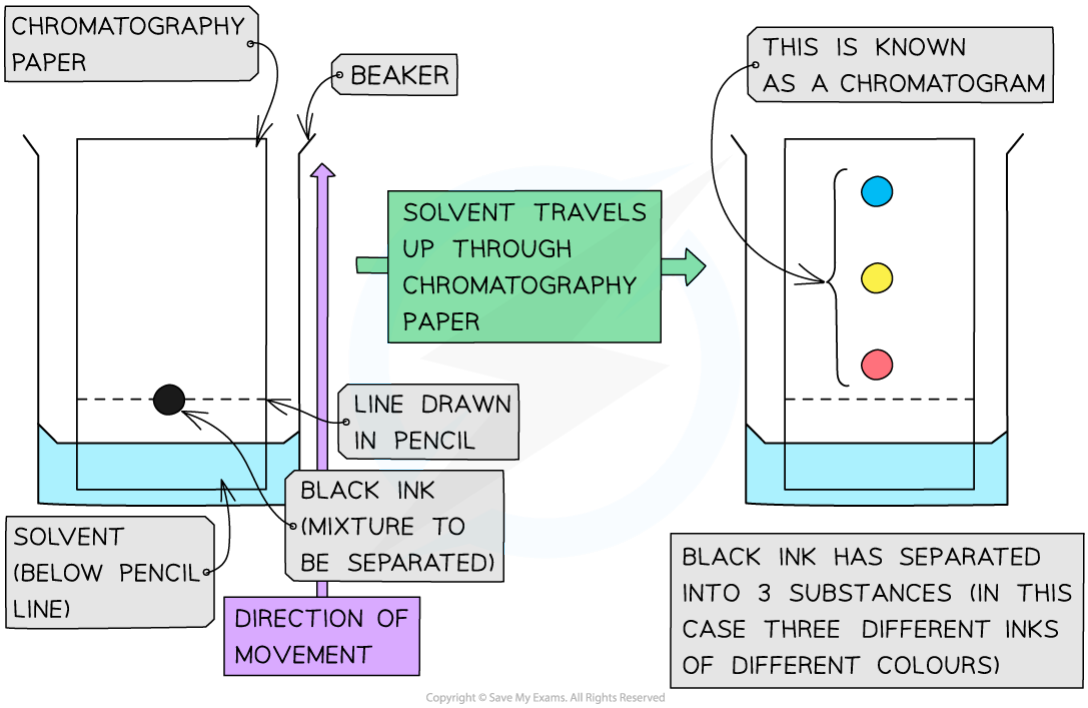
- **Paper chromatography** is one specific form of chromatography
- In paper chromatography:
  - The mobile phase is the **solvent** in which the sample molecules can move, which in paper chromatography is a liquid e.g. water or ethanol
  - The stationary phase in paper chromatography is the **chromatography paper**

Paper chromatography method

- A spot of the mixture (that you want to separate) is placed on chromatography paper and left to dry
- The chromatography paper is then **suspended in a solvent**
- As the solvent travels up through the chromatography paper, the different components within the mixture begin to move up the paper at different speeds
  - **Larger molecules move slower than smaller ones**
  - This causes the original mixture to separate out into different spots or bands on the chromatography paper
- This produces what is known as a **chromatogram**

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES



An example of a chromatogram that has been produced by using paper chromatography to separate a spot of ink.

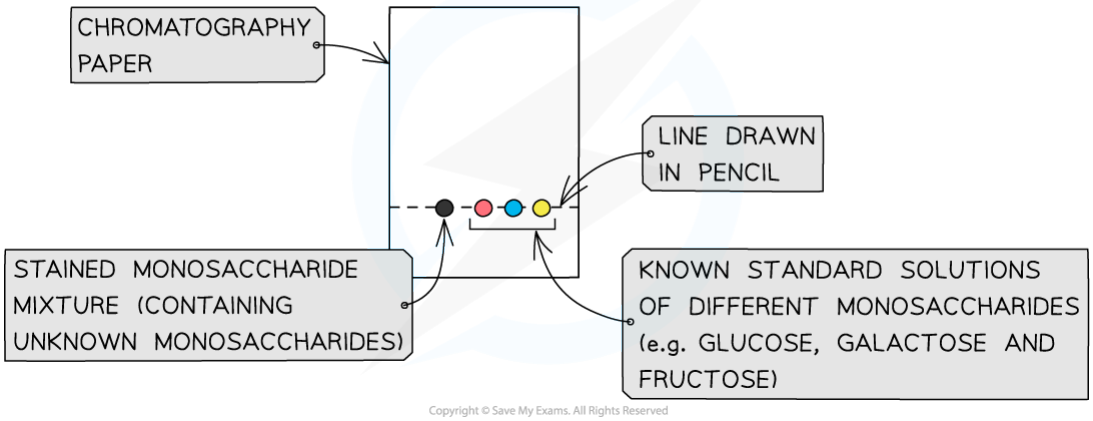
Using chromatography to separate a mixture of monosaccharides

- Paper chromatography can be used to **separate a mixture of monosaccharides**
- Mixtures containing coloured molecules, such as ink or chlorophyll, do not have to be stained as they are already coloured
- Mixtures of colourless molecules, such as a mixture of monosaccharides, **have to be stained first**
- A spot of the stained monosaccharide sample mixture is placed on a line at the bottom of the chromatography paper
- Spots of **known standard solutions** of different monosaccharides are then placed on the line beside the sample spot
- The chromatography paper is then **suspended in a solvent**
- As the solvent travels up through the chromatography paper, **the different monosaccharides** within the mixture **separate out** at different distances from the line
- The unknown monosaccharides can then be identified by **comparing** and **matching** them with the chromatograms of the known standard solutions of different monosaccharides
    - If a spot from the monosaccharide sample mixture is at the **same distance from the line** as a spot from one of the known standard solutions, then **the mixture must contain this monosaccharide**

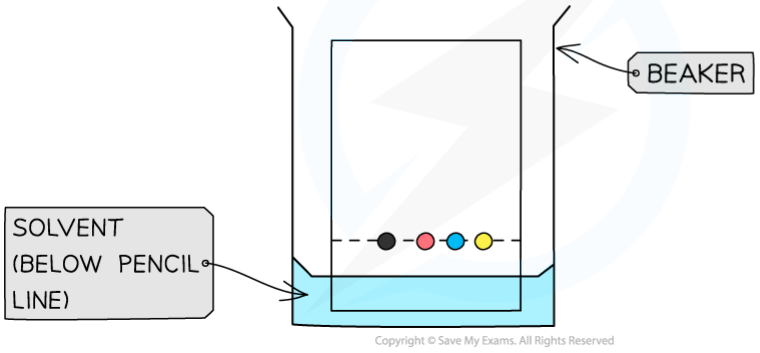
1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

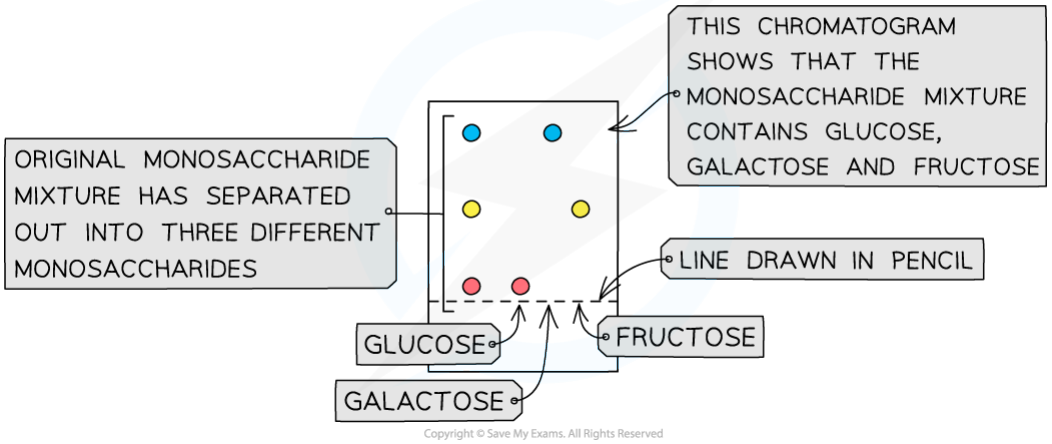
1 SET UP CHROMATOGRAPHY PAPER AS SHOWN



2 LOWER PAPER INTO A BEAKER WITH APPROPRIATE SOLVENT. WAIT FOR SOLVENT TO TRAVEL UP THE PAPER.



3 ANALYSE CHROMATOGRAM



1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

How chromatography can be used to separate a mixture of monosaccharides and identify the individual components.

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

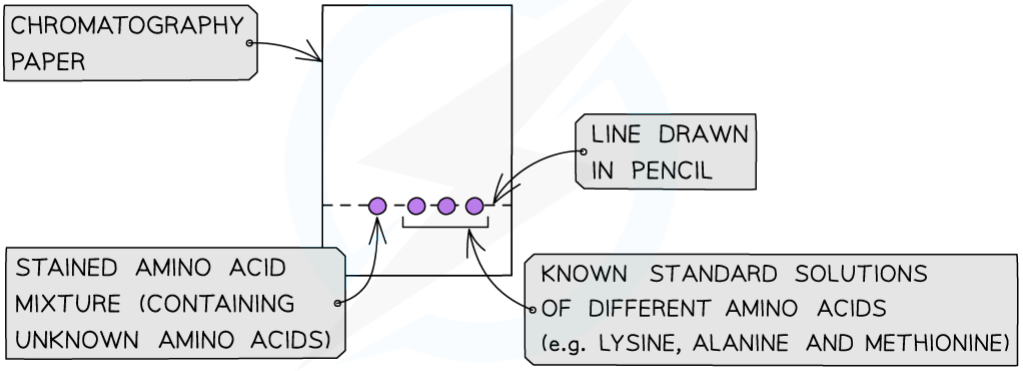
Using chromatography to separate a mixture of amino acids

- Paper chromatography can be used to **separate a mixture of amino acids**
- A spot of the unknown amino acid sample mixture is placed on a line at the bottom of the chromatography paper
- Spots of **known standard solutions** of different amino acids are then placed on the line beside the unknown sample spot
- The chromatography paper is then **suspended in a solvent**
- Each amino acid will be **more or less soluble** in the mobile phase than others and will therefore **separate out** of the mixture travelling with the solvent at **different times/distances** from the line, depending on their:
  - **Charge**
  - **Size**
- The unknown amino acid(s) can then be identified by **comparing** and **matching** them with the chromatograms of the **known standard solutions** of different amino acids
  - If a spot from the amino acid sample mixture is at the **same distance from the line** as a spot from one the known standard solutions, then **the mixture must contain this amino acid**
- In order to view the spots from the different amino acids, it may be necessary to first **dry** the chromatography paper and then **spray it** with **ninhydrin solution** (this chemical **reacts with amino acids**, producing an **easily visible blue-violet colour**)

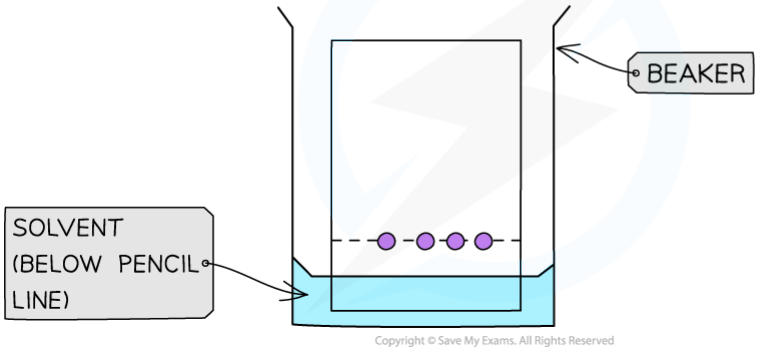
1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

1 SET UP CHROMATOGRAPHY PAPER AS SHOWN

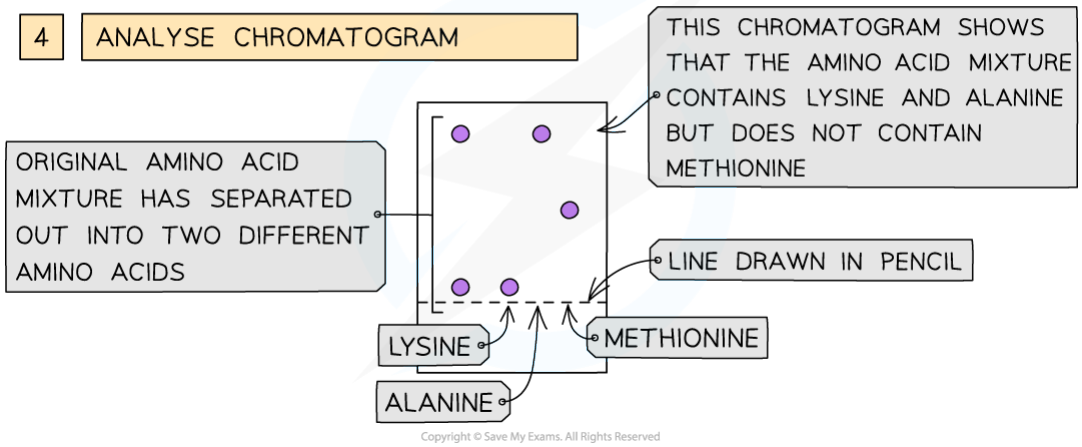


2 LOWER PAPER INTO A BEAKER WITH APPROPRIATE SOLVENT. WAIT FOR SOLVENT TO TRAVEL UP THE PAPER.



3 DRY CHROMATOGRAPHY PAPER AND SPRAY WITH NINHYDRIN SOLUTION

4 ANALYSE CHROMATOGRAM



How chromatography can be used to separate a mixture of amino acids and identify the individual components.

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Calculating the Rf value

- After a chromatogram has been obtained the molecules present in the sample mixture can be identified by calculating their retardation factor (Rf)
- In order to calculate Rf values, a line must be drawn across the chromatogram to show how far the solvent travelled
  - This line is known as the **solvent front**
  - The distance between the **origin line** and the solvent front is the distance moved by the solvent
  - The origin line is the line at the **bottom** of the paper on which the samples were placed at the beginning of the experiment
- The R_f value demonstrates how far a dissolved molecule travels during the mobile phase
  - A **smaller R_f value** indicates the molecule is **less soluble** and **larger** in size
- The Rf value of each solute (each spot on the chromatogram) is calculated and then compared to the Rf values of known molecules/substances
- The equation is:

Rf = distance moved by solute ÷ distance moved by solvent

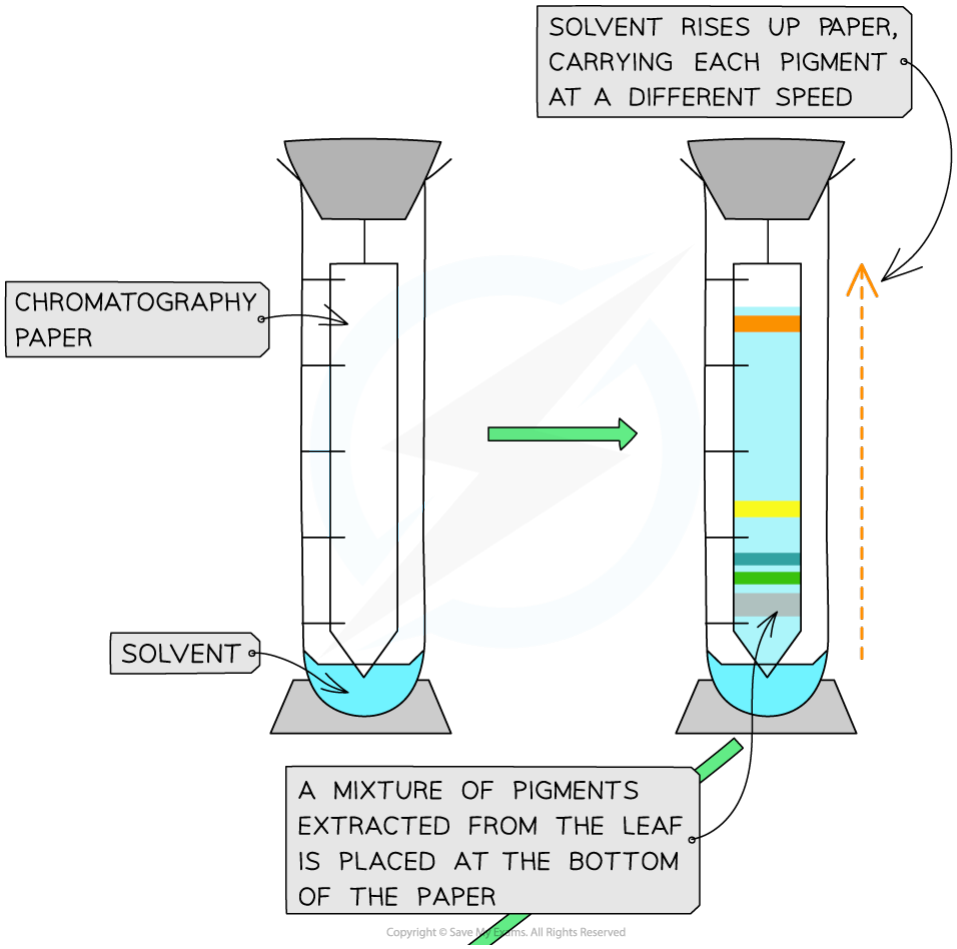
- The Rf value is a ratio so it is **always lower than one**
  - It has **no units**

Using Rf values to identify chloroplast pigments

- Chromatography can be used to separate and identify chloroplast pigments that have been extracted from a leaf as **each pigment will have a unique Rf value**
- Although specific Rf values depend on the solvent that is being used, in general:
  - **Carotenoids** have the **highest Rf values** (usually close to 1)
  - **Chlorophyll B** has a **much lower Rf value**
  - **Chlorophyll A** has an Rf value somewhere **between** those of carotenoids and chlorophyll B
  - **Small Rf values** indicate the pigment is **less soluble** and **larger** in size

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES



PIGMENT	Rf VALUE
CAROTENE	0.95
XANTHOPHYLL	0.71
CHLOROPHYLL A	0.65
CHLOROPHYLL B	0.45
LEAF STAIN	

PIGMENTS SEPARATE, AS THEY MOVE DIFFERENT DISTANCES

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Paper chromatography can be used to separate the photosynthetic pigments found within chloroplasts. Rf values can then be calculated for each pigment and compared to known Rf values for the different pigments.

Exam Tip

It is always worth trying to understand **why** a certain practical technique is useful. An example of when chromatography would be used is if you have an unknown liquid and you have determined it contains protein using a Biuret test. Chromatography will then show you which amino acids are present so you can better understand the potential use or function of the sample. This could be useful in crime scene investigations or in detecting additives or spoilage in foods.

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

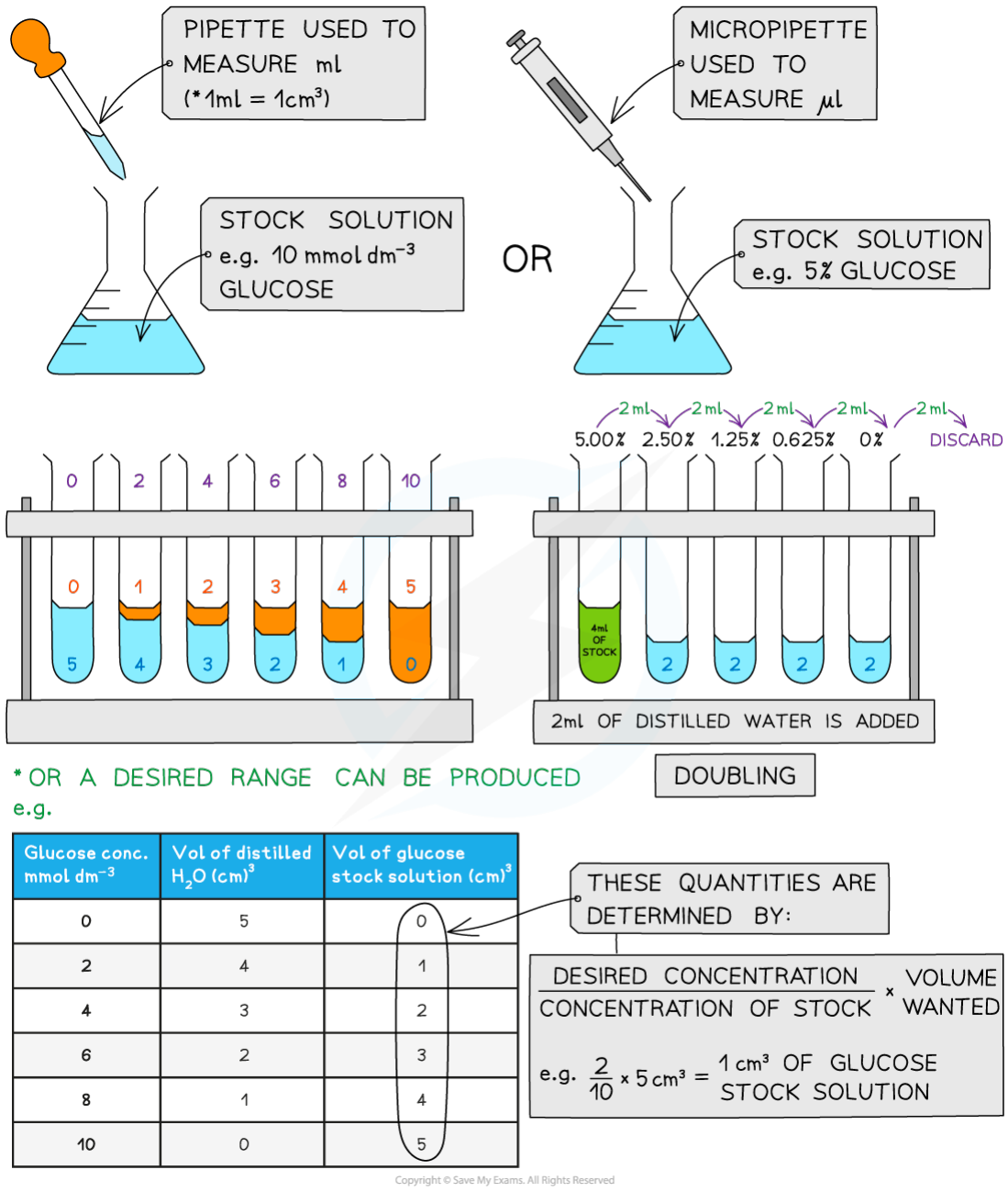
1.2.14 SERIAL DILUTIONS

Serial Dilutions

- Serial dilutions are created by taking a series of dilutions of a stock solution. The concentration decreases by the **same** quantity between each test tube
  - They can either be 'doubling dilutions' (where the concentration is halved between each test tube) or a desired range (e.g. 0, 2, 4, 6, 8, 10 mmol dm^{-3})
- Serial dilutions are completed to create a standard to compare unknown concentrations against
  - The comparison can be:
    - Visual
    - Measured through a calibration/standard curve
    - Measured using a colourimeter
  - They can be used when:
    - Counting bacteria or yeast populations
    - Determining unknown glucose, starch, protein concentrations

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇



*OR A DESIRED RANGE CAN BE PRODUCED

e.g.

Glucose conc. mmol dm^{-3}	Vol of distilled H_2O $(cm)^3$	Vol of glucose stock solution $(cm)^3$
0	5	0
2	4	1
4	3	2
6	2	3
8	1	4
10	0	5

THESE QUANTITIES ARE DETERMINED BY:

$$\frac{\text{DESIRED CONCENTRATION}}{\text{CONCENTRATION OF STOCK}} \times \text{VOLUME WANTED}$$

e.g. $\frac{2}{10} \times 5\,cm^3 = 1\,cm^3$ OF GLUCOSE STOCK SOLUTION

Making serial dilutions

YOUR NOTES ⬇

1.2 Practical Skills: Endorsement Assessment

1.2.15 PRACTICAL: INVESTIGATING THE RATE OF TRANSPIRATION

Practical: Investigating the Rate of Transpiration

Using a potometer

- Air movement, humidity, temperature and light intensity all have an effect on the rate at which transpiration occurs
- The table below explains how these four factors affect the rate of transpiration when they are all high; the opposite effect would be observed if they were low

Transpiration Rate Factors Table

Factor	Condition	Effect on the rate of transpiration (more/less)
Air movement	High	More – good airflow removes water vapour from the air surrounding the leaf which sets up a concentration gradient between the leaf and the air, increasing water loss
Humidity	High	Less – humidity is a measure of moisture (water vapour) in the air; when the air is saturated with water vapour the concentration gradient is weaker so less water is lost
Light intensity	High	More – guard cells are responsive to light intensity; when it is high they are turgid and the stomata open allowing water to be lost
Temperature	High	More – at higher temperatures, particles have more kinetic energy so transpiration occurs as a faster rate as water molecules evaporate from the mesophyll and diffuse away faster than at lower temperatures

- A **potometer** can be used to investigate the effect of environmental factors on the rate of transpiration

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Apparatus

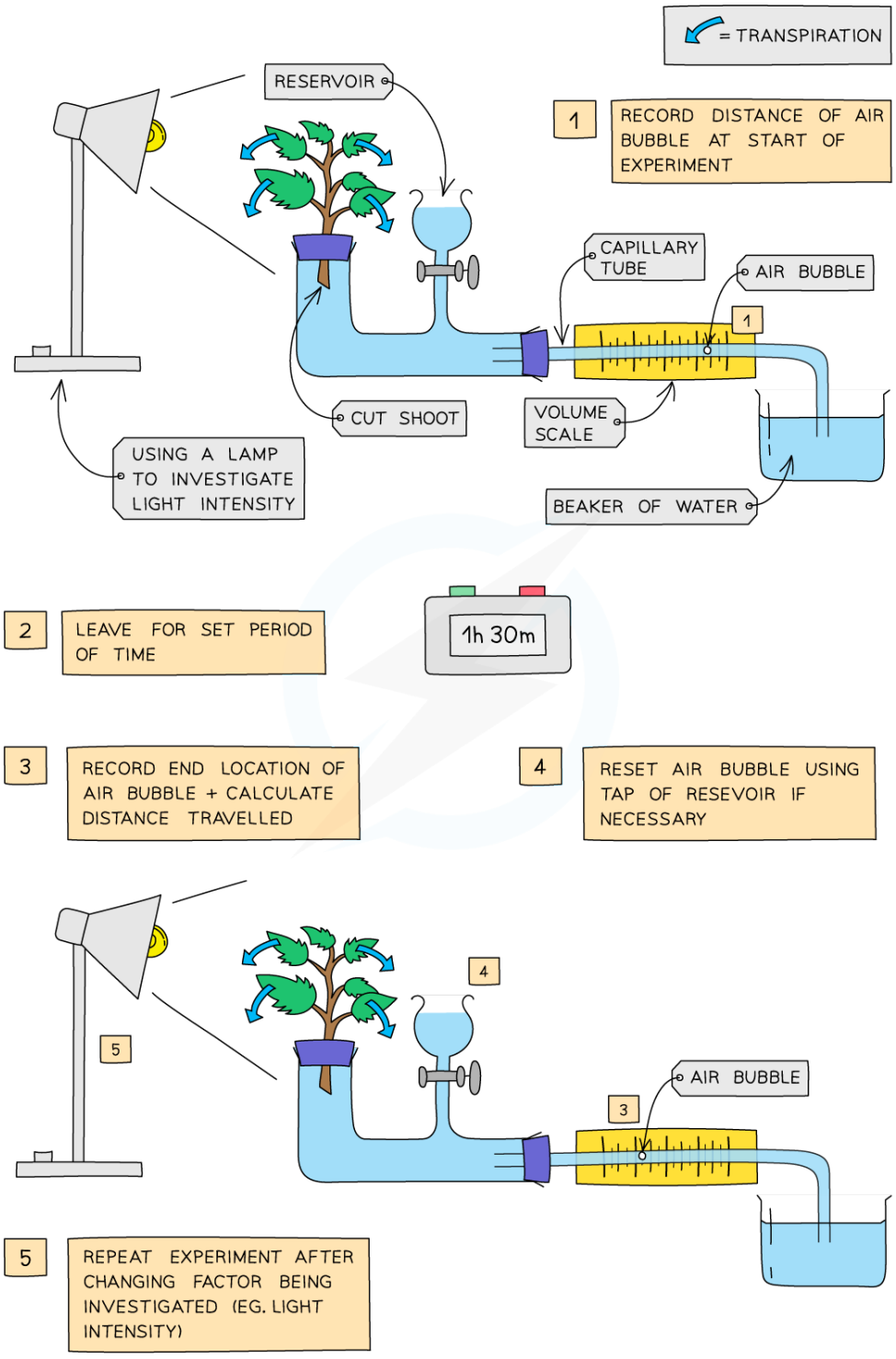
- Plant shoot
- Cutting board
- Scalpel/scissors
- Paper towels
- Potometer
- Volume scale
- Beaker
- Capillary tube
- Stopwatch
- Vaseline

Method

- Cut a shoot underwater
  - This is done to **prevent air from entering the xylem**
- Place the shoot in the tube
- Set up the apparatus as shown in the diagram
- Make sure it is airtight, using vaseline to seal any gaps
  - If air enters the apparatus the readings will be affected
- Dry the leaves of the shoot
  - Any moisture present on the leaves will affect the rate of transpiration
- Remove the capillary tube from the beaker of water to allow a single **air bubble** to form and place the tube back into the water
- Set up the environmental factor you are investigating
- Allow the plant to adapt to the new environment for 5 minutes
- Record the **starting location** of the air bubble
- Leave for a set period of time
- Record the **end location** of the air bubble
- Change the light intensity or wind speed or level of humidity or temperature (only one – whichever factor is being investigated)
- Reset the bubble by opening the tap below the reservoir
- Repeat the experiment
- The **further the bubble travels in the same time period, the faster transpiration is occurring** and vice versa

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES



Investigating transpiration rates using a potometer

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Environmental factors can be investigated in the following ways:

- **Airflow**: Set up a fan or hairdryer
- **Humidity**: Spray water in a plastic bag and wrap around the plant
- **Light intensity**: Change the distance of a light source from the plant
- **Temperature**: Temperature of room (cold room or warm room)

Exam Tip

Remember when designing an investigation to ensure a fair test you must keep all factors the same other than the one you are investigating.

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

1.2.16 PRACTICAL: USING A LIGHT MICROSCOPE

Practical: Using a Light Microscope

- Many biological structures are too small to be seen by the naked eye
- Optical microscopes are an invaluable tool for scientists as they allow for tissues, cells and organelles to be seen and studied
- For example, the movement of chromosomes during mitosis can be observed using a microscope

How optical microscopes work

- Light is directed through the thin layer of biological material that is supported on a glass slide
- This light is focused through several lenses so that an image is visible through the eyepiece
- The magnifying power of the microscope can be increased by rotating the higher power objective lens into place

Apparatus

- The key components of an optical microscope are:
  - The eyepiece lens
  - The objective lenses
  - The stage
  - The light source
  - The coarse and fine focus
- Other tools used:
  - Forceps
  - Scissors
  - Scalpel
  - Coverslip
  - Slides
  - Pipette

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES

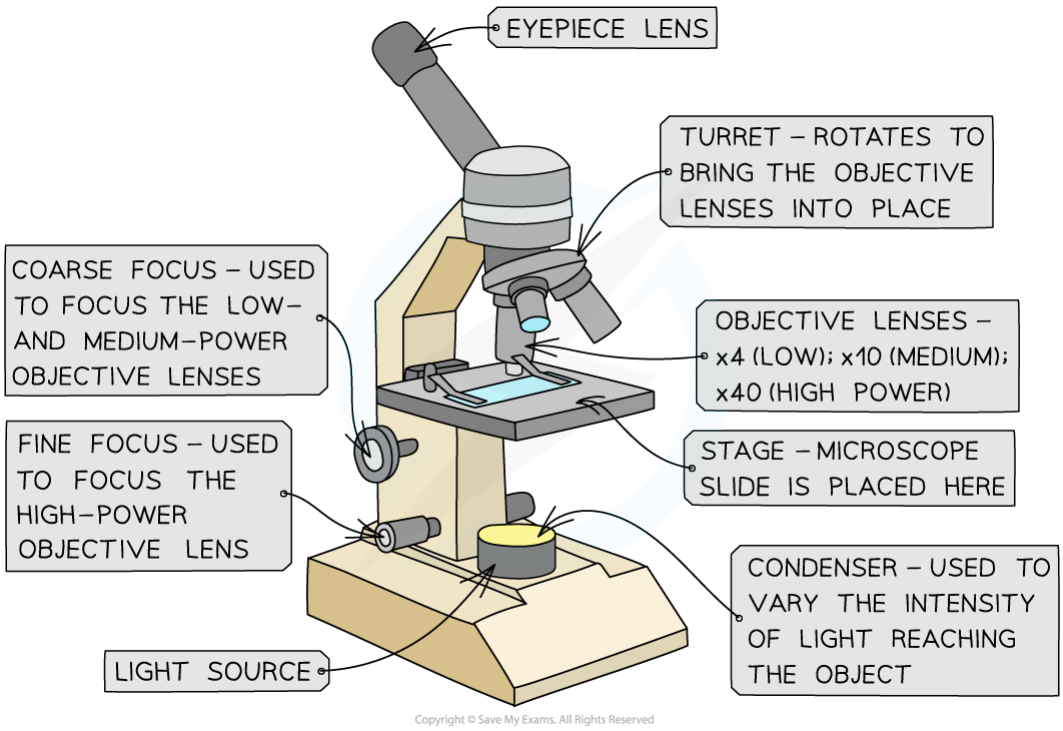


Image showing all the components of an optical microscope

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Method

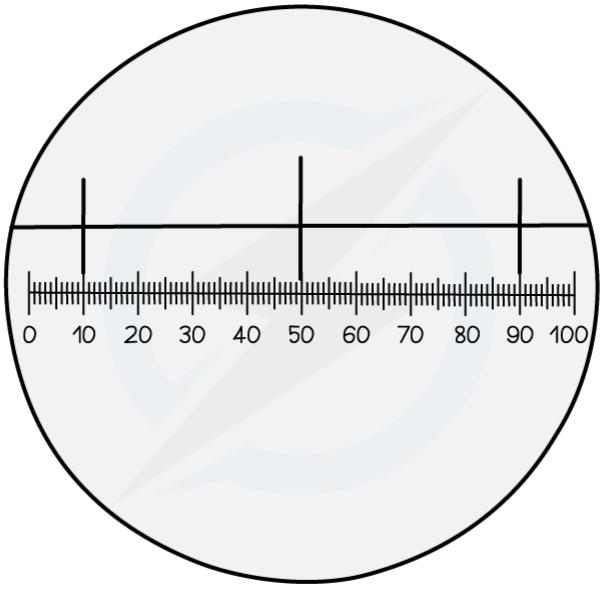
- Preparing a slide using a **liquid specimen**:
  - Add a few drops of the sample to the slide using a pipette
  - Cover the liquid/smear with a coverslip and gently press down to **remove air bubbles**
  - **Wear gloves** to ensure there is no cross-contamination of foreign cells
- Preparing a slide using a **solid specimen**:
  - Use scissors to cut a small sample of the tissue
  - Peel away or cut a **very thin layer** of cells from the tissue sample to be placed on the slide (using a scalpel or forceps)
  - Some tissue samples need be treated with chemicals to kill/make the tissue rigid
  - Gently place a coverslip on top and press down to remove any air bubbles
  - A **stain** may be required to make the structures visible depending on the type of tissue being examined
  - Take care when using sharp objects and wear gloves to prevent the stain from dying your skin
- When using an optical microscope always **start with the low power objective lens**:
  - It is **easier to find** what you are looking for in the field of view
  - This helps to **prevent damage** to the lens or coverslip incase the stage has been raised too high
- Preventing the dehydration of tissue:
  - The thin layers of material placed on slides can **dry up rapidly**
  - Adding a drop of water to the specimen (beneath the coverslip) can prevent the cells from being damaged by dehydration
- Unclear or blurry images:
  - Switch to the lower power objective lens and try using the **coarse focus** to get a clearer image
  - Consider whether the specimen sample is **thin enough** for light to pass through to see the structures clearly
  - There could be **cross-contamination** with foreign cells or bodies
- Use a **calibrated** graticule to take measurements of cells

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Using a graticule

- An **eyepiece graticule** and **stage micrometer** are used to measure the size of the object when viewed under a microscope
- Each microscope can vary slightly so **needs to be calibrated** when used
- The calibration is done with a stage micrometer, this is a slide with a very accurate scale in **micrometres (µm)**, it is usually in 10 µm divisions, so 1 mm divided into 100 divisions
- The eyepiece graticule is a disc placed in the eyepiece with 100 divisions, this has no scale
- To know what the divisions equal at each magnification the eyepiece graticule is calibrated to the stage micrometer at each magnification



- In the diagram, the stage micrometer has three lines each 10 µm apart
- Each 10 µm division has 40 eyepiece graticule divisions
- **40 graticule divisions = 10 µm**

1 graticule division = number of micrometres ÷ number of graticule division

- 1 graticule division = 10 ÷ 40 = 0.25 µm this is the **magnification factor**
- The specimen slide would be used to replace the stage micrometer and the eyepiece graticule at the same magnification would be used to measure the length of the object
- The number of graticule divisions can then be multiplied by the magnification factor:

graticule divisions x magnification factor = measurement (µm)

1.2 Practical Skills: Endorsement Assessment

YOUR NOTES ⬇

Limitations

- The size of cells or structures of tissues may appear inconsistent in different specimen slides
  - Cell structures are 3D and the different tissue samples will have been **cut at different planes** resulting in inconsistencies when viewed on a 2D slide
- Optical microscopes do not have the same magnification power as other types of microscopes and so there are some structures that can not be seen
- The treatment of specimens when preparing slides could alter the structure of cells

Exam Tip

The calculations involving stage micrometers and eyepiece graticules are often seen in exam questions, so make sure that you are comfortable with how to calibrate the graticule and calculate the length of an object on the slide.