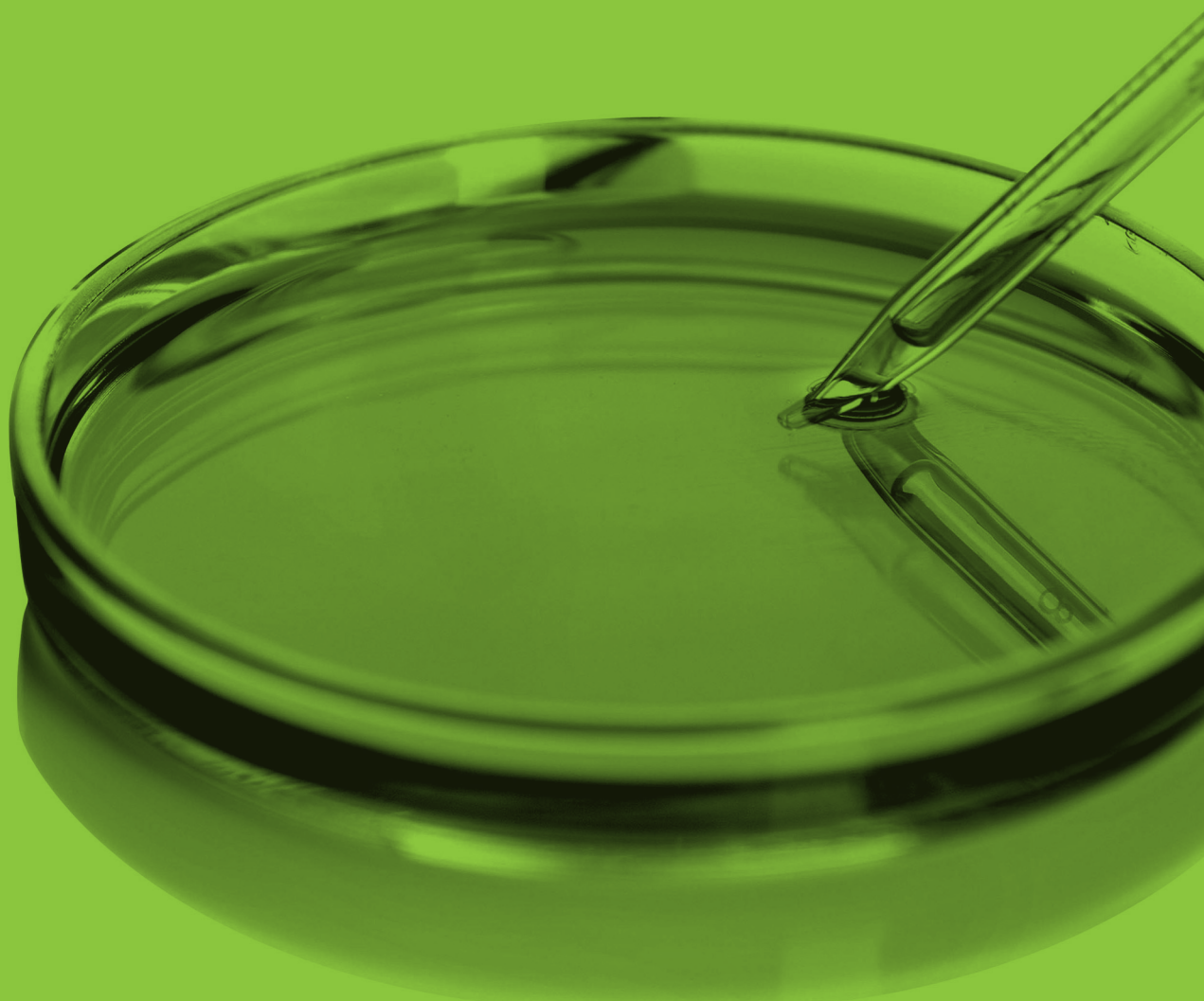
WJEC AS/A LEVEL

BIOLOGY

LAB BOOK

Name: .....



Student Record

AS/A Level Biology

Candidate Name .....

Topic	Specified practical work	Date completed	Teacher / Student comments
Biological Compounds	Food tests		
Cell structure	Calibration of the light microscope		
	Preparation and scientific drawing of a slide of living cells		
Cell membranes and transport	Determination of water potential by measuring changes in mass or length		
	Determination of solute potential by measuring the degree of incipient		
	Investigation into the permeability of cell membranes using beetroot		
Enzymes	Investigation into the effect of temperature or pH on enzyme activity		
	Investigation into the effect of enzyme or substrate concentration		
Nucleic Acids	Simple extraction of DNA from living material		
Genetic information is copied and passed on	Scientific drawing of cells from slides of root tip to show stages of mitosis		
	Scientific drawing of cells from prepared slides of developing anthers to show stages of meiosis		
All organisms are linked through their evolutionary history	Investigation into biodiversity in a habitat		
Adaptations for gas exchange	Investigation into stomatal numbers in leaves		
	Dissection of fish head to show the gas exchange system		
	Scientific drawing of a low power plan of a prepared slide of T.S. leaf		

Student Record

Topic	Specified practical work	Date completed	Teacher / Student comments
Adaptations for transport	Investigation into transpiration using a simple potometer		
	Scientific drawing of a low power plan of a prepared slide of T.S artery and vein		
	Dissection of mammalian heart		
Importance of ATP	Investigation of dehydrogenase activity using artificial hydrogen acceptors		
Respiration	Investigation into factors affecting the rate of respiration in yeast		
Photosynthesis	Investigation into the separation of chloroplast pigments by chromatography		
	Investigation into factors affecting the rate of photosynthesis		
	Investigation into the role of nitrogen and magnesium in plant growth		
Microbiology	Investigation into the numbers of bacteria in fermented milk		
Population size	Investigation into the abundance and distribution of organisms in a habitat		
Homeostasis and the kidney	Dissection of kidney		
Sexual reproduction in plants	Investigation of the digestion of starch agar using germinating seeds		
	Dissection of wind and insect-pollinated flowers		
	Scientific drawing of a low power plan of a prepared slide of an anther, including calculation of actual size and magnification of drawing		
Inheritance	Experiment to illustrate gene segregation including the use of the chi squared test in testing the significance of genetic outcomes		
Variation and evolution	Investigation of continuous variation in a species (including use of the Student's t-test)		

Guidance Notes

There are three types of specified practical work in this specification

- Investigative work
- Microscopy
- Dissection

In general you should be able to:

- apply investigative approaches and methods to practical work and think independently when undertaking practical work;

- use a wide range of experimental and practical instruments, equipment and techniques appropriate to the knowledge and understanding included in the specification.

Experimental Design

You should be able to :

- identify the independent variable – the factor you will test/ change

- identify the dependent variable- the factor which you are measuring

- identify the controlled variables – the factors that you need to keep constant

- use the correct units for all your variables

- identify a suitable range for your independent variable, this would normally be at least five values

- explain why repeat readings would be needed – a mean is more reliable than an individual reading and it will help identify anomalous results

- design a suitable control experiment

- assess the main risks of your experiment

Hazard	Risk	Control measure

Hazard - an object or chemical and the nature of the hazard

Risk - an action in the method that can create a risk from the hazard

Control measure - must be practicable in the context of the practical

Table of results

Your table should have:

- correct column headings
- appropriate units in headings (not in body of table)
- columns for sufficient repeats
- appropriate recording of readings, time to the nearest second, same number of decimal places throughout table except 0

Exemplar table of results

Independent variable (unit)	Dependent variable (unit)			
	Trial 1	Trial2	Trial 3	Mean
Value 1				
Value 2				
Value 3				
Value 4				
Value 5				

Graphs

Your graph should have:

- the independent variable plotted on the x axis
- the dependent variable plotted on y axis
- the axes labelled correctly
- used at least half of the grid should have been used on both axes
- the correct units on both axes
- a suitable linear scale used on each axis, including a figure at the origin for both axes
- all plots accurately plotted
- the points accurately joined with a suitable line with no extrapolation. Point to point using a ruler through centres is advised for most graphs
- range bars correctly drawn

Analysis of results

You should be able to :

- identify a trend in the results
- comment on the consistency of the readings
- comment on the accuracy of the readings
- suggest improvements for any inaccuracies identified
- give an explanation of results using relevant and sound biological knowledge
- draw a suitable valid conclusion

Calibration of microscope

In order to measure the size of a structure on a microscope slide it is necessary to calibrate the microscope. Inside the eyepiece of the microscope there is an eye piece graticule. It is graduated 1-10 with 10 subdivisions between each number therefore the eyepiece graticule has 100 eyepiece units [epu] along its length.

With different magnifications, the divisions on the eyepiece graticule will cover different actual lengths of the specimen on the slide.

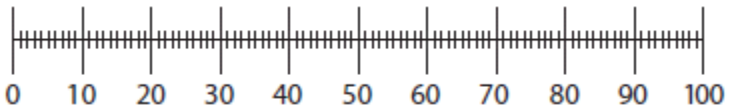
A stage micrometer is used to measure the length of each division at different magnifications. There are two types of stage micrometer available, check which you are using.

Either

The stage micrometer is a slide with a line **1 mm** long on it. The line is also marked for tenths and hundredths of a mm. There are 100 stage micrometer units [smu] on the 1 mm line. Each stage micrometer unit = 0.01 mm or 10 μm.

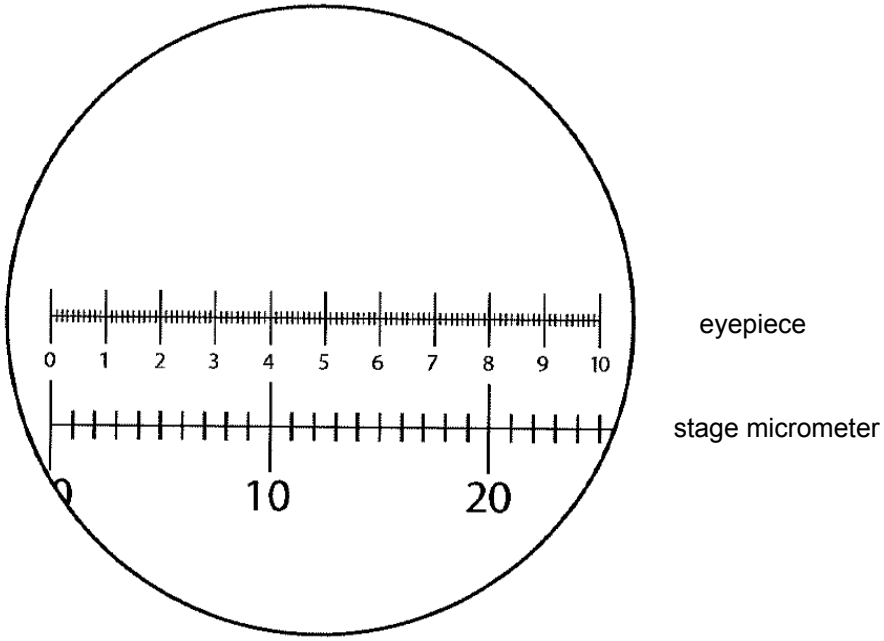
Or

The stage micrometer is a slide with a line **10 mm** long on it. The line is also marked for tenths and hundredths of a mm. There are 100 stage micrometer units [smu] on the 10 mm line. Each stage micrometer unit = 0.1 mm or 100 μm .



To calibrate the microscope

- Line up the zero of the eyepiece graticule and the zero of the stage micrometer.
- Make sure the scales are parallel.
- Look at the scales and see where they are in line again.



Using this x40 objective lens, 20 stage micrometer units make up 80 eyepiece units.

80 eyepiece units = 20 stage micrometer units

If 1 stage micrometer unit = 0.01 mm

1 eye piece unit = $\frac{20}{80}$ = 0.25 stage micrometer units

1 stage micrometer unit = 0.01 mm

1 eye piece unit = 0.25 x 0.01 mm
= 0.0025 mm or 0.0025 x 1000μm
= 2.5μm

If 1 stage micrometer unit = 0.1 mm

1 eye piece unit = $\frac{20}{80}$ = 0.25 stage micrometer units

1 stage micrometer unit = 0.1 mm

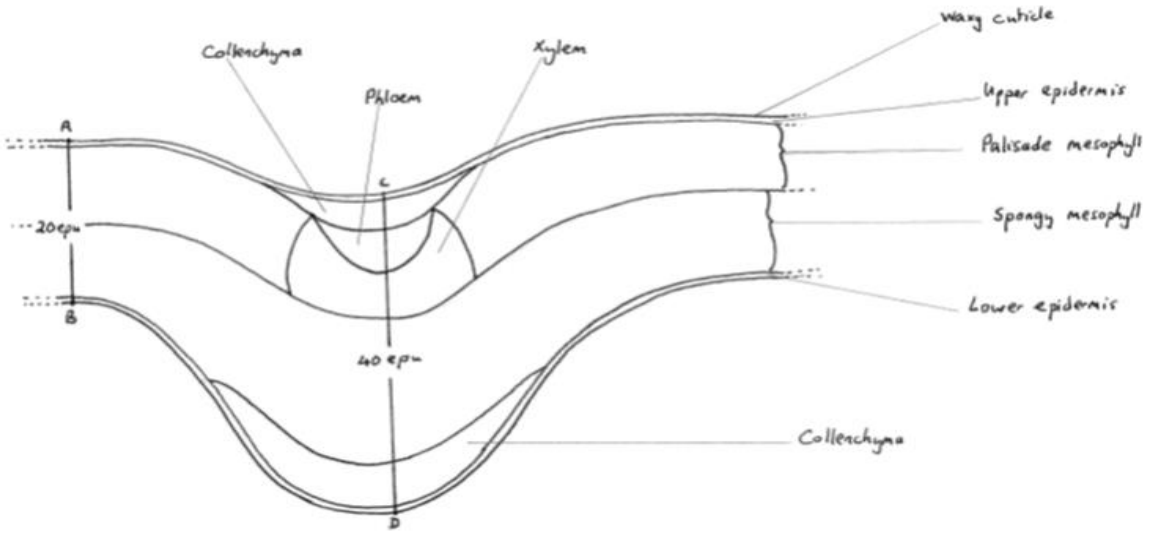
1 eye piece unit = 0.25 x 0.1 mm
= 0.025 mm or 0.025 x 1000 μm
= 25 μm

Microscope drawing

Low power plan

This shows the distribution of tissues in a transverse section (TS) or longitudinal section (LS) of a structure.

T.S. Leaf of *Ligustrum* – Low power plan



It is not always necessary to draw a plan of the entire structure but if a part is drawn it should be indicated that it is a part of a structure. This is usually done by drawing dotted lines to show where the tissues continue.

When completing low power plans, you should:

- use a sharp pencil.
- not use any shading
- not draw any individual cells
- make your drawing at least half a page of A4 in size and position the labels to the side of the drawing
- make all lines clear, complete and not overlapping
- draw label lines with a ruler to the centre of the tissue layer, they should not cross each other
- ensure tissue layers are all drawn to the correct proportion
- draw a line across two tissues and give the width of this line in eyepiece units. If one line across tissue A has been given 48 epu and the second line across tissue B has been given 12 epu, the correct proportion should show that tissue A is 4 times the width of tissue B at that point.
- check tissue boundaries by using a higher objective lens than that being used to draw the plan

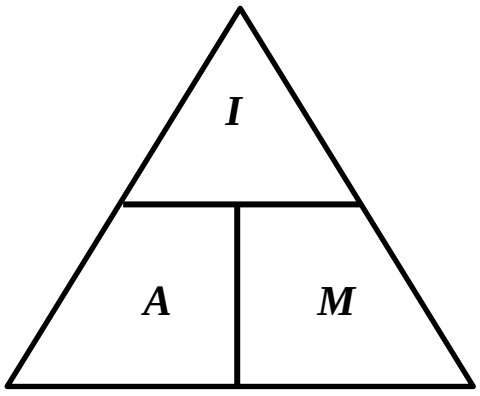
High power drawing of individual cells

When completing high power drawings of individual cells, you should:

- use a sharp pencil
- not use any shading
- draw two or three cells
- make your drawing at least half a page of A4 in size and position the labels to the side of the drawing
- make all lines clear, complete and not overlapping
- use single lines to represent the tonoplast membrane or the cell membrane. A double line should be used to represent the cell wall
- calculate the actual length or diameter of the cells
- not draw structures which you cannot see for example details of the structure of the chloroplast or mitochondria using a x40 objective

Magnification of a drawing

Magnification shows us the size of a drawing or image in relation to the size of the actual object.
The magnification, size of object or size of image can be calculated using the triangle method.



I = Size of image

A = Actual size of object

M = magnification.

Cover what you wish to calculate and the equation is given.

$$I = A \times M.$$

$$M = \frac{I}{A}$$

$$A = \frac{I}{M}$$

Check that the units for the size of the object and image are the same.

Microscopy

Throughout the A level course you will observe the following slides. Ensure that you are able to identify the structures and tissues given below.

Leaf e.g. Privet

cuticle; upper epidermis; palisade mesophyll/cells; spongy mesophyll; xylem; phloem; collenchyma; lower epidermis

Marram grass leaf

cuticle; adaxial epidermis/upper epidermis; fibres/sclerenchyma; palisade mesophyll; xylem; phloem; abaxial epidermis/lower epidermis; stomata

Water lily leaf

cuticle; adaxial epidermis/upper epidermis; palisade mesophyll; xylem; phloem; spongy mesophyll; air spaces; collenchyma; abaxial epidermis / lower epidermis; stomata

Root

epidermis/exodermis; cortex; endodermis; pericycle; xylem; phloem

Stem

Epidermis; cortex; medulla/pith; xylem; phloem; vascular bundle; cambium; collenchyma; sclerenchyma

Artery and Vein

Endothelium; tunica interna/tunica intima; tunica media; tunica externa/tunica adventitia; lumen

Ileum

Columnar epithelium; mucosa/lamina propria; muscularis mucosa/mucosal muscle; submucosa; circular muscles; longitudinal muscles; serosa; villus; goblet cells

Trachea

Ciliated epithelium; lamina propria; blood vessel/artery/vein/arteriole/venule; mucous glands; submucosa; muscle; cartilage; connective tissue; perichondrium

Lung

Bronchus/bronchiole with ciliated epithelium; alveoli with squamous epithelia; blood vessels

Tapeworm

Scolex with hooks and suckers; proglottids/segments; zone of proglottid formation behind scolex;

Anther
Epidermis; tapetum/inner wall; fibrous layer/outer wall; area of dehiscence/stomium; pollen sac; xylem; phloem; parenchyma

Testis
Seminiferous tubule; spermatozoa; spermatids; spermatogonia; area of Leydig cells; Sertoli cell

Ovary
Germinal epithelium; primary follicle; graafian follicle; secondary follicle; primary oocyte; secondary oocyte; blood vessels; stroma; corpus luteum; theca; cumulus cells; antrum

Spinal cord
Dorsal root; ventral root; grey matter; white matter; central canal; pia mater; dura mater; ventral median fissure; meninges

Kidney
Bowman's capsule; glomerulus; capillaries; distal / proximal convoluted tubule

Food tests

Specification reference: **1.1**

Chemical elements are joined together to form biological compounds

Introduction

The chemical composition of different foods can be determined by performing chemical tests. There are five tests which you need to know and understand how to use.

- Reducing sugars
- Non-reducing sugars
- Proteins
- Starch
- Fats and oils

These tests can be used on pure chemicals to demonstrate the positive results from each and then used to investigate the composition of different foods.

Apparatus and reagents

5 boiling tubes
2 test tubes
1 beaker
3 dropping pipettes
Benedict's reagent
Dilute hydrochloric acid (0.5 mol dm^{-3})
Dilute sodium hydroxide/ sodium bicarbonate
Biuret reagent
Iodine- potassium iodide solution
Absolute alcohol
Glucose solution
Sucrose solution
Albumen solution
Starch solution
Oil
Water bath

Method

Reducing sugars

1. Mix 2 cm^3 of the test solution with an equal volume of Benedict's reagent.
2. Heat the mixture in a water bath to between 70 ^oC and 90 ^oC for 5 minutes
3. Record your observations.

Non-reducing sugars

1. Mix 2 cm^3 of the test solution with an equal volume of Benedict's reagent.
2. Heat the mixture in a water bath to between 70 °C and 90 °C.
3. Observe and record colour change.
4. Put another 2 cm^3 of the test solution into a boiling tube, add 2 drops of hydrochloric acid and heat in a water bath to 70 °C and 90 °C for 2 minutes.
5. Add 2 drops of sodium hydroxide/ small spatula of sodium bicarbonate.
6. Add 2 cm^3 Benedict's reagent.
7. Heat the mixture in a water bath to between 70°C and 90 °C for 5 minutes.
8. Record your observations.

Proteins

1. Mix 2 cm^3 of the test solution with 2 cm^3 of Biuret reagent in a boiling tube.
2. Cover the top of the boiling tube and invert it once.
3. Record your observations.

Starch

1. Mix 2cm^3 of the test solution with 2 drops of iodine in potassium-iodide solution.
2. Record the colour change

Fats and oils

1. Mix the fat or oil with 5 cm^3 of absolute alcohol in a boiling tube.
2. Shake the tube.
3. Pour the mixture into another boiling tube half full of cold water.
4. Record your observations.

Use the methods given above to investigate the chemical content of some foods or unknown mixtures.

Calibration of the light microscope at low and high power, including calculation of actual size of a structure and the magnification of a structure in a drawing

Specification reference: **1.2**

Cell structure and organisation

Introduction

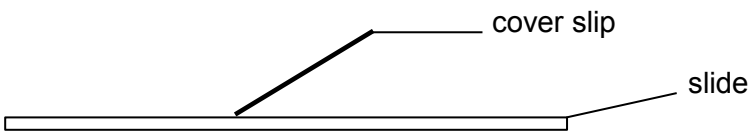
This practical is a simple introduction to the use of a microscope and how it can be used to take measurements.

Apparatus

Microscope fitted with an eye piece graticule
Stage micrometer
Microscope slide
Microscope slide cover slip
Paper towel

Method

1. Using the method given in the guidance section, calibrate your microscope at low, medium and high power.
2. Take one human hair and place it on a microscope slide.
3. Put a small drop of water on the hair.
4. Hold a cover slip on the slide as shown in the diagram.



5. Gently lower the cover slip onto the hair and press it gently with a piece of paper towel.
6. Use the x40 objective to measure the width of the hair in eye piece units and use the method given in the guidance section and your calibration to calculate the actual size.
7. Draw a section of the hair.
8. Calculate the magnification of your drawing by using the method given in the guidance section.

Calibration at low power x ……..

Calibration at medium power x ……..

Calibration at high power x ……..

Preparation and scientific drawing of a slide of onion cells including calibration of actual size and magnification of drawing

Specification reference: **1.2**

Cell structure and organisation

Introduction

An onion is made up of swollen leaf bases separated by thin membranes of cells. In this exercise you will make a wet mount on a microscope slide, look at the cells using each objective lens and identify the features of the cell visible under the light microscope. Using a calibrated eye piece graticule you will calculate the size of a cell and draw a group of at least three cells in the correct proportion. You should calculate the magnification of your drawing.

Apparatus

Microscope fitted with an eye piece graticule
Microscope slide and cover slip
Onion
Paper towel
Scalpel
White tile
Mounted needle
Iodine solution
Forceps

Method

1. Place two drops of water onto a microscope slide.
2. Take a small piece of onion and using forceps peel off the membrane from the underside (the rough side).
3. Lay a piece of the membrane flat on the surface of the slide taking care that it is a single layer and not folded back on itself.
4. Add three drops of iodine solution.
5. Place one edge of a coverslip onto the slide and lower it gently using a mounted needle, making sure that there are no air bubbles.
6. Gently press the coverslip down using a piece of paper towel.
7. Using the x4 objective position the slide and focus on the section.
8. Swing the x10 objective into place and move the slide carefully until a clear area of cells are observed i.e. no large bubbles, no folds and a single layer of cells.
9. Draw a group of at least three cells in the correct proportion. Indicate the length of one cell in eye piece units on the drawing.
10. You should use the x40 objective to help you identify and label structures in the cells.
11. Calculate the actual size of one of your cells and the magnification of your drawing.

Determination of water potential by measuring changes in mass or length

Specification reference: **1.3**

Cell membranes and transport

Introduction

If two solutions of different water potentials are separated by a selectively permeable membrane, water will move into the solution with the lower water potential. The cytoplasm and cell sap are solutions and the tonoplast and cell membrane are selectively permeable. The cell can therefore be considered to be an osmotic system in which a solution is surrounded by a selectively permeable membrane. It will lose or gain water by osmosis depending on the water potential of the adjacent cell or bathing solution. Where there is no change in mass or length the water potential of the bathing solution is equal to that of the tissue.

A tissue sample, such as a cylinder of potato or fragment of leaf, contains millions of cells. If it gains water by osmosis, the mass increases. The cells will stretch by a small amount, until prevented from doing so by the cell wall, and so the length of a cylinder of tissue will increase. The converse is also true – if the tissue sample loses water, its mass decreases and the length of a cylinder of tissue decreases.

Apparatus

Vegetable e.g. potato
Chopping board/ white tile
Cork borers: sizes 3 and 4 are suitable
Ruler graduated in mm
Fine scalpel
Fine forceps
5 x boiling tubes
Boiling tube rack
50 cm^3 measuring cylinder
Distilled water
Sodium chloride solutions (0.2, 0.4, 0.6, 0.8 mol dm^{-3})

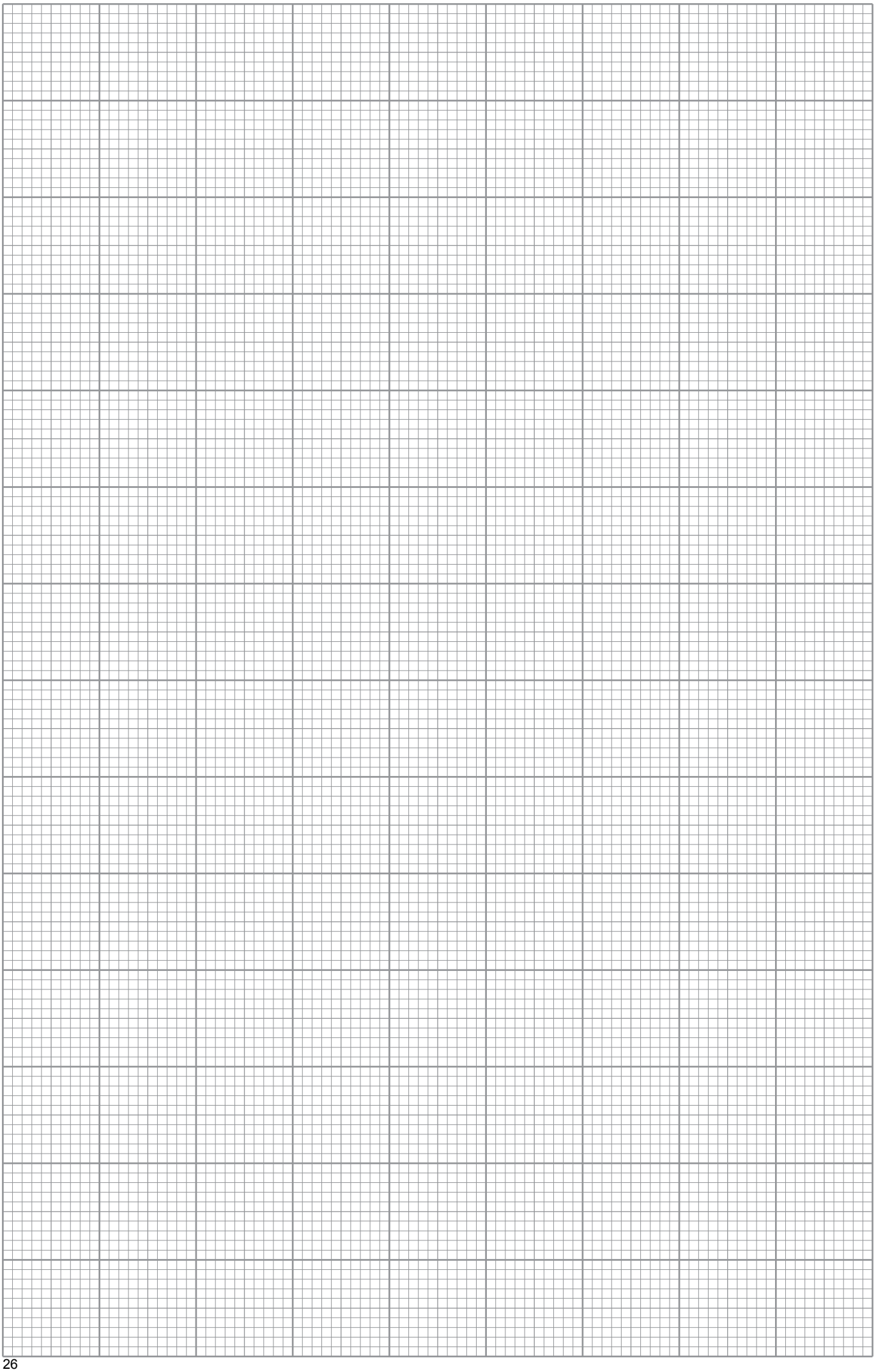
Method

1. Cut 15 cylinders of tissue, each approximately 50mm long, on the chopping board and use the scalpel to remove any periderm (skin) as its suberin makes it waterproof, and would prevent osmosis.
2. Place 30cm^3 distilled water or solution in to each test tube. Make sure you label each tube.
3. Using the scalpel and forceps, ensure the ends of the cylinder are at 90° to its length.

4. Measure the length of the cylinder to the nearest mm or the mass to the nearest 0.01 g.
5. Using the forceps, place 3 cylinders into each boiling tube.
6. Leave at room temperature for a minimum of 45 minutes, or overnight at 4 °C.
7. Gently blot the cylinders and re-measure the length or re-weigh the cylinders.
8. Record your results in a table.
9. Plot the mean percentage change against the concentration of solution.
10. Estimate the solute potential of the tissue.

You may wish to use this information when designing your results table or analysing your readings:

Molarity / mol dm^{-3}	Solute potential / kPa
0.05	-130
0.10	-260
0.15	-410
0.20	-540
0.25	-680
0.30	-860
0.35	-970
0.40	-1120
0.45	-1280
0.50	-1450
0.55	-1620
0.60	-1800
0.65	-1980
0.70	-2180
0.75	-2370
0.80	-2580
0.85	-2790
0.90	-3000
0.95	-3250
1.00	-3500



Determination of the solute potential by measuring the degree of incipient plasmolysis

Specification reference: **1.3**

Cell membranes and transport

Introduction

When the water potential of plant tissue and its surroundings are equal, there is no net movement of water in or out of the cell. The cell is neither turgid nor plasmolysed and is at incipient plasmolysis. The cell membrane is withdrawn from the cell wall in places and the cell contents exert no pressure on the cell wall.

In principle, when examined under the microscope, every cell in the tissue would be expected to show its cytoplasm withdrawn in places from the cell wall. Cells, however, show variation in their behaviour. Incipient plasmolysis is the point where plasmolysis just takes place and for practical purposes this is taken to be when half of the cells are plasmolysed and half are not plasmolysed.

The equation describing water potential is $\psi_{cell} = \psi_S + \psi_P$
At incipient plasmolysis, the pressure potential, $\psi_P = 0$
$\therefore \ \psi_{cell} = \psi_S + 0$
$\therefore \ \psi_{cell} = \psi_S$
Thus the water potential of the cells is equal to their solute potential.

The water potential of the cells is also equal to the solute potential of the bathing solution, which is known. Thus, ψ_S of the bathing solution $= \psi_{cell} = \psi_S$.

Apparatus

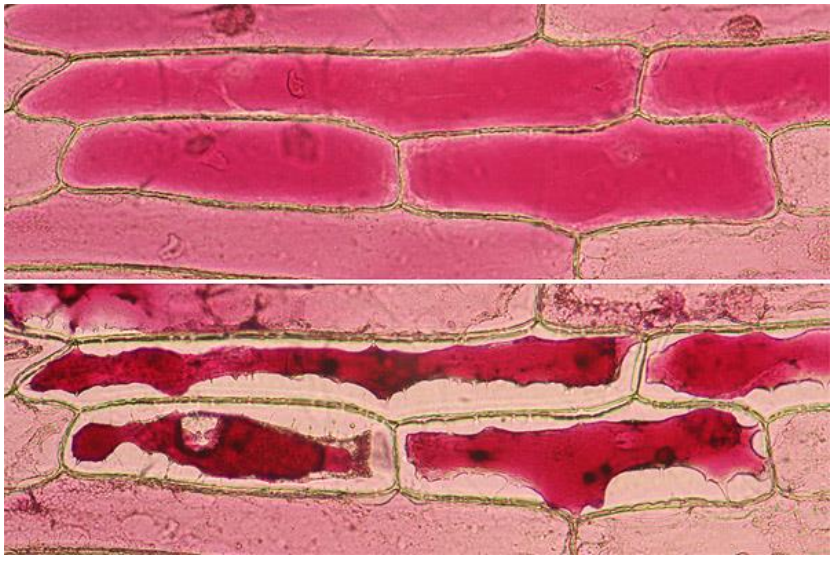
White tile
Fine forceps
Fine scissors
Red onion
5 x 9 cm Petri dishes/ small bottles
Distilled water
Sodium chloride solution (0.2, 0.4, 0.6, 0.8 mol dm^{-3})
Stopclock
Microscope slides
Cover slips
Microscope
Dropping pipettes

Method

1. Set up five labelled Petri dishes/ small bottles each containing 10cm^3 of one of the following solutions: distilled water, 0.2, 0.4, 0.6, 0.8 mol dm^{-3} sodium chloride solution.

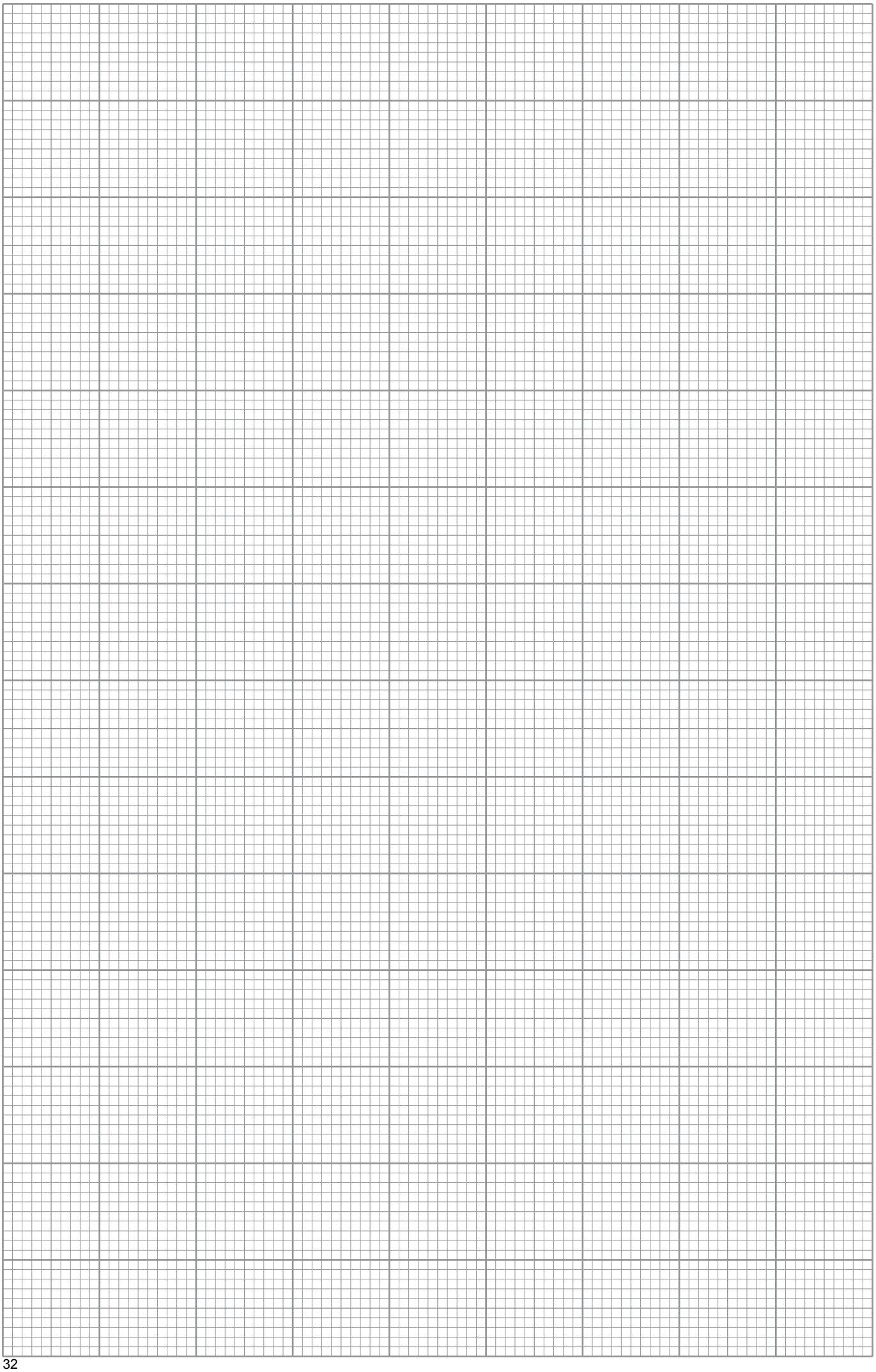
2. Insert the fine forceps tip just under the upper epidermis of the onion leaf.
3. Keeping the forceps handles parallel with the epidermis, so as not to penetrate the underlying mesophyll, grip the epidermis and, maintaining the tension in the tissue, pull the epidermis off the mesophyll, away from you and place into distilled water.
4. When several square centimetres of epidermis have been peeled, place one square into each labelled petri dish/small bottle.
5. Leave at room temperature for a minimum of 30 minutes.
6. Carefully spread the tissue out on a microscope slide, so that it is not folded. Take a scalpel and rock the blade backwards and forwards over the tissue in order to cut out a 0.5 x 0.5 cm square.
7. Add two drops of bathing solution and apply a cover slip.
8. If any solution exudes from the cover slip, blot it with filter paper to dry the slide.
9. Using a x 10 and then a x 40 objective lens, examine all the cells in a field of view and count the number that are turgid and the number plasmolysed.
10. Repeat the counts at all concentrations of bathing solution.
11. Record your results in a table.
12. Plot a graph of % cells plasmolysed against the concentration of the bathing solution.
13. Using the graph, read the concentration of bathing solution that would produce plasmolysis in 50 % of the cells.
14. From the table given in the previous experiment, determine the solute potential of this solution. This is equal to the solute potential of the cells.

The photograph below shows the appearance of turgid and plasmolysed cells.



turgid cells

plasmolysed cells



Investigation into the permeability of cell membranes using beetroot

Specification reference: **1.3**

Cell membranes and transport

Introduction

Cell membranes are fluid structures which control the exit and entry of materials into cells. Some substances cross the membrane through proteins, others diffuse through the phospholipid layer. The molecules within the membrane are constantly moving and their movement is greatly affected by temperature. Heating the membrane can cause gaps to form between the phospholipid molecules and the membrane will become more permeable. The protein in the membrane can be denatured by heat.

Beetroot cells contain betalain, a bright red, water soluble pigment, in the cell vacuoles. If the cell membranes are damaged the pigment can escape from the cells and can be detected in an aqueous medium around the tissue. Beetroot grows in soil at a temperature of between $10-15\,^{o}C$.

Apparatus

Beetroot cylinders
White tile
10 test tubes
Scalpel
250 cm^3 beaker
Forceps
Water baths at (25, 35,45,55,65 ^{o}C)
Thermometer
Stop clock
Colorimeter with a blue filter / colour chart

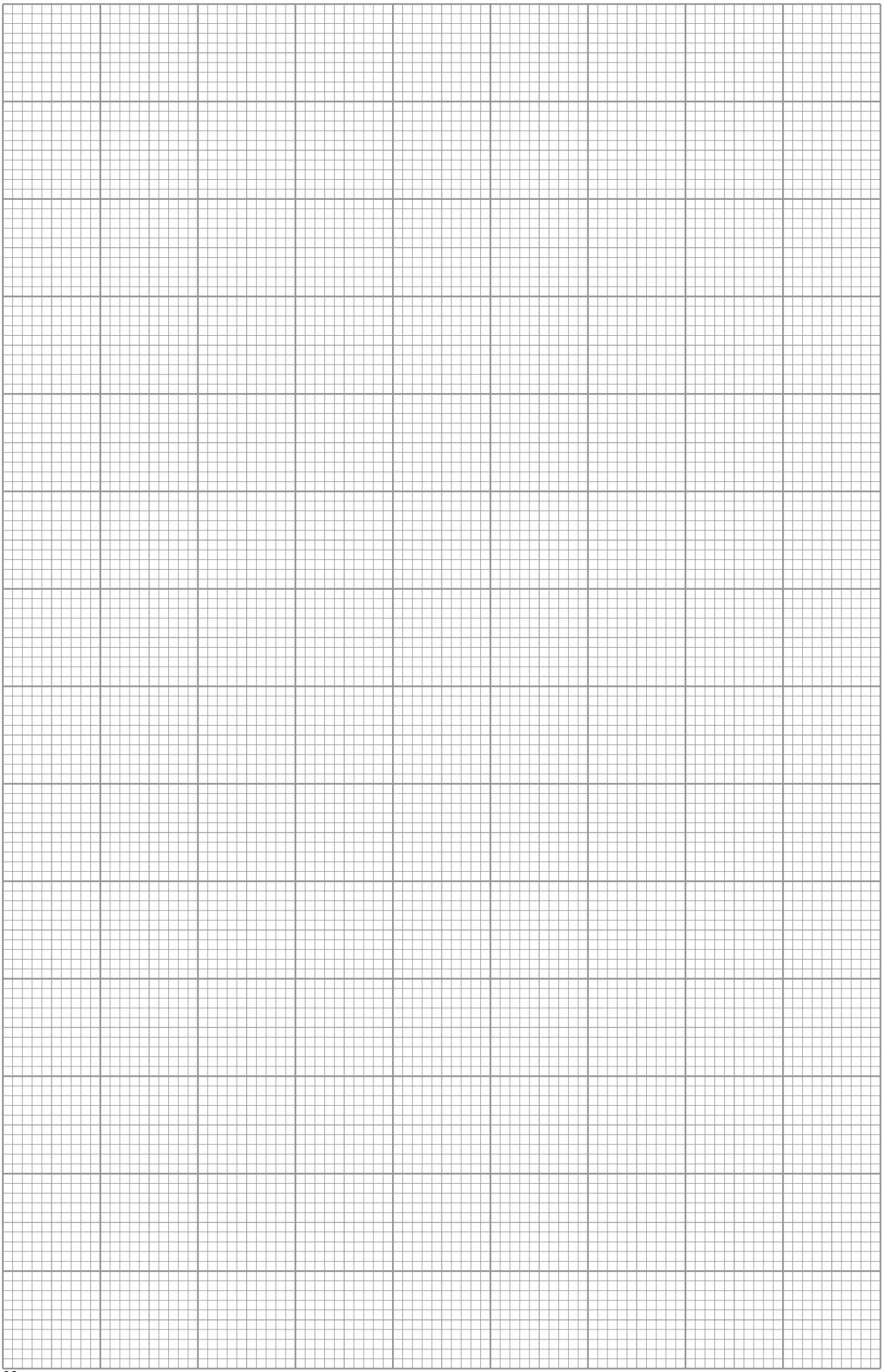
Method

1. Cut 5 pieces of beetroot, 1 cm long, from the cylinders provided.
2. Wash under running water to remove the pigment released from cells during cutting.
3. Place a test tube containing 5 cm^3 of distilled water into each water bath to equilibrate for 5 minutes.
4. Place 1 piece of beetroot into each test tube for 30 minutes.
5. After 30 minutes, shake the test tubes gently to make sure any pigment is well-mixed into the water, then remove the beetroot cores.

6. Describe the depth of colour in each test tube. A piece of white card behind the tubes will make this easier to see. Arrange the tubes in order of temperature of the water bath.

7. If you have access to a colorimeter, set it to respond to a blue/ green filter (or wavelength of 530 nm) and measure absorbance/ percentage transmission. Check the colorimeter reading for distilled water.

8. Measure the absorbance/percentage transmission of each tube and plot a graph of absorbance/percentage transmission against temperature.



Investigation into the effect of temperature or pH on enzyme activity

Specification reference: **1.4**

Biological reactions are regulated by enzymes

Introduction

Phenolphthalein is an indicator that is pink in alkaline solutions of about pH10, but turns colourless in pH conditions less than 8.3. In this investigation, an alkaline solution of milk, lipase and phenolphthalein will change from pink to colourless as the fat in milk is broken down to form fatty acids (and glycerol) thus reducing the pH to below 8.3. The time taken for this reaction to occur is affected by temperature.

Apparatus

Milk, full-fat or semi-skimmed
Phenolphthalein in a dropper bottle
lipase solution (5g/ 100cm^3)
Sodium carbonate solution (0.05 mol dm^{-3})
5 x Test tubes and rack
2 x 10cm^3 syringes/measuring cylinders
2cm^3 syringe
Stirring rod
Thermometer
Water baths set to 15°C, 25 °C, 35°C, 45°C and 55°C.
Ice

Method

1. Place a beaker of lipase solution in the 25 °C water bath.
2. Place 5 cm^3 milk, in a test tube.
3. Add 5 drops of phenolphthalein to the test tube.
4. Add 7 cm^3 of sodium carbonate solution.
5. Place the test tube in the 25°C water bath for 10 minutes to equilibrate.
6. Add 1 cm^3 of lipase from the beaker in the water bath and start the stop clock.
7. Stir the contents of the test tube until the solution loses its pink colour, record the time taken.
8. Repeat steps 1 – 7 for 15°C, 35°C, 45°C and 55°C.

Investigation into the effect of enzyme or substrate concentration on enzyme activity

Specification reference: **1.4**

Biological reactions are regulated by enzymes

Introduction

The substrate concentration in an enzyme catalysed reaction affects how much product is made.

Catalase is an enzyme that increases the breakdown of hydrogen peroxide, a toxin produced as a by-product of reactions in cells. The reaction of catalase is shown below.

$$2\ H_2O_2 \longrightarrow 2H_2O + O_2$$

Catalase is found in high concentration in raw potatoes.

Use the method below to carry out an investigation into the effect of enzyme of substrate concentration on enzyme activity

Apparatus and reagents

Freshly cut potato cylinders
Pestle and mortar
Specimen tubes/test tubes
Stock solution of hydrogen peroxide
Filter paper discs
Forceps
Stopwatch
Syringe
Distilled water
Paper towel

Method

1. Grind a 2 cm piece of potato cylinder with 5 cm^3 of distilled water to make a smooth paste containing the enzyme.
2. Place 10cm^3 of H_2O_2 in a specimen tube/ test tube.
3. Using forceps, dip a filter paper disc into the enzyme suspension, tap off the excess.
4. Drop the filter paper disc into the hydrogen peroxide solution and measure the time, to the nearest second, that it takes from striking the surface to sink to float up to the surface again.
5. Remove the disc from the tube using forceps and discard.

Simple extraction of DNA from living material

Specification reference: **1.5**

Simple extraction of DNA from living material

Introduction

DNA can be extracted in visible amounts from a wide source of material such as onions and strawberries. Strawberries are an excellent source because they have very large genomes, and can have up to eight copies of each chromosome (octoploid). Strawberries are soft and, when ripe, they produce pectinase and cellulase enzymes which break down the cell walls helping the release of DNA.

Apparatus and reagents

1 resealable plastic bag
1 Strawberry
10cm^3 of washing up liquid (detergent)
1g of salt
100 cm^3 water
2 x 250 cm^3 beakers (one beaker will be used for the filtering apparatus below)
Filtering apparatus: coffee filter paper and beaker
Ice cold 90 % alcohol
1 ice lolly stick or plastic coffee stirrer
Acetic-orcein stain

Method

1. Remove the green calyx from the strawberries.
2. Put the strawberry into the plastic bag, seal it and crush it for about two minutes. Make sure that the strawberry is completely crushed.
3. In a beaker, mix together 10cm^3 of detergent, 1g of salt and 100 cm^3 of water. This mixture is the DNA extraction liquid.
4. Add 10cm^3 of this extraction liquid to the bag with the strawberry.
5. Reseal the bag and gently mix the extraction liquid with the strawberry for 1 minute. (Avoid making too many soap bubbles).
6. Place the coffee filter inside the beaker.
7. Open the bag and pour the strawberry liquid into the filter. You can twist the filter just above the liquid and gently squeeze the remaining liquid into the beaker.

8. Pour down the side of the beaker an equal amount of cold 90 % alcohol as there is strawberry liquid. Do not mix or stir.
9. Within a few seconds, watch for the development of a white cloudy substance (DNA) in the top layer above the strawberry extract layer.
10. Tilt the beaker and pick up the DNA using the plastic coffee stirrer or wooden stick.
11. Test your sample with acetic-orcein stain. A red colour will show that it does contain nucleic acids.

Scientific drawing of cells from slides of root tip to show stages of mitosis

Specification reference: **1.6**

Genetic material is copied and passed on to daughter cells

Introduction

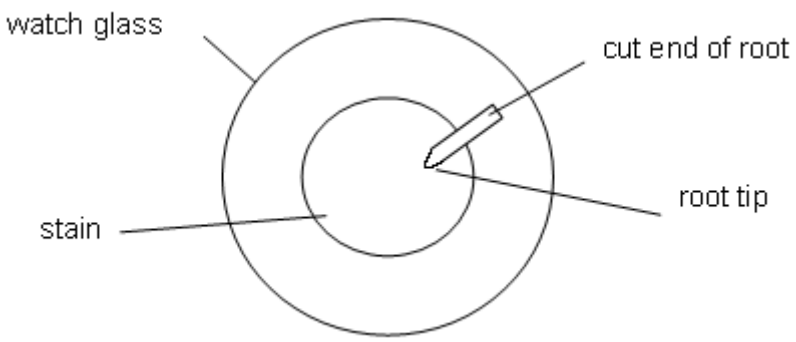
Mitosis is a process of cell replication needed for growth and repair. Onion (*Allium* sp.) is very useful for root tip preparation to study the different stages in mitosis. At the tip of the root there is an apical meristem where cells divide by mitosis. To observe the stages of mitosis the root tissues must be well fragmented. Hydrochloric acid is used to separate the cells by breaking down the tissue which binds cells together (maceration).

Apparatus and reagents

Microscope	Bunsen burner / hot plate
Garlic or onion with developing roots	Microscope slide and cover slit
Scalpel	Fine forceps
Watch glass	Mounted needle
acetic-orcein stain	Dropping pipette
1 M hydrochloric acid	Paper towel

Method

1. Cut 10 mm from the tip of a growing root.
2. Place 20 drops of acetic-orcein stain and 2 drops of 1 M hydrochloric acid into a watch glass.
3. Place the root tip so that the tip is in the stain and the cut end facing the outside of the watch glass as shown in the diagram.



4. Remove 2 – 3 mm from the tip of the root and place it on a microscope slide.
5. Add 2 – 3 drops of acetic-orcein stain.
6. Warm the slide under gentle heat for 4 – 5 seconds.
7. Completely break up the tissue with a mounted needle.
8. Apply a cover slip, place the slide and coverslip on a layer of paper towel and fold the paper towel over the coverslip. Make sure that the slide is on a flat surface and squash down on the coverslip with a strong vertical pressure using your thumb. Do not twist or roll the thumb from side to side.
9. Use a microscope with the x 10 objective to locate the zone of cell division (apical meristem). The cells in this region are 'square' in shape with nuclei which are large relative to the whole cell area. If you see xylem vessels with their characteristic spiral thickening you are looking in the wrong area ....not at the tip!
10. If the cells are overlapping, squash again.
11. Using the x 40 objective lens, observe and draw, cells at interphase, prophase, metaphase, anaphase and telophase.

Scientific drawing of cells from prepared slides of developing anthers to show stages of meiosis

Specification reference: **1.6**

Genetic material is copied and passed on to daughter cells

Introduction

Each anther of a flower contains four pollen producing chambers called pollen sacs. Inside the pollen sacs a large number of diploid pollen mother cells are produced by mitosis. Each pollen mother cell divides by meiosis to give a tetrad of four haploid cells. These cells separate from each other and become the pollen grains. The production of the pollen grains usually takes place at an early stage in flower development, usually when the flower is in the bud stage.

Apparatus

Prepared slides of T.S anther
Microscope

Method

1. Using the x10 objective focus on the cells at the centre of one of the pollen sacs.
2. Use the x40 objective and identify as many stages of meiosis as you can.
3. Repeat this for all four pollen sacs.
4. Make drawings to show all the stages which you have identified.
5. Calculate the size of one cell and the magnification of one of your drawings.

Investigation into biodiversity in a habitat

Specification reference: **2.1**

All organisms are related through their evolutionary history

Introduction

Biodiversity refers to the number of species (species richness) and the relative number of individuals within each species (species evenness). The type of index and the techniques used to estimate it will depend upon the habitat being studied. The method depends on counting species richness and evenness and substituting the data into a formula to generate a number which is a diversity index. These methods could be used to look at the diversity of invertebrates in a clean and a polluted stream or the ground flora (vegetation) in a woodland compared to a field.

Calculating Simpson's index:

Simpson's diversity index, D $= 1 - \frac{\Sigma n(n-1)}{N(N-1)}$

Where: N = number of organisms sampled;
n = number of individuals of each species.

Assessing biodiversity of ground flora

Apparatus

Quadrat/
Identification key for selected species
Random number generator or 20 sided dice
2 x 20 Metre tape

Method

1. Using tape measures set up at pair of axes at right angles to each other in the selected area
2. Generate random numbers/ roll the dice to produce the figures for the x and y co-ordinates to identify the sampling position.
3. Place the quadrat at the generated sampling position.
4. Identify all species present and record the numbers of each present.
5. Repeat for a total of 10 quadrats.
6. Calculate the Simpson's index for the area.

Assessing biodiversity of invertebrates

Apparatus

Flat bottomed net
Quadrat
Identification key for selected species

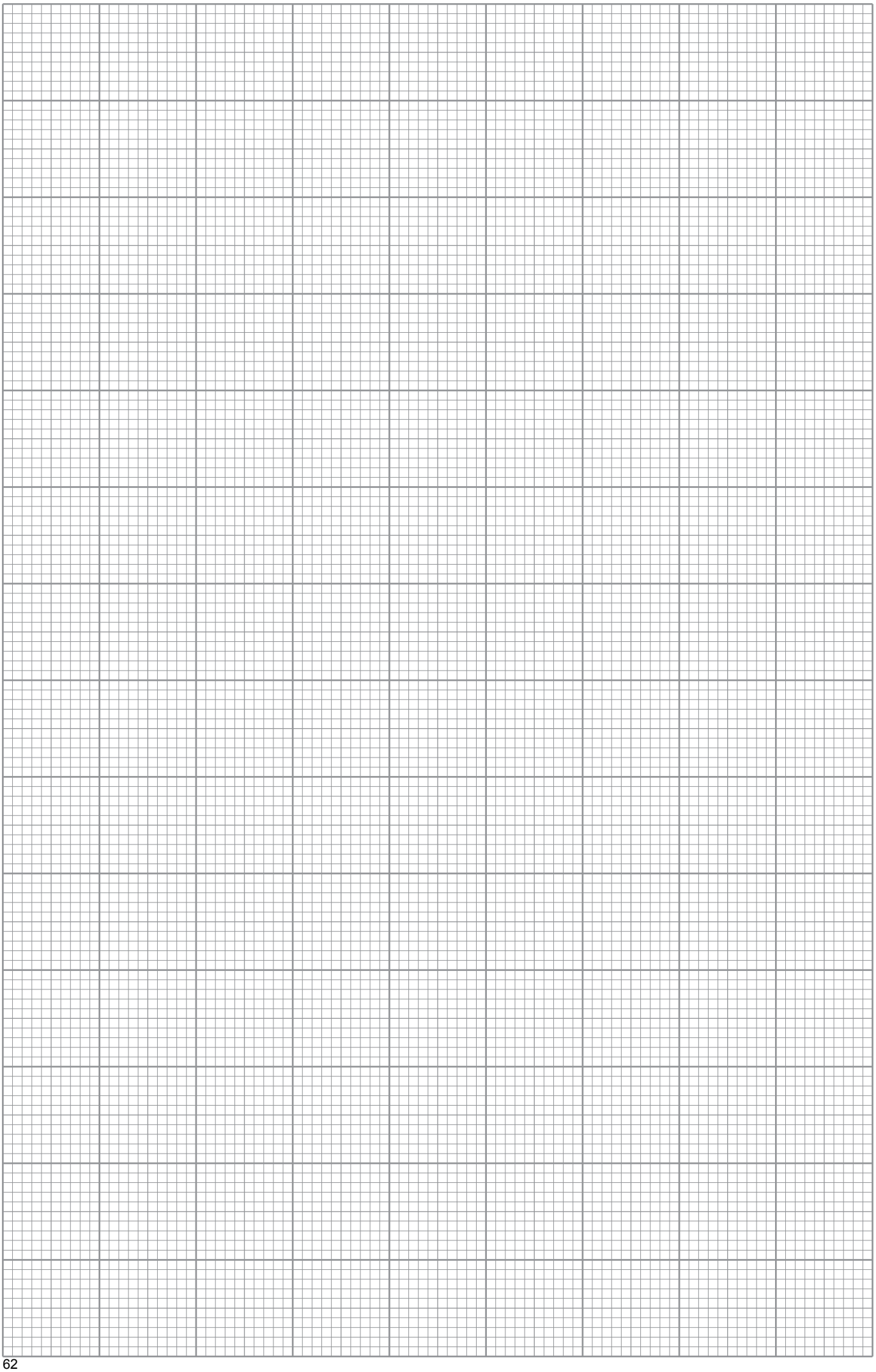
Method

1. Use a fixed area sampler or mark an area with a quadrat
2. Place flat edged net held against the stream bottom downstream from the quadrat.
3. Agitate the stream bed with a boot (or use a stick or tool) and 'kick' for two minutes, covering the whole area evenly.
4. Place the catch in a shallow white tray with 2 cm depth of fresh water from the stream or river.
5. Identify all species present and record the numbers of each present.
6. Return animals about 1m upstream from where they were caught.
7. Repeat for a total of 10 quadrats (or share results from other groups).
8. Calculate the Simpson's index for the stream.

Use the following table to calculate Simpsons Diversity Index for the chosen area

Species	n	$n-1$	$n(n-1)$
Total	N =		$\sum n(n-1)$ =

Simpson's diversity index, D $= 1 - \frac{\Sigma n(n-1)}{N(N-1)}$



Investigation into stomatal numbers in leaves

Specification reference: **2.2**

Adaptations for gas exchange

Introduction

Stomata are pores surrounded by two guard cells on the aerial parts of plants. They are most densely packed in leaves, which therefore make suitable experimental material. This technique describes how to measure their density in the lower epidermis of a leaf.

Apparatus

Leaves
White tile
Fine forceps
Fine scissors/scalpel
Clear nail varnish/PVA glue
Microscope slides
Cover slips
Dropping pipette
Distilled water
Microscope

Method

1. Making a replica of the epidermis:

(i) Place a leaf on a white tile with its lower epidermis facing upwards.

(ii) Stretch the leaf between two fingers of one hand. With the other hand, apply a layer of colourless nail varnish between the veins, and allow it to dry.

(iii) Apply a second layer of nail varnish/PVA glue and allow it to dry.

(iv) Hold a pair of fine forceps horizontally and insert one point between the epidermis and the nail polish/PVA glue layer. Grip the layer and peel it away from you, maintaining tension in the peeled layer. This produces a replica of the lower epidermis.

(v) Place the replica on a microscope slide and use scissors/scalpel to cut a sample, taking care that the replica does not fold.

(vi) Apply two drops of water and cover with a cover slip.

2. Counting the stomata:

(i) Focus on the replica using the x10 objective and then refocus using the x40 objective.

(ii) Count the number of stomata in the field of view.

(iii) Repeat for three fields of view and calculate a mean.

3. Calculating stomata distribution:

(i) From your microscope calibration, calculate the area of the field of view by:

- Measuring the diameter of the field of view
- Converting to an actual size in mm
- Using πr^2 to calculate the area of the field of view

(ii) Calculate the distribution of stomata where

mean number of stomata per mm^2 = $\frac{mean\ number\ of\ stomata\ per\ field\ of\ view}{area\ of\ field\ of\ view\ in\ mm^2}$

Dissection of fish head to show the gas exchange system

Specification reference: **2.2**

Adaptations for gas exchange

Introduction

A bony fish takes in water at the mouth. When the fish closes its mouth and raises the floor of the buccal cavity, the volume is decreased, and consequently the pressure is increased. Water is then driven over the gills, where gas exchange takes place, and out through the operculum.

Apparatus

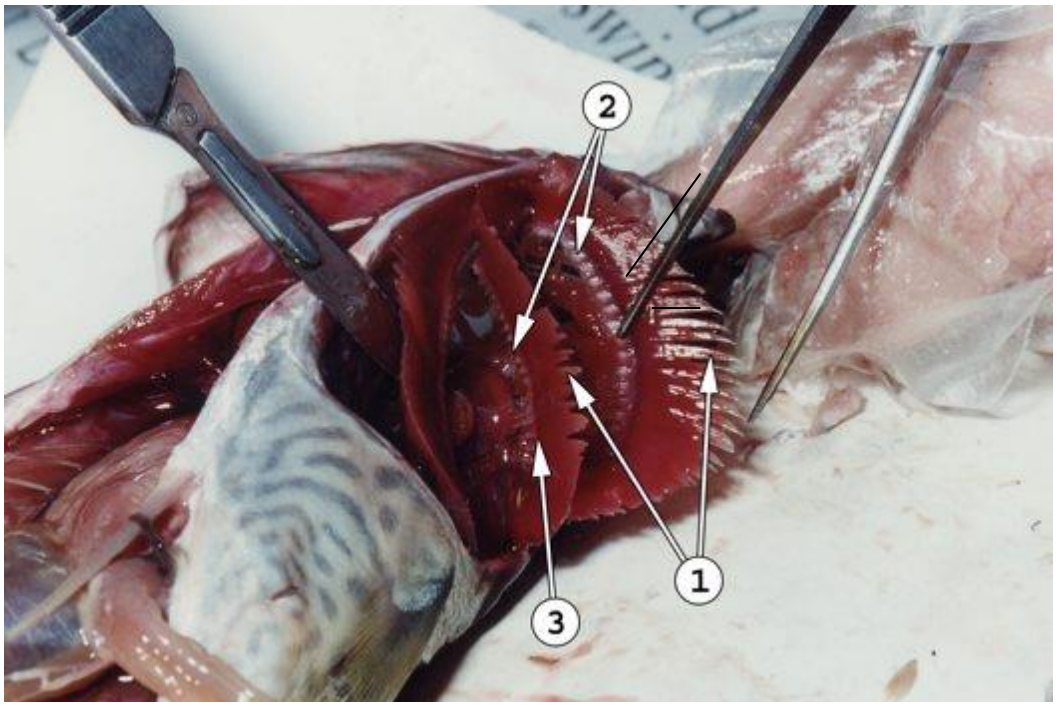
Large fish head	Glass rod
Dissecting board	Microscope slide
Fine scissors	Cover slip
Large scissors	Microscope
Fine forceps	Water
Fine scalpel	Gloves

Method

You should make labelled drawings or take photographs at each stage for your records.

1. Rinse the fish head thoroughly under cold, running water and run water through the gills, to remove mucus. If the salmon is fresh, the gills will be bright red and should not have any mucus on them. Older material is duller and may be covered with mucus.
2. Open the salmon's mouth and note the teeth on both jaws and the tongue, which has taste buds, at the bottom of the mouth. The lower jaw moves up and down to take in water and prey. The salmon does not chew and there is limited sideways movement of the lower jaw.
3. Use forceps to move the operculum in and out, showing how it moves during ventilation. The operculum may be stiff to move, but this is expected because it needs to close very firmly for the ventilation mechanism to be effective in maintaining pressure differences.
4. Lift the operculum and identify the gill filaments and the gill slits, which are the spaces between the gills.

5. Submerge the fish head in cold water. The gills should 'fluff up' - notice the large surface area.
6. Push onto the bottom of the mouth to see the floor of the cavity.
7. Gently push the glass rod into the mouth, through the buccal cavity and through a gill slit to show the pathway of water during ventilation.
8. Use scissors to cut the operculum off where it is attached to the head. This may be hard work as the operculum is a strong structure. You will see four gills, each of which is supported by a bony gill arch.
9. With large scissors, cut through the first gill arch where it attaches to the head at the bottom. As with the operculum, cutting through the gill arch may require considerable strength.
10. Cut through the first gill arch where it attaches to the head at the top.
11. Note the gill rakers attached to the gill arch. They filter solids, preventing damage to the gill filaments.
12. With fine scissors, cut off a few mm from a gill filament and place on a microscope slide. Place 2 drops of water on the material and cover it with a cover slip. Examine it under the microscope using a x4 and then a x10 objective.



1. Gill filaments
2. Gill rakers
3. Gill arches

Scientific drawing of low power plan of a prepared slide of T.S. leaf, including calculation of actual size and magnification of drawing

Specification reference: **2.2**

Adaptations for gas exchange

Introduction

This practical requires you to observe and draw a prepared slide of a dicotyledonous leaf. You should draw in proportion as described in the guidance notes.

Apparatus

Microscope
Slide of TS leaf (dicotyledon)

Method

Examine the slide using the x10 objective lens.

1. Draw a small outline of the leaf.
2. Position the slide to include the mid rib.
3. Show on the outline drawing the position of the section which will be shown as a plan.
4. Draw a plan to show the distribution of tissues in the correct proportion.
5. You may need to use the x40 objective to identify some of the tissue layers.
6. Identify and label the following: upper and lower epidermis; palisade mesophyll; spongy mesophyll; xylem; phloem; cuticle; collenchyma; sclerenchyma (if present); guard cells
7. Draw 2 lines, measured in eye piece units, on the plan.
8. Calculate the actual size of one dimension of the leaf and the magnification of your drawing.

Investigation into transpiration using a simple potometer

Specification reference: **2.3**

Adaptations for transport

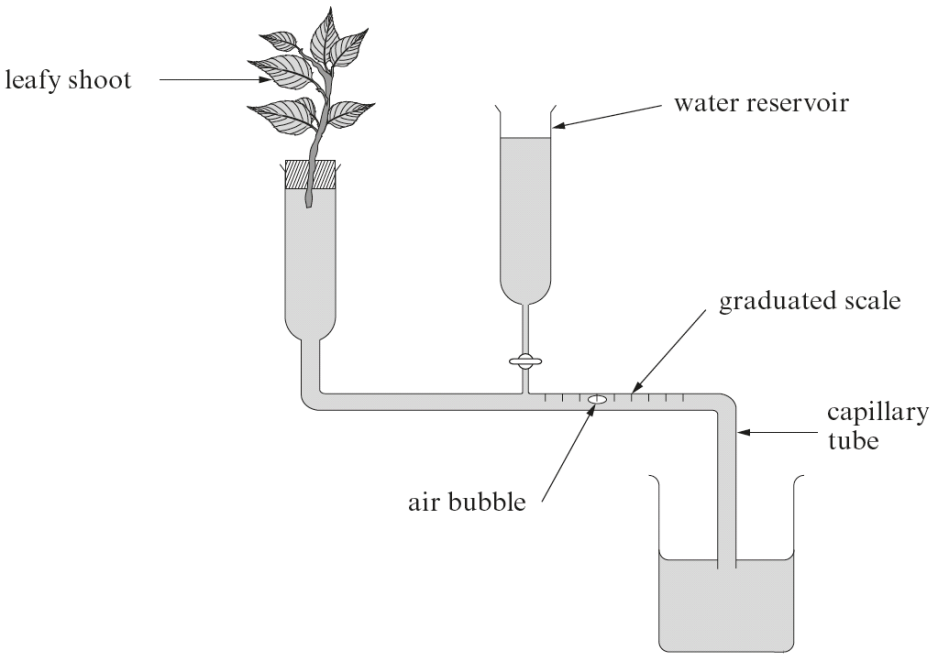
Introduction

The loss of water from the leaves of a plant causes water to be absorbed by the plant and moved through the xylem vessels to the leaves. This upward movement of water through the plant is called the transpiration stream. The evaporation of water from the leaves (mainly through the stomata) to the atmosphere is called transpiration. The assumption is made that the rate of evaporation from the leaf is equal to the rate of uptake. Although, a very small volume of water is used in physiological processes (e.g. photosynthesis typically uses less than 1% of total water as a reactant), the assumption is broadly true and this method gives a reasonable approximation of the transpiration rate.

The rate of transpiration is affected by temperature, light, humidity, wind and atmospheric pressure.

Apparatus

Potometer
Freshly cut plant stems (cut end in water)
Scissors/sharp knife
Vaseline
Bowl of water
Small beaker of water
Clamp stand
Stopclock
Paper towel



Method

1. Immerse the potometer completely under water and assemble it under water to prevent any air bubbles entering.
2. Put the cut end of the stalk (not the leaves)of your plant under water and cut off the last centimetre of the stalk diagonally underwater.
3. With the potometer and stalk still underwater push the stalk into the potometer as shown in the diagram of the apparatus. The stalk should fit tightly into the potometer. It is important that there are no air bubbles in the system and that no air can be sucked into the apparatus around the stalk.
4. Remove the plant and potometer from the water.
5. Apply Vaseline to the joints to prevent air entering.
6. Gently dab the leaves with a paper towel to remove excess water.
7. Clamp the potometer in an upright position with the end of the capillary tube under the water in the beaker.
8. Remove the capillary tube from the water and let an air bubble form.
9. Replace the end of the capillary tube under water.
10. When the air bubble reaches the scale record how far the air bubble travels in a known time.
11. Repeat stages 8, 9 and 10 twice.
12. Record your results in a table.

13. You should record the internal diameter of the capillary tube so that results can be expressed in the form of cm^3 water lost per minute.
    Volume in mm^3 is $\pi r^2 h$
    π= 3.14
    r = radius
    h = distance moved by air bubble.
14. Remove the leaves, place onto graph paper, draw around the edge of each leaf and calculate the total surface area of the leaves.
15. Express your final rate of transpiration as the volume of water lost per cm^2 per minute.

Scientific drawing of low power plan of a prepared slide of T.S. artery and vein, including calculation of actual size and magnification of drawing

Specification reference: **2.3**

Adaptations for transport

Introduction

This practical requires you to observe and draw a prepared slide of an artery and vein. You should draw in proportion as described in the guidance.

Apparatus

Microscope
Slide of T.S. artery/vein

Method

1. Examine a T.S of an artery using the x10 objective lens.
2. Draw a plan to show the distribution of tissues in the correct proportion.
3. You may need to use the x40 objective to identify some of the tissue layers.
4. Identify and label: endothelium; tunica intima/interna; tunica media; tunica externa / adventitia; lumen.
5. Draw 2 lines, measured in eye piece units, on the plan.
6. Calculate the actual size of the tissues and the magnification of the drawing.
7. Repeat steps 1-6 using a T.S. vein.

Dissection of a mammalian heart

Specification reference: **2.3**

Adaptations for transport

Introduction

By dissecting a mammalian heart, you should:

- see that it is a hollow organ comprising four chambers
- see that the associated blood vessels have different structures
- appreciate the differences in the thickness of the muscle in different chambers
- recognise the valves, both within the heart and in the blood vessels, and the tendons that contribute to the heart's functioning.

Apparatus

Lamb's heart
Chopping board
Scalpel
Scissors
Forceps
Glass rod

Method

You should make labelled drawings or take photographs at each stage for your records.

1. Observe the outside of the heart.
   - Note if it is covered in fat.
   - Note any large blood vessels. The widest is the aorta and it has thick walls. You may also see the pulmonary artery. Look down these blood vessels into the heart and note the semi-lunar valves at their bases. The vena cava and pulmonary veins have thinner walls than the arteries.
   - Note any blood vessels on the surface of the heart. These are likely to be the coronary vessels bringing blood to the muscle of the heart wall.
   - Note the apex of the heart, the pointed end. This is the base of the ventricles, from where their contraction starts.

2. Look down through the atria from the top – using forceps find the tricuspid and bicuspid valves.

3. Use scissors to cut through the heart about 3 cm from the apex and look at the cut end. If you have cut far enough up, you will be able to see the ventricles. You can distinguish them as the left ventricle has a much thicker wall than the right ventricle.

4. Insert a glass rod into the left ventricle and gently push it upwards. You may see it emerge through the aorta. Reinsert the glass rod and alter its angle to allow it to pass up through the atrio-ventricular (bicuspid) valve into the left atrium and out through the pulmonary vein.

5. Using scissors, cut from the base of the ventricle up through the atrium and pulmonary vein, using the glass rod as your guide.

6. Identify the semilunar valves in the aorta and pulmonary artery.

7. Open up the heart to observe:
   - the wall of the left ventricle is much thicker than the wall of the left atrium.
   - the bicuspid valve
   - the cordae tendineae (tendons) that attach the atrio-ventricuar (bicuspid) valve to the ventricle wall.
   - the inner surface of the ventricle is not flat. The shape ensures streamlined blood flow through the heart.
   - Blood clots may be present in the chambers of the heart but these can be removed with forceps.

8. An equivalent exercise may be done using the right side of the heart, exposing the tricuspid valve.

Investigation of dehydrogenase activity in yeast

Specification reference: **3.1**

Importance of ATP

Introduction

Yeast performs aerobic respiration when there is access to oxygen, towards the top of a suspension in a test tube, and anaerobic respiration lower down. Dehydrogenase activity removes hydrogen atoms from intermediates in both types of respiration and transfers them to hydrogen acceptors. If an artificial hydrogen acceptor is added to the suspension, it will accept the hydrogen atoms and undergo a colour change on being reduced. The time taken for the indicator to change colour can be used as a measure of the rate of dehydrogenase activity.

Use the method below to carry out an investigation into dehydrogenase activity in yeast

Apparatus

Redox indicator: methylene blue (0.05 g/100cm^3)
Yeast suspension (100 g/dm^{-3})
30 °C Water bath
Test tube
Cork for test tube
10 cm^3 syringe
1 cm^3 syringe
Stop clock

Method

1. Place 10 cm^3 of the yeast suspension into a test tube.
2. Place test tube in water bath for 5 minutes, to equilibrate to 30 °C.
3. Add 1 cm^3 indicator.
4. Invert the test tube once, to mix.
5. Replace the test-tube in the water bath.
6. Time how long the indicator takes to change colour.

Investigation into the separation of chloroplast pigments by chromatography

Specification reference: **3.2**

Photosynthesis uses light energy to synthesise organic molecules

Introduction

Chloroplast pigments, located on the membranes of the thylakoids and grana, harvest light in the light-dependent reactions of photosynthesis, and transfer its energy into the light-independent reactions, in the synthesis of complex organic molecules.

In flowering plants, there are two major groups of chloroplast pigments:

- The chlorophylls
  - Chlorophyll a is the commonest and is found in all photosynthetic organisms studied.
  - Chlorophyll b is found in flowering plants.
  - Phaeophytin, a breakdown product of chlorophyll a molecule, lacking the central magnesium ion is seen in flowering plants and in purple sulphur bacteria.

- Carotenoids comprise
  - Carotenes: α- and β-carotene are orange but lycopene, found in tomatoes, is bright red.
  - Xanthophylls, such as lutein and zeaxanthin, appear yellow.

Chloroplast pigments will be extracted from the leaves of an angiosperm, separated by chromatography and identified. The distance each pigment travels on the chromatogram depends on

- its solubility in the solvent – more soluble pigments travel further
- its absorption by the paper or silica gel – pigments that are absorbed less travel further

Apparatus

Dark green leaves e.g. spinach, stinging nettle
Scissors
Sand
Pestle
Mortar
Propanone
Distilled water
2 x Stopper
Pipette
Chromatography paper or silica gel chromatography plates
Pencil
Ruler
Capillary tube
Hair drier
2 x Boiling tube
Petroleum ether
Vial

Method

Preparing the pigment solution

1. Chop 2 g leaf material finely with scissors and place in the mortar.
2. Add a pinch of sand and 5 cm^3 propanone.
3. Grind the leaf fragments to a slurry.
4. Place slurry in a boiling tube.
5. Add 3 cm^3 distilled water, shake vigorously and stand for 8 minutes.
6. Add 3 cm^3 petroleum ether mix by gentle shaking and allow layers to separate.
7. Collect the upper, petroleum ether layer, which contains the chloroplast pigments, in a pipette and transfer to a vial.

Preparing the chromatography paper

1. Draw a pencil line across the chromatography paper or plate approximately 2 cm from one end.
2. Draw chloroplast pigment solution into a capillary tube and put a small spot in the centre of the pencil line. Ensure that the capillary tube does not pierce or tear the chromatography paper.
3. Dry the spot as quickly as possible, preventing its spread.
4. Repeat steps 2 and 3 until there is a small but intense spot of pigment.

Running the chromatogram

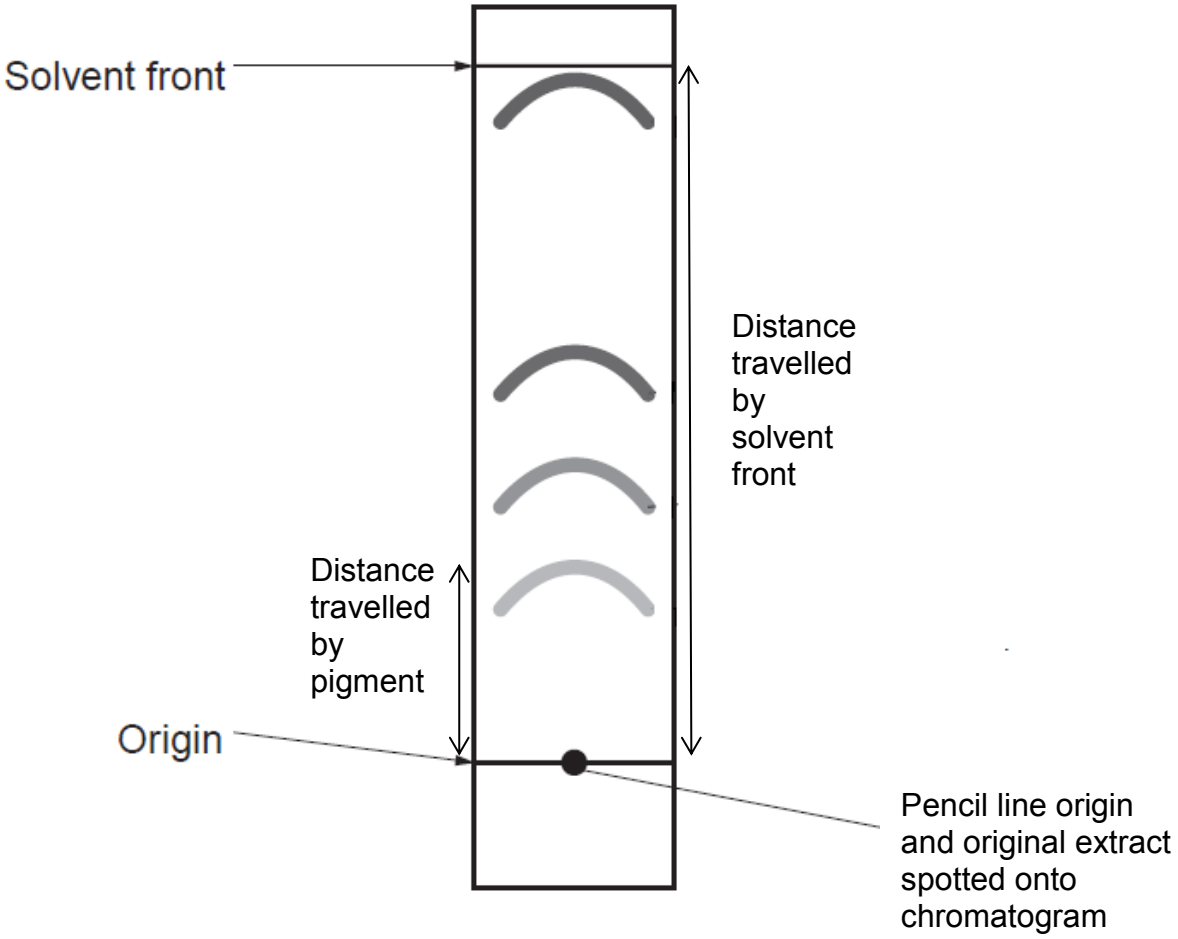
1. Place freshly-made 1:2 propanone : petroleum ether solvent mixture in a boiling tube until it is approximately 5 mm deep.
2. Without touching the sides of the boiling tube, slide the chromatography paper into the boiling tube so that its end is below the surface of the ethanol but the spot is above, and not touching it.
3. Hold the chromatography paper in place with the stopper, folding the paper over the rim of the boiling tube at the top.
4. Leave the boiling tube ensuring that it is not moved, until the solvent has climbed up the paper to within 10 mm of the top.
5. Remove the chromatography paper from the boiling tube and immediately, draw a pencil line across the paper to mark the solvent front.
6. Mark the position of the top of each pigment spot with a pencil.

Identifying the pigments

1. Use a ruler to measure the distance from the origin to the solvent front.
2. Measure the distance from the origin to the top of each pigment spot.
3. Calculate R_f for each pigment, where $R_f = \frac{distance\ travelled\ by\ pigment}{distance\ travelled\ by\ solvent\ front}$

4. Published data allow the pigments to be identified as R_f is constant for each pigment in a given solvent. The table here shows data for separation in 1:2 propanone : petroleum ether.

Spot colour	Pigment	R_f
yellow	ß-carotene	0.96
grey	phaeophytin	0.70
blue-green	chlorophyll a	0.58
green	chlorophyll b	0.48
yellow-brown	xanthophyll	0.44 (TLC) 0.75 (paper chromatography)



Investigation into the effect of light on the rate of photosynthesis

Specification reference: **3.2**

Photosynthesis uses light energy to synthesise organic molecules

Introduction

Photosynthesis can be summarised as

$$\text{carbon dioxide} + \text{water} \xrightarrow{\text{light}} \text{glucose} + \text{oxygen}$$

To measure its rate, in principle, the mass of carbon dioxide or water used, the light energy absorbed or the mass of sugar or oxygen produced could be assessed. But in practice, an easier method involves assessing the colour change in a pH indicator. pH increases when carbon dioxide is absorbed from solution in the photosynthesis of aquatic organisms, i.e. becomes less acidic.

When carbon dioxide dissolves in water the carbonic acid produced dissociates releasing hydrogen ions, which lower the pH of water:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

As photosynthesis removes carbon dioxide from the solution, the concentration of hydrogen ions decreases and so the pH increases. This can be visualised by noting the colour change of hydrogen carbonate indicator:

$$\underset{\text{yellow}}{\text{acid}} \rightleftharpoons \underset{\text{red}}{\text{neutral}} \rightleftharpoons \underset{\text{purple}}{\text{alkali}}$$

Scenedesmus quadricauda, a photosynthetic protoctistan, immobilised in alginate beads, is a suitable experimental material. If it turns the indicator purple, its rate of photosynthesis exceeds its rate of respiration; if it turns yellow its respiration exceeds its photosynthesis; if it remains red, photosynthesis and respiration are occurring at equal rates and the algae are at the compensation point.

Use the method below to carry out an investigation into the effect of light on the rate of photosythesis

Apparatus

For making algal balls

5 cm^3 *Scenedesmus quadricauda* culture
3 cm^3 sodium alginate solution (3 %)
10 cm^3 syringe without needle
200 cm^3 calcium chloride (2 g /100 cm^3)
Glass rod
Beakers
Distilled water
Tea strainer

For running the experiment

Algal balls
Glass vial + stopper 10 cm^3
Hydrogen carbonate indicator
Colour chart for indicator (*School Science Review* **85** (312) 37–45) or colorimeter with 550 nm filter
Metre ruler
Fluorescent lamp
Timer

Method

Making algal balls

1. Stir a mixture of 5 cm^3 *Scenedesmus* culture and 3 cm^3 3 % sodium alginate solution gently with the glass rod until they are well mixed.
2. Draw the mixture into a 10 cm^3 syringe barrel.
3. With constant pressure on the plunger, drop the mixture, one drop at a time, into 200 cm^3 calcium chloride solution.
4. Leave the balls for 20 minutes.
5. Strain the balls through the tea strainer.
6. Return the balls to the beaker swirl them in distilled water.
7. Repeat steps 5 and 6 twice more.
8. Use immediately or store at 4 ^oC, but bring to room temperature for approximately 20 minutes before use.

Running the experiment

1. Place 20 algal balls in a vial.
2. Add 10cm^3 hydrogen carbonate indicator.
3. Place the vials at a distance from a light source
4. After a given time assess the pH of the indicator in the vial using the colour chart or read its absorbance at 550 nm in a colorimeter.

Investigation into the role of nitrogen and magnesium in plant growth

Specification reference: **3.2**

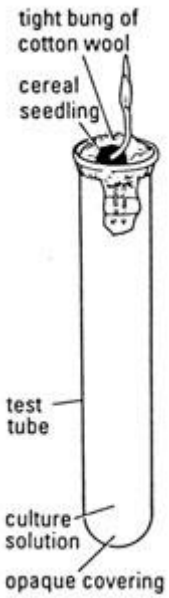
Photosynthesis uses light energy to synthesise organic molecules

Introduction

Plants require nitrogen in the form of nitrate ions absorbed by the roots to make amino acids, chlorophyll and nucleotides. Magnesium ions are also absorbed by the roots of a plant and are required by the plant as a component of chlorophyll. The effect of a lack of nitrate ions or magnesium ions on plant growth can be demonstrated by comparing seeds grown in a culture solution containing all of the plants essential nutrients with seedlings grown in a culture solution lacking nitrate and also to seedlings grown in a culture solution lacking magnesium.

Apparatus and chemicals

Sach's complete water culture solution
Sach's water culture solution lacking nitrate ions
Sach's water culture solution lacking magnesium ions
Test tubes (1 per culture solution)
Cotton wool
Aluminium foil
Dropping pipette
Germinated barley seedlings



Method

1. Select equal size barley seedlings.
2. Set up 5 test tubes, as shown in the diagram, with Sach's complete culture solution.
3. Set up another 5 test tubes with Sach's culture solution lacking nitrate.
4. Set up a further 5 test tubes with Sach's culture solution lacking magnesium.
5. Solutions should be topped up when necessary and completely replaced weekly.
6. All 15 barley seedlings should be placed in the same conditions for example light and temperature.
7. After a month examine the seedlings, record any differences between them and measure the length of the roots and shoot.
8. Dry the seedlings in an oven and record the dry mass.

Investigation into factors affecting respiration in yeast

Specification reference: **3.3**

Respiration releases chemical energy in biological processes

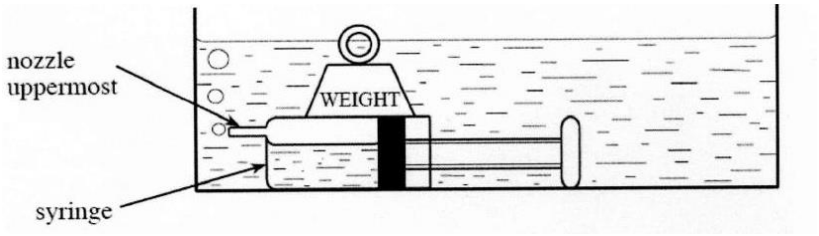
Introduction

Yeast is a unicellular fungus, which respires producing carbon dioxide. Respiration is controlled by enzymes. The rate of formation of carbon dioxide can be used as a measure of the rate of respiration.

Use the method below to carry out an investigation into factors affecting respiration in yeast

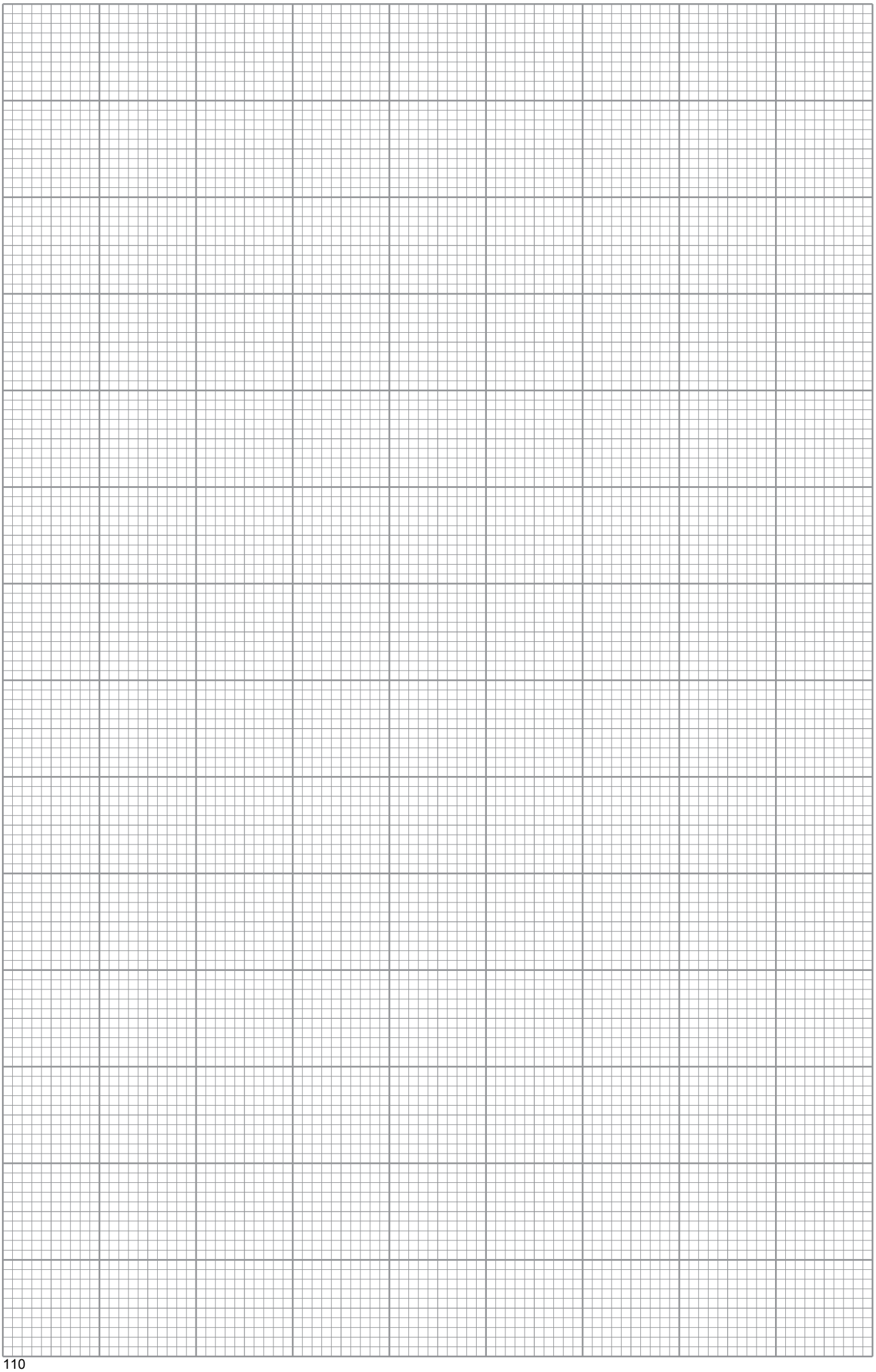
Apparatus

Yeast (100 g/dm^3)
Sucrose solution (0.4 mol dm^{-3})
Thermometer
Access to hot and cold water
1 dm^3 beaker for carrying water
20 cm^3 syringe
Weight
Trough
Marker pen
Glass rod
Timer



Method

1. Mix hot and cold water in the trough to attain the chosen temperature. The temperature should be monitored throughout the experiment: mix in hot water mixed as necessary to maintain the temperature to ± 1 °C.
2. Stir the yeast suspension and draw 5 cm^3 into the 20 cm^3 syringe.
3. Wash the outside of the syringe with running water to remove any yeast solution on outside of syringe.
4. Draw into the syringe an additional 10 cm^3 sucrose solution.
5. Pull the plunger back until it almost reaches the end of the syringe barrel.
6. Invert the syringe gently to mix the contents.
7. Place the syringe horizontally in the water bath, ensuring the nozzle is uppermost and place the weight on top of the syringe to hold it in place.
8. Allow 2 minutes for the yeast and sucrose to equilibrate to temperature.
9. When gas bubbles emerge regularly from the nozzle of the syringe, count the number released in one minute.



Investigation into the numbers of bacteria in milk

Specification reference: **3.4**

Microbiology

Introduction

Raw milk is unpasteurised. It carries potentially pathogenic bacteria, including *Salmonella*, *E. coli* and *Listeria* from the cow's udder and other sources. It is produced by only 2% of UK dairy farms, and it is not legal to sell it in UK high street shops. Pasteurisation does not sterilise milk. It reduces the number of pathogens below that likely to cause disease, but it does not kill bacterial spores. Under certain conditions, remaining viable cells will reproduce and spores may germinate. Bacterial metabolism can convert lactose into lactic acid, which, being acid, makes the milk taste sour. Depending on the concentration of lactic acid, the soluble casein proteins can solidify and the milk separates into curds, which are solid, and whey, which is liquid.

For the last 10000 years, before Pasteur was ever heard of, people consumed raw milk and milk that was fermented, under the influence of its lactic acid bacteria, *Lactobacillus spp*, and the bifidobacteria, *Bifido sp*. The product has many names around the world, including kefir, leben, acidophilus milk and cultured buttermilk. Now there are many commercially available fermented milks. The benefits of these include increased shelf life, high digestibility, and the consumption by people with "lactose intolerance" without harm. In addition, it is claimed that the bacteria present contribute to restoring a healthy gut flora, compromised by a western diet.

Fermented milks are not pasteurised and bacteria continue to divide in them as they age. This experiment will investigate the bacterial count of fermented milk of different ages. Experiments must be done under sterile conditions, such that no other microbes are introduced and those from the milk are assessed.

Apparatus

All equipment must be sterile and all manipulations must be carried out using aseptic technique.

Preparing dilutions

2 samples of fermented milk e.g. Yakult or Actimel one with a distant use-by date and one at its use-by date
1 cm^3 syringes
9 cm^3 syringe
Distilled water
Screw-cap bottles e.g. universal bottles

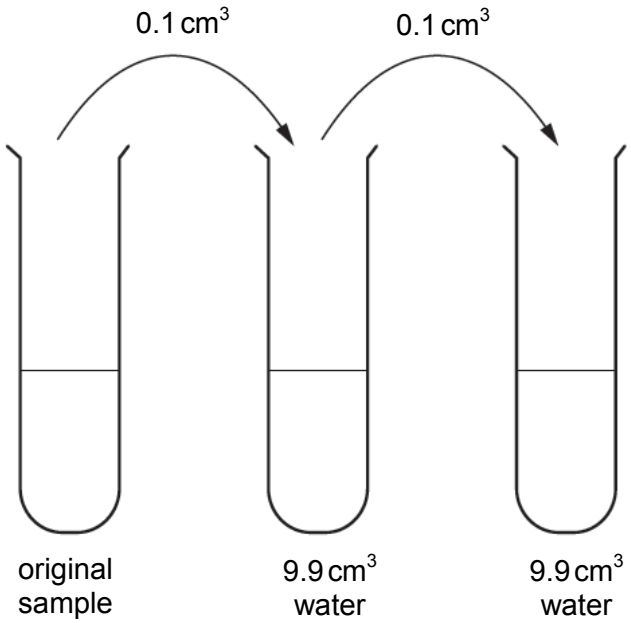
Plating and growing samples
9 cm Petri dishes
Molten MRS agar, maintained at approximately 50°C
Sticky tape
25°C incubator

Outline method

Preparing dilutions

For each sample of fermented milk:

1. Using sterile technique, transfer 9.9 cm^3 water to each of 5 sterile universal bottles, with a 10 cm^3 syringe.
2. Transfer 0.1 cm^3 fermented milk to the first bottle and mix to produce a 10^{-2} dilution. Label this 10^{-2}.
3. Transfer 0.1 cm^3 of the 10^{-2} dilution to the second bottle and mix to produce a 10^{-4} dilution. Label this 10^{-4}.
4. Transfer 0.1 cm^3 of the 10^{-4} dilution to the second bottle and mix to produce a 10^{-6} dilution. Label this 10^{-6}.
5. Continue until a 10^{-10} dilution has been produced.



Plating samples

1. For each dilution of fermented milk, transfer 1 cm^3 to approximately 12 cm^3 molten MRS agar.
2. Add the milk and agar to the centre of a 9 cm Petri dish.
3. Swirl the dish gently so that the sample is well distributed in the agar and the bottom of the Petri dish is covered, taking care not to transfer any agar to the sides of the Petri dish.
4. Tape the lid to the base, on opposite sides, with two small pieces of sticky tape.
5. Leave at room temperature, until the agar has solidified.
6. Invert the dishes and label underneath.
7. Incubate at 25°C for 5 days.

Determining the bacterial count

1. Choose the dilution that produces easily countable colonies.
2. Count the number of colonies in each plate.
3. Calculate the bacterial count of the initial fermented milk sample.

Investigation into the abundance and distribution of plants in a habitat

Specification reference: **3.5**

Population size and ecosystems

Introduction

The plant species that grow in any habitat depend upon the soil, which itself depends upon the bedrock. Physical factors also influence the plant species that grow, as do biological factors such as predation and infection. The plants that grow determine the animals of the habitat. Thus, plants are fundamental to the community in any habitat and their abundance and distribution are paramount in any environmental study.

Abundance refers to the number of any species present.
The distribution refers to how widespread they are.

Apparatus

0.25 m x 0.25 m open frame or gridded quadrat
2 x 10 m tape measures
20 m tape measure
plant identification keys

Method

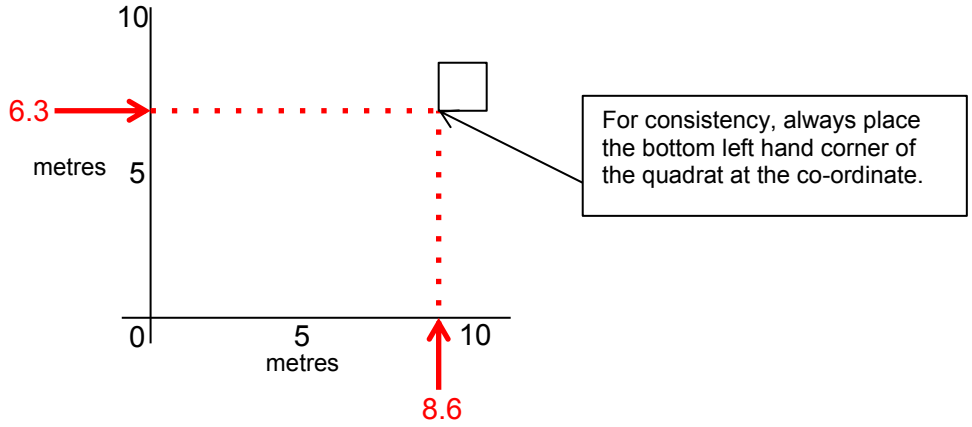
1. In an area where the abiotic variables are uniform e.g. an open field

A representative of the whole area is used, which is an "open frame quadrat", a square frame with sides of e.g. 0.5 m, giving an area of 0.25 m^2. The plants in the frame are identified and a measurement for each species recorded as either

- A direct count
- Percentage cover
- A value using the ACFOR system

Setting up coordinates for sampling

In a uniform grassland, set up a pair of 10 m long axes at right angles to each other and use random numbers e.g. from a random number generator to find co-ordinates for the quadrat. If, for example, the random numbers were 63 and 81, the co-ordinates would be where the lines from 6.3 m and 8.1 m along the axes intersected:



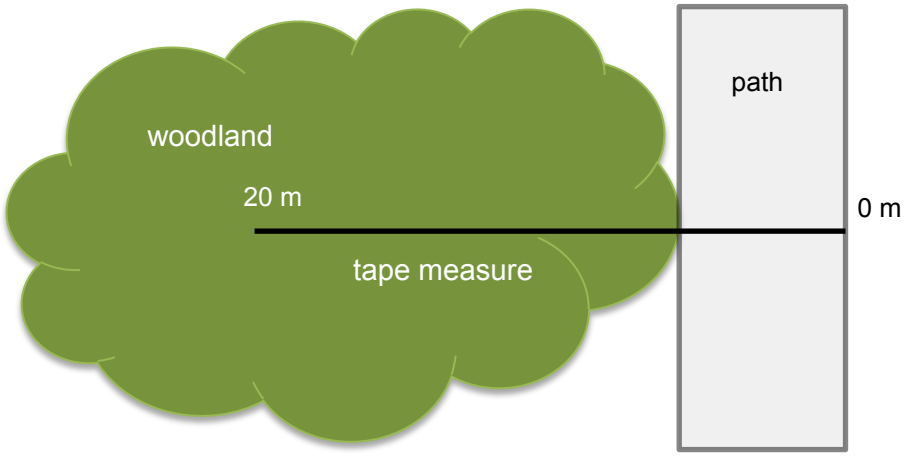
If it is difficult to count individual plants, estimate the percentage area cover.

Take readings at 10 pairs of random co-ordinates and calculate a mean for each species.
Compare with an area with different abiotic factors.

2. In an area where there is a change in an environmental factor

If there is an environmental gradient i.e. a distinct change, such as light intensity e.g. under the shade of a tree or the edge of a woodland, a line along the gradient, a 'transect' is used.

Line transect: run a 20 m tape measure into the wood and identify the plants touching the tape every two metres.



Belt transect: Place a 0.5 m square quadrat every metre along the tape measure and estimate the density, percentage frequency or percentage area cover.

Dissection of a mammalian kidney

Specification reference: **3.7**

Homeostasis and the kidney

Introduction

The urinary system consists of two kidneys, two ureters, the bladder and urethra. The kidney's functions are to remove nitrogenous wastes, such as urea from the body and to maintain the water content, and thus the pH and ion balance of the blood. The wastes constitute urine, which moves from the kidneys, through the ureters to the bladder, from where it leaves the body through the urethra.

By dissecting a mammalian kidney, you will recognise aspects of the gross structure e.g. the cortex and medulla, pyramids and pelvis, and will understand how collecting ducts join to drain urine into the ureter.

Lambs' kidneys from the butcher are suitable to show the kidney's anatomy.

Apparatus

Kidney
Chopping board
Scalpel
Scissors
Forceps
Lens on a stand

Method

These instructions are written for people who are right-handed. If you are left-handed, you may wish to work with left and right reversed.

1. Observe the outside of the kidney.
    - Note if it is covered in fat. Mammals tend to lay down fat around internal organs and fat around the kidney is common.
    - Note if blood vessels and the ureter are attached to the kidney, and note the connective tissue that sheaths and binds them. They emerge at a recess in the kidney called the hilum.
    - Note that the kidney, like all other organs, is covered in a thin membrane. This is the tough and fibrous renal capsule.
    - Organs bought from a butcher may have approximately 1 cm deep slashes which show that they have been examined by meat inspectors, as is required by law.

2. Remove the fat from the outside of the kidney. It can be removed by hand.
3. Place the kidney flat on the chopping board with the hilum on the right hand side.
4. Keeping the blade of a fine scalpel horizontal, pierce the kidney on the right hand side in front of the hilum, and make repeated small cuts, bringing the blade towards you each time. Rotate the kidney anti-clockwise after every few cuts, so that you cut right round the organ.
5. Extend the cuts through to the centre so that the kidney can be separated into two halves.
6. Note that the cortex is red-brown and that the medulla is deeper red. Note the pyramids and the pelvis. Note that the pelvis extends into the ureter.
7. Using one half of the kidney, place on the chopping board so that the place where the ureter emerges from the kidney is towards you. With forceps in your left hand, lift the connective tissue and cut through it with fine scissors, away from you, to uncover tubules leading into the pelvis.
8. Continue cutting towards the cortex, exposing finer tubules. More can be seen if the dissection is done through a lens on a stand. The tubules are continuous with the microscopic collecting ducts of the nephrons.
9. Further detail can be observed by taking a small sample from the cortex and medulla and crushing them under a coverslip. The kidney tubules can be seen using the x10 objective lens.

Investigation of the digestion of starch agar using germinating seeds

Specification reference: **4.2**

Sexual reproduction in plants

Introduction

There are large quantities of starch stored in the endosperm of seeds such as maize (*Zea mays)* and cotyledons of seeds such as broad bean *(Vicia faba)* . During germination of a seed the starch is broken down by the enzyme amylase into maltose which is transported in the phloem to the growing points in the plumule and radical.

Apparatus and reagents

Soaked maize seeds
Starch agar plates
White tile
Scalpel
Iodine-potassium iodide solution
Boiling tube
Water bath

Method

1. Cut the maize seed in half.
2. Place one half cut surface down onto the starch agar.
3. Place the other half of the maize seed into the boiling tube add water and place in a water bath at 80 ^{o}C for 10 minutes. This will denature any enzymes present.
4. Place this half of the maize seed onto another starch agar plate cut surface down. This will act as a control.
5. Incubate both plates at 25 ^{o}C overnight.
6. Remove the maize seeds and flood each plate with iodine-potassium iodide solution.
7. Observe and record any difference between the two plates.
8. Calculate the area of any ‘clear’ zone on the plates.

Dissection of wind and insect-pollinated flowers

Specification reference: **4.2**

Sexual reproduction in plants

Introduction

Pollination is the transfer of pollen grains from the anther to the stigma. Some species of plants have flowers adapted for pollination by birds, mammals and water but most use insects or wind. There are two types of flowering plant, the monocotyledons and dicotyledons. The monocotyledons have leaves with parallel veins e.g. grasses, barley, maize, lily and plantain. In monocotyledons the floral parts are in threes or multiples of three. The dicotyledons have leaves with netted veins e.g. geranium, primrose and buttercup.

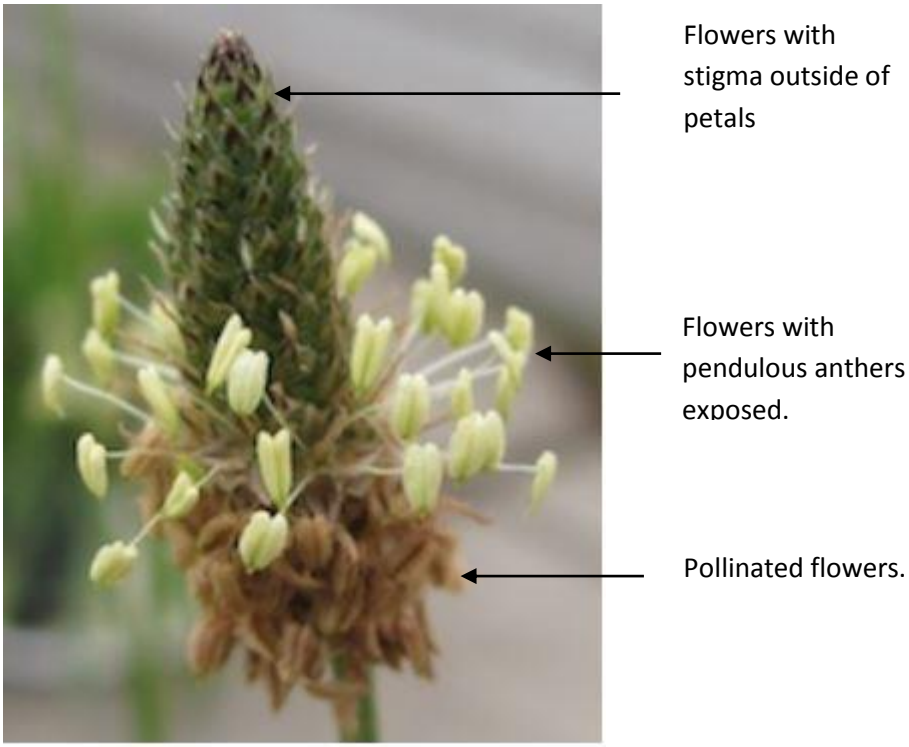
Apparatus

Insect pollinated flower
Wind pollinated flower
White tile
Fine forceps
Scalpel
Magnifying glass

Method for insect pollinated flower

1. Examine the flower, identify the floral parts and count the numbers of sepals and petals.
2. Place the flower onto the tile and from the stalk end cut the flower in half.
3. Using the magnifying glass examine one of the halves.
4. Identify the floral parts at the centre of the flower which may have been covered by the petals.
5. Draw the flower as seen in section, identify and label the parts.

Method for wind pollinated flower, for example Plantain



1. Using the fine forceps remove one of the flowers with protruding anthers.
2. Examine using the magnifying glass.
3. Draw and label the flower.
4. Separate the floral parts using mounted needles.
5. Using fine forceps remove one of the flowers with a protruding stigma. Observe using a magnifying glass.
6. Using mounted needles separate the floral parts.

Scientific drawing of cells from prepared slides of anther

Specification reference: **4.2**

Sexual reproduction in plants

Introduction

This practical requires you to observe and draw a prepared slide of an anther. You should draw in proportion as described in the guidance notes.

Apparatus

Microscope
Slide of TS anther

Method

1. Examine the slide using the x10 objective lens.
2. Draw a plan to show the distribution of tissues in the correct proportion.
3. You may need to use the x40 objective to identify some of the tissue layers.
4. The entire structure need not be drawn but if it is not a complete representation of the entire structure a small drawing should be made and the area drawn in the plan shown.
5. Draw two lines measured in eye piece units on the plan.
6. Label the following structures: epidermis; tapetum/ inner wall; fibrous layer/ outer wall; area of dehiscence/ stomium; pollen sac; xylem; phloem; parenchyma.
7. Calculate the actual size of the plan and the magnification of the drawing.

Experiment to illustrate gene segregation

Specification reference: 4.3

Inheritance

Introduction

Mendelian genetics explains how different rations of phenotypes can be produced when the genetic composition of the parents is known. Such ratios can be observed if a large enough sample of progeny is counted. The numbers counted may be subjected to statistical testing, using the χ^2 test, which indicates whether or not the observed ratio is statistically equivalent to the theoretical Mendelian ratio.

In maize, *Zea mays, a* single cob is covered in very many kernels, each of which contains a seed which is the result of a single fertilisation.

The kernels show a number of characteristics, for example texture and colour, as is shown below.

Apparatus

Zea mays corn cob

Method

1. Identify the phenotypes shown by the cob and count how many kernels of each phenotype there are.
2. Identify the Mendelian ratio that is closest to the counts that you have made.
3. Use the χ^2 test and probability table to test if the sample shows that ratio you have identified.
4. Deduce the genotype of the parent plants.

Phenotype	Observed number (O)	Expected number (E)	(O – E)	$(O - E)^2$	$\frac{(O - E)^2}{E}$

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Degrees of freedom	Probability		
	P = 0.10	P = 0.05	P = 0.02
1	2.71	3.84	5.41
2	4.61	5.99	7.82
3	6.25	7.82	9.84
4	7.78	9.49	11.67
5	9.24	11.07	13.39

Investigation of continuous variation in a species

Specification reference: **4.4**

Variation and evolution

Introduction

Polygenic characters often show continuous variation, which can be demonstrated by plotting a frequency histogram, producing an approximately normal curve. Counts or measurements of samples are made and if their distribution is approximately normal, their means may be compared using Student's t test.

Apparatus

Ruler in mm
15 ivy leaves from each of two contrasting sites e.g. growing in bright sun and growing in the shade.

Method

1. Measure the maximum width of each leaf.
2. Calculate the mean width of each sample.
3. Plot frequency histograms for the two samples, to determine whether they are approximately normally distributed.
4. Calculate the standard deviation for each sample.

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

5. Test that the means are different using Student's t test.

$$t = \frac{\left|\bar{x}^1 - \bar{x}^2\right|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

where,

$\left|\bar{x}^1 - \bar{x}^2\right|$ = the difference in mean values of sample 1 and sample 2

s_1^2 and s_2^2 are the squares of the standard deviation of the samples

n_1 *and* n_2 are the number of readings in each sample.

Sample 1	Width (mm)	Deviation from the mean $(x-\bar{x})$	Deviation squared $(x-\bar{x})^2$
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
Mean			Σ =
Sample 2			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
Mean			Σ =

Degrees of freedom	p = 0.1	p = 0.05	p = 0.02	p = 0.01	p = 0.002	p = 0.001
1	6.314	12.706	31.821	63.657	318.310	636.620
2	2.920	4.303	6.965	9.925	22.327	31.598
3	2.353	3.182	4.541	5.841	10.214	12.924
4	2.132	2.776	3.747	4.604	7.173	8.610
5	2.015	2.571	3.365	4.032	5.893	6.869
6	1.943	2.447	3.143	3.707	5.208	5.959
7	1.895	2.365	2.998	3.499	4.785	5.408
8	1.860	2.306	2.896	3.355	4.501	5.041
9	1.833	2.262	2.821	3.250	4.297	4.781
10	1.812	2.228	2.764	3.169	4.144	4.587
11	1.796	2.201	2.718	3.106	4.025	4.437
12	1.782	2.179	2.681	3.055	3.930	4.318
13	1.771	2.160	2.650	3.012	3.852	4.221
14	1.761	2.145	2.624	2.977	3.787	4.140
15	1.753	2.131	2.602	2.947	3.733	4.073
16	1.746	2.120	2.583	2.921	3.686	4.015
17	1.740	2.110	2.567	2.898	3.646	3.965
18	1.734	2.101	2.552	2.878	3.610	3.922
19	1.729	2.093	2.539	2.861	3.579	3.883
20	1.725	2.086	2.528	2.845	3.552	3.850
21	1.721	2.080	2.518	2.831	3.527	3.819
22	1.717	2.074	2.508	2.819	3.505	3.792
23	1.714	2.069	2.500	2.807	3.485	3.767
24	1.711	2.064	2.492	2.797	3.467	3.745
25	1.708	2.060	2.485	2.787	3.450	3.725
26	1.706	2.056	2.479	2.779	3.435	3.707
27	1.703	2.052	2.473	2.771	3.421	3.690
28	1.701	2.048	2.467	2.763	3.408	3.674
29	1.699	2.045	2.462	2.756	3.396	3.659
30	1.697	2.042	2.457	2.750	3.385	3.646
40	1.684	2.021	2.423	2.704	3.307	3.551
60	1.671	2.000	2.390	2.660	3.232	3.460
120	1.658	1.980	2.358	2.617	3.160	3.373
∞	1.645	1.960	2.326	2.576	3.090	3.291