GCE A LEVEL

eduqas
Part of WJEC

WJEC Eduqas GCE A LEVEL in

BIOLOGY

ACCREDITED BY OFQUAL

GUIDANCE FOR TEACHING

Teaching from 2015

Version 2 - February 2024



wjec cbac

This Ofqual regulated qualification is not available for candidates in maintained schools and colleges in Wales.

SUMMARY OF AMENDMENTS

Version	Description	Page number
2	Chromatography method updated.	53 and 54

INTRODUCTION

The **WJEC Eduqas A level Biology** qualification, accredited by Ofqual for first teaching from September 2015, is available to:

- All schools and colleges in England and private schools in Wales
- Schools and colleges in independent regions such as Northern Ireland, Isle of Man and the Channel Islands
- Independent schools in Wales.

It will be awarded for the first time in Summer 2017, using grades A*–E.

The qualification provides a broad, coherent, satisfying and worthwhile course of study. It encourages learners to develop confidence in, and a positive attitude towards, biology and to recognise its importance in their own lives and to society.

The specification lends itself to a variety of teaching and learning styles and offers learners of all abilities an enjoyable and positive learning experience. The optional topics have been developed to allow learners to gain an insight into a range of biological topics and their applications.

Practical work is an intrinsic part of biology, and is greatly valued by higher education. It is imperative that practical skills are developed throughout the course and that an investigative approach is promoted wherever possible.

Additional ways that WJEC Eduqas can offer support:

- Specimen assessment materials
- Face-to-face CPD events
- Question paper database
- Examiners' reports on each question paper
- Free access to past question papers and mark schemes via the secure website
- Direct access to the subject officer
- Free online resources
- Exam Results Analysis
- Online Examination Review

The document contains resources to support the teaching of A level biology in England. Resources are contributed by the Society of Biology, Nuffield Foundation, Science and Plants for Schools, British Ecological Society, Biochemical Society, Field Studies Council, Society for General Microbiology and the Wellcome Trust.

If you have any queries please do not hesitate to contact:

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AIMS OF THE TEACHERS' GUIDE

The principal aim of the Teachers' Guide is to support teachers in the delivery of the new **WJEC Eduqas A level Biology** specification and to offer guidance on the requirements of the qualification and the assessment process.

The guide is **not intended as a comprehensive reference**, but as support for professional teachers to develop stimulating and exciting courses tailored to the needs and skills of their own learners in their particular institutions.

The guide offers assistance to teachers with regard to the depth of coverage required as well as links to useful digital resources (both our own, freely available, digital materials and some from external resources) to provide ideas for engaging lessons.

	SPECIFICATION STATEMENT	COMMENT
(a)	the key elements are present as inorganic ions in living organisms: Mg^{2+}, Fe^{2+}, Ca^{2+}, $PO4^{3-}$	The roles of Mg^{2+}, Fe^{2+}, PO_4^{3-} in cell metabolism in plants and animals. This should include their role as components of biological molecules, e.g. chlorophyll, haemoglobin, nucleic acids and phospholipids. The role of Ca^{2+} in strengthening tissues e.g. bones and teeth in animals and cell walls in plants.
(b)	the importance of water in terms of its polarity, ability to form hydrogen bonds, surface tension, as a solvent, thermal properties, as a metabolite	Thermal properties should include understanding of the importance of high specific heat capacity and high latent heat of vaporisation. The role of water as a metabolite should include: as a reactant in photosynthesis and hydrolysis and as a product of aerobic respiration and condensation reactions. An understanding that water provides support and buoyancy.



"Food tests to include: iodine-potassium iodide test for starch; Benedict's test for reducing and non-reducing sugars; biuret test for protein; emulsion test for fats and oils" practical work

Continued on next page

General guidance

ADDITIONAL RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(c)	the structure, properties and functions of carbohydrates: monosaccharides (triose, pentose, hexose sugars); disaccharides (sucrose, lactose, maltose); polysaccharides (starch, glycogen, cellulose, chitin)	Candidates should be able to recognise examples of monosaccharides (formula – $C_n(H_2O)_n$) to include: triose (glyceraldehyde), pentose (ribose, deoxyribose) and hexose (α– and β–glucose, fructose, galactose). Candidates should be able to recognise examples of disaccharides (formula - $C_{12}H_{22}O_{11}$) to include: sucrose (glucose-fructose), maltose (α–glucose-α–glucose) and lactose (glucose-galactose). Candidates should be able to recognise examples of polysaccharides to include: starch, a polymer of α–glucose (composed of straight-chain amylose and branched amylopectin), glycogen, a polymer of α–glucose (branched structure), cellulose a polymer of β–glucose and chitin a polymer of β monomers with some –OH groups replaced by nitrogen-containing acetylamine groups. Cellulose and chitin are similar structural polysaccharides with the adjacent monomers twisted through 180° to each other, allowing hydrogen bonds between chains, forming microfibrils. Candidates should be able to link the properties and structures of these molecules to their functions. This should include solubility, strength, energy content and osmotic effect.
(d)	alpha and beta structural isomerism in glucose and its polymerisation into storage and structural carbohydrates, illustrated by starch, cellulose and chitin	
(e)	the chemical and physical properties which enable the use of starch and glycogen for storage and cellulose and chitin as structural compounds	



Select the image (left) for " Food tests to include: iodine-potassium iodide test for starch; Benedict's test for reducing and non-reducing sugars; biuret test for protein; emulsion test for fats and oils" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(f)	the structure, properties and functions of lipids as illustrated by triglycerides and phospholipids	Candidates should be able to recognise examples of: triglycerides, phospholipids and give the structural formula for glycerol and general formula for an unsaturated fatty acid. Saturated fatty acids have only single carbon-to-carbon bonds. Monounsaturated fatty acids have one carbon-to-carbon double bond and polyunsaturated fatty acids contain two or more carbon-to-carbon double bonds. Candidates should understand how the functions of lipids and phospholipids in cells and organisms are related to their hydrophilic and hydrophobic properties. Other functions of lipids should include insulation, energy storage and protection.
(g)	the implications of saturated and unsaturated fat on human health	A high intake of fat by humans, notably saturated fats, is a contributory factor in heart disease. It raises the low density lipoprotein (LDL) cholesterol level, which increases the incidence of atheromas in coronary arteries (and in other arteries). Detailed knowledge of atheroma formation is not expected.



Select the image (left) for " Food tests to include: iodine-potassium iodide test for starch; Benedict's test for reducing and non-reducing sugars; biuret test for protein; emulsion test for fats and oils" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(h)	the structure and role of amino acids and proteins	Candidates should be able to draw the general formula for amino acids and recognise amino (basic), carboxylic (acidic) and R (variable) groups. Proteins are polymers of amino acids of which there are twenty types which are coded for in proteins and which differ by the R group. Candidates are not expected to recall names of amino acids but can be expected to identify them, given a structural formula and a suitable table showing R groups. Candidates should be able to identify peptide, disulphide, ionic, hydrogen bonds and hydrophobic interactions between R groups at the various levels of protein structure. Candidates should be familiar with different ways of representing protein structures, including ribbon diagrams and recognise regions of molecules as having a primary structure e.g. sequence of amino acids, a secondary structure e.g. α helices, β pleated sheets, a tertiary structure e.g. further folding of the polypeptide chain and a quaternary structure as more than one polypeptide chain bonded together. The bonding within a protein affects the three dimensional structure of the molecule and therefore affects its function within cells and organisms e.g. fibrous proteins (e.g. keratin) - a structural function and globular proteins (e.g. enzymes) - a metabolic function.
(i)	the primary, secondary, tertiary and quaternary structure of proteins	
(j)	the relationship of the fibrous and globular structure of proteins to their function	
	Candidates should be able to use given structural formulae (proteins, triglycerides and carbohydrates) to show how bonds are formed and broken by condensation and hydrolysis, including peptide, glycosidic and ester bonds. (Candidates should be able to recognise and understand but not reproduce the structural formulae of the above molecules.)	



Select the image (left) for " Food tests to include: iodine-potassium iodide test for starch; Benedict's test for reducing and non-reducing sugars; biuret test for protein; emulsion test for fats and oils" practical work

General guidance

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EDUQAS > A LEVEL Biology > Specification from 2015

Food tests

Specification reference: Core Concepts 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 °C and 90 °C for 5 minutes.
3. If the blue Benedict's has changed colour to an orange / red precipitate the test solution is a reducing sugar.

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. If it is not a reducing sugar there will be no colour change and the solution will remain blue.
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.
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. If the blue Benedict's has changed colour to orange / red precipitate, the test solution is a non-reducing sugar.

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. If the solution turns purple the test is positive.

Starch

1. Mix 2 cm^3 of the test solution with 2 drops of iodine in potassium-iodide solution.
2. If starch is present the solution will change colour to blue/ black.

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. If lipids are present a cloudy white emulsion will form.

Risk Assessment

Hazard	Risk	Control measure
Biuret is an irritant	Could splash onto hands or into eyes when transferring biuret to test tube	Wear gloves/ eye protection
Ethanol is flammable	Could catch fire if used near a Bunsen burner	Ensure all Bunsen burners are turned off before ethanol is used

Benedict's reagent and Iodine solution are classed as low hazard by CLEAPSS at these concentrations.

Teacher/ Technician's notes

Starch
Iodine is only sparingly soluble in water (0.3 g per litre); it is usual to dissolve it in potassium iodide solution (KI) to make a 0.01 M solution (by tenfold dilution of a 0.1 M solution) to use as a starch test reagent. Refer to CLEAPSS Recipe card 33.

Reducing/ non reducing sugar
Benedict's reagent can be purchased from a laboratory supplier or can be made.

1 dm^3 of Benedict's reagent contains:

100 g anhydrous sodium carbonate
173 g sodium citrate
17.3 g copper(II)sulphate pentahydrate

Protein
Biuret reagent can be purchased from a laboratory supplier or potassium hydroxide and dilute copper(II)sulphate solution can be used as an alternative.

Fats and oils
Absolute alcohol should be used because if it has been diluted an emulsion of fats will be produced in the alcohol before it is poured into the water.

Further work

- Testing a variety of foods.
- The Benedict's test is semi-quantitative, 0.5 g % concentration reducing sugar gives a green precipitate, 1 g% concentration a yellow precipitate, 1.5 g % an orange precipitate and above 2 g% a red precipitate. Students could assess the % concentration of reducing sugar by comparing the colour to standard solutions of reducing sugars. They would need to plan which variables needed to be controlled to carry out such a semi-quantitative study.
- The Biuret test is also semi-quantitative. The deeper the violet colour the more peptide bonds (hence protein) which is present.

Practical techniques

- Use of qualitative reagents to identify biological molecules.

COMPONENT: CC2 CELL STRUCTURE AND ORGANISATION — EXAM LEVEL: A LEVEL

	SPECIFICATION STATEMENT	COMMENT
(a)	the structure and function of the following: mitochondria; endoplasmic reticulum (rough and smooth); ribosomes; Golgi body; lysosomes; centrioles; chloroplasts; vacuoles; nucleus; chromatin; nuclear envelope; nucleolus; plasmodesmata	Candidates should be able to recognise the listed organelles on a diagram or electron micrograph of eukaryotic cells, and draw them onto a generalised diagram of a cell, understanding their relative size. Candidates should understand that organelles work together to carry out functions within cells, e.g. in the synthesis and transport of biological molecules such as glycoproteins.
(b)	the structure of prokaryotic cells and viruses	The cell theory states that new cells are formed from other existing cells and that the cell is a fundamental unit of structure, function and organisation in all living organisms. Candidates should know that viruses do not show a typical cell structure and compare the structure of a virus with prokaryotic and eukaryotic cells. Candidates should know some differences between prokaryotic cells and eukaryotic cells, including: lack of membrane-bound organelles, structure and location of genetic material, structure of ribosomes, cell wall structure, sites of respiration. Candidates should know some differences between plant and animal cells.
(c)	Cell theory and the similarities and differences in the cell structures of eukaryotes(animal and plant), prokaryotes and viruses, including the examination of a range of electron micrographs of prokaryote and eukaryote cells to show structure	
(d)	the levels of organisation including aggregation of cells into tissues, tissues into organs and organs into organ systems and also the examination of a range of prepared slides showing examples of epithelia, muscle and connective tissue	Candidates should be able to recognise: ciliated, columnar and squamous epithelia; striated, smooth and cardiac muscle and connective tissue.



Select the image (left) for "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" practical work



Select the image (left) for "Preparation and scientific drawing of a slide of living cells e.g. onion/ rhubarb/ *Amoeba* including calculation of actual size and magnification of drawing " practical work

General guidance | **Microscope calibration**

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EDUQAS > A LEVEL Biology > Specification from 2015

List of Related External Resources

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: Core Concepts 2

Cell structure and organisation

Introduction

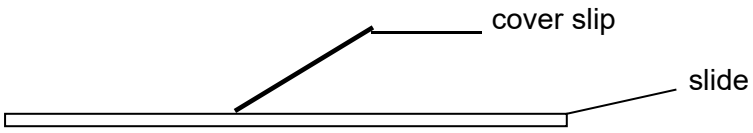
This practical is a simple introduction to the use of a microscope and how it can be used to take measurements.

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.

Risk Assessment

Hazard	Risk	Control measure
Microscope lamp/ bulb is hot	Could burn skin when trying to move lamp/ microscope	Leave lamp to cool before moving

Teacher/ Technician notes

Microscopes must be fitted with eye piece graticules. These can be bought separately and fitted to most microscopes retrospectively.

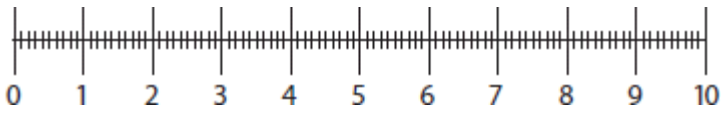
Stage micrometers can be bought readymade or can be made by buying film reticules from scientific suppliers and fixing them to microscope slides. Stage micrometers come in two sizes and the calibration of both are given below.

Please make sure that students are aware that there are **100 eyepiece** units in the eyepiece graticule and they should **not** be working in **fractions** of an eyepiece or stage micrometer unit e.g. 2.8. instead it should always be **whole numbers,** e.g. 28.

There is space for the students to record the calibration for their microscopes in this practical. It is best if they use the same microscope for each piece of microscope work, there is then no need for them to calibrate the microscope every time. Instead they can refer to that page in their lab book for the calibration for that objective. The microscopes can be labelled for ease of identification.

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.

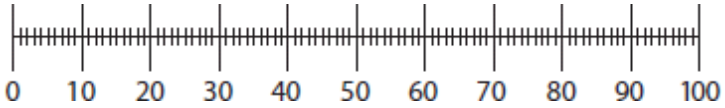
A stage micrometer is used to measure the length of each division at different magnifications. There are two types of stage micrometer available, check which one you are using.

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.

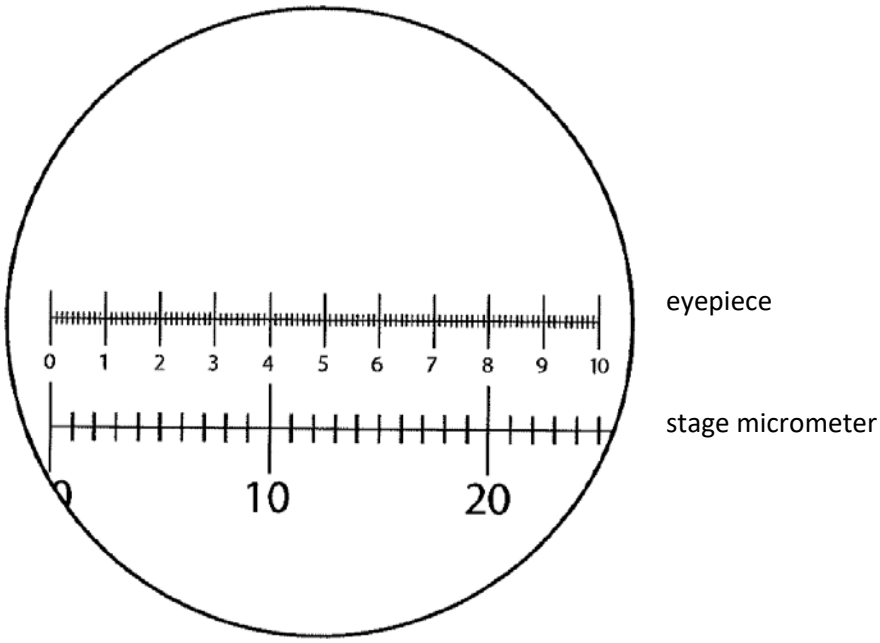
Or

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.



If 1 stage micrometer unit = 0.01 mm

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

80 eyepiece units = 20 stage micrometer units

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

1 stage micrometer unit = 0.01 mm

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

If 1 stage micrometer unit = 0.1 mm

Using this x40 objective lens, 2 stage micrometer units would make up 80 eyepiece units.

80 eyepiece units = 2 stage micrometer units

$$\text{1 eye piece unit} = \frac{2}{80} = 0.025 \text{ stage micrometer units}$$

1 stage micrometer unit = 0.1 mm

1 eye piece unit = 0.025 x 0.1 mm
= 0.0025 mm or 0.025 x 1000 μm
= 2.5 μm

Practical techniques

- use of light microscope at high power and low power, including use of a graticule.

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

Specification reference: Core concepts 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.

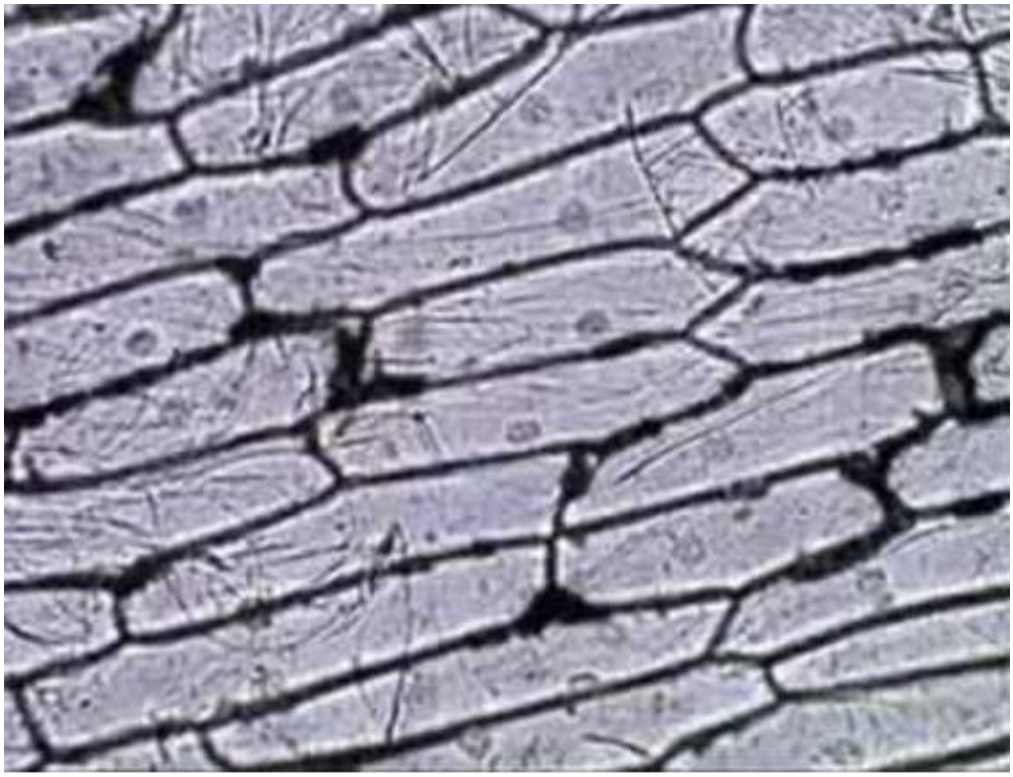
Risk Assessment

Hazard	Risk	Control measure
Scalpel blade is sharp	Could cut skin when cutting onion	Cut away from body onto a white tile
Iodine in potassium iodide solution is an irritant	Could be transferred from skin to eyes	Wear safety glasses
Broken glass is sharp	Cover slips could shatter and cut skin	Place a paper towel over the coverslip before pressing down onto it

Teacher/ Technician's notes

Iodine is only sparingly soluble in water (0.3 g per litre); it is usual to dissolve it in potassium iodide solution (KI) to make a 0.01 M solution (by tenfold dilution of a 0.1 M solution). Refer to CLEAPSS Recipe card 33.

Red onions or rhubarb epidermis will give a clearer definition of the cytoplasm.



See guidance notes in the Lab book with regard to quality of drawing, calculation of actual size and magnification.

Further work

- Observation of *Amoeba*
- Observation of alga

Practical techniques

- use of light microscope at high power and low power, including use of a graticule
- produce scientific drawing from observation with annotations

	SPECIFICATION STATEMENT	COMMENT
(a)	the principal components of the plasma membrane and understand the fluid-mosaic model	The principal biochemical constituents of the cell membrane including: intrinsic and extrinsic proteins, glycoproteins, phospholipids and, in animal cells, cholesterol. The polarity of protein molecules affects their position in the membrane. Intrinsic proteins include channel proteins and carrier proteins. The extracellular surfaces of the proteins can be glycosylated to form a glycocalyx. Candidates should be able to draw a simple diagram to illustrate the fluid mosaic model.
(b)	the factors affecting permeability of the plasma membrane	This should include temperature and organic solvents.
(c)	the following transport mechanisms: diffusion and factors affecting the rate of diffusion; osmosis and water potential; pinocytosis; facilitated diffusion; phagocytosis; secretion (exocytosis); active transport and the influence of cyanide	Transport across cell membranes is affected by surface area, the concentration gradient, temperature, the size of the molecule, lipid solubility and thickness of the membrane. This then determines how different molecules are transported across the membrane. Facilitated diffusion (involving channel or carrier proteins, but not ATP) and active transport (carrier proteins with ATP) provide a mechanism to increase the rate of transport across the membrane for some molecules (e.g. polar molecules). Co-transport is a type of facilitated diffusion by which two substances are simultaneously transported across a membrane by a carrier protein. Candidates should understand the process of osmosis in plant and animal cells in terms of water potential. Exocytosis and endocytosis provide a mechanism for bulk transport across a cell membrane and these processes change the surface area of cells as they occur.



Select the image (left) for "Determination of water potential by measuring changes in mass or length" practical work



Select the image (left) for " Determination of solute potential by measuring the degree of incipient plasmolysis I" practical work



Select the image (left) for "Investigation into the permeability of cell membranes using beetroot " practical work

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A Level Biology > Specification from 2015

Determination of water potential by measuring changes in mass or length

Specification reference: Core concepts 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 large enough to extract 50 mm cylinders: potatoes, sweet potatoes, yams, beetroots, swede, turnip, parsnip and carrot are suitable.
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 of distilled water or solution into 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.

Risk assessment

Hazard	Risk	Control measure
Scalpel blades are sharp	May cut skin when cutting cylinders	Cut away from body onto a white tile
Cork borers are sharp	May cut skin when cutting cylinders	The cylinders of tissue must be cut on the chopping board with the force directed downwards

Technician's notes

Making solutions – masses per dm^3 are given in the table below. The weighed solutes should be dissolved in a minimum volume of water and then the solution made up to 1 dm^3 with distilled water.

concentration of solution / mol dm^{-3}	mass sodium chloride per dm^3 solution / g
0	0
0.2	11.7
0.4	23.4
0.6	35.1
0.8	46.8

Alternatively, a 1 mol dm^{-3} solution may be made and diluted as needed. To make up a 1 mol dm^{-3} solution, 58.5 g of sodium chloride is required.

Sample results

Concentration of bathing solution mol dm^{-3}	Initial length / mm	Final length / mm	Length change / mm	% length change	mean % length change
0	52	57	5	10	10
	49	55	6	12	
	50	54	4	8	
0.2	48	49	1	2	3
	50	52	2	4	
	50	51	1	2	
0.4	58	55	-3	-5	-2
	50	48	-2	-4	
	53	54	1	2	
0.6	50	48	-2	-4	-6
	52	48	-4	-8	
	50	47	-3	-6	
0.8	49	41	-8	-16	-13
	50	43	-7	-14	
	52	48	-4	-8	

When there is no change in length, the concentration of the cell contents is equal to that of the bathing solution, which is read at the x-intercept. The equivalent solute potential can be read from the table.
At incipient plasmolysis, $\psi_P = 0$, $\therefore \psi_{cell} = \psi_S$ $\therefore$ this figure gives the water potential of the cells.

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

- If cylinders are left for too short a time before re-measuring, results are still valid. The intercept would be the same but less water will have entered or left the cells, giving a smaller gradient. The error in measurement is proportionally greater and the experiment, therefore, less accurate.

- In biology, it is normal to join data points to construct a line on a graph. Where an intercept is to be read, a line/ curve of best fit may be used. This takes into account all the data, rather than only the two points either side of the intercept.

Further work

- Different plant material may be used to test a hypothesis relating to dissolved sugar concentration and relative sweetness of the vegetables. An example would be to compare the intercepts of using cylinders of potato and sweet potato. It could be hypothesised that the line for sweet potato would intercept the horizontal axis at a higher concentration and lower solute potential because sweet potato has a higher concentration of dissolved sugars.

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).

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

Specification reference: Core Concepts 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 $= \psi_{cell} = \psi_S$.

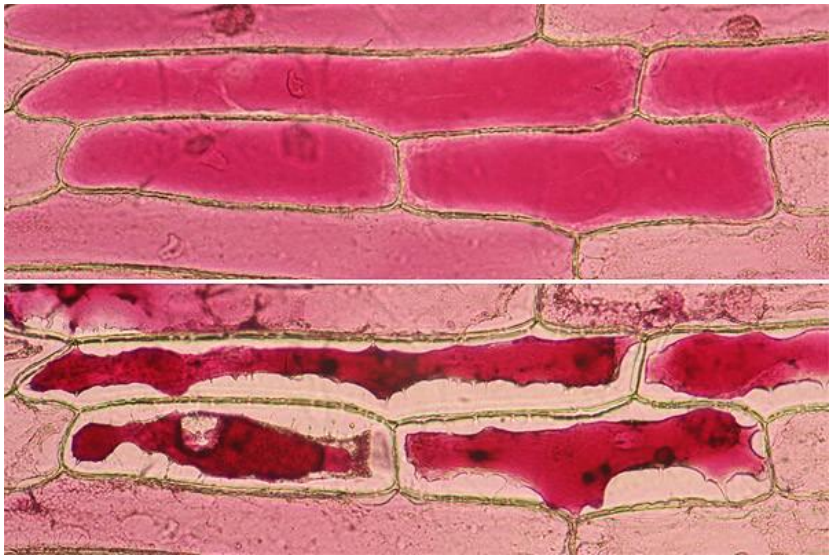
Apparatus

White tile
Fine forceps
Fine scissors
Rhubarb petioles or red onion
5 x 9 cm Petri dishes, 100 cm^3 beakers or watch glasses
Distilled water
Sodium chloride solutions 0.2, 0.4, 0.6, 0.8 mol dm^{-3}: Instructions for making these solutions are given in the previous experiment.
Stop clock
Microscope slides
Cover slips
Microscope
Dropping pipettes

Method

1. Set up five labelled Petri dishes/ small bottles each containing 10 cm^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

Risk assessment

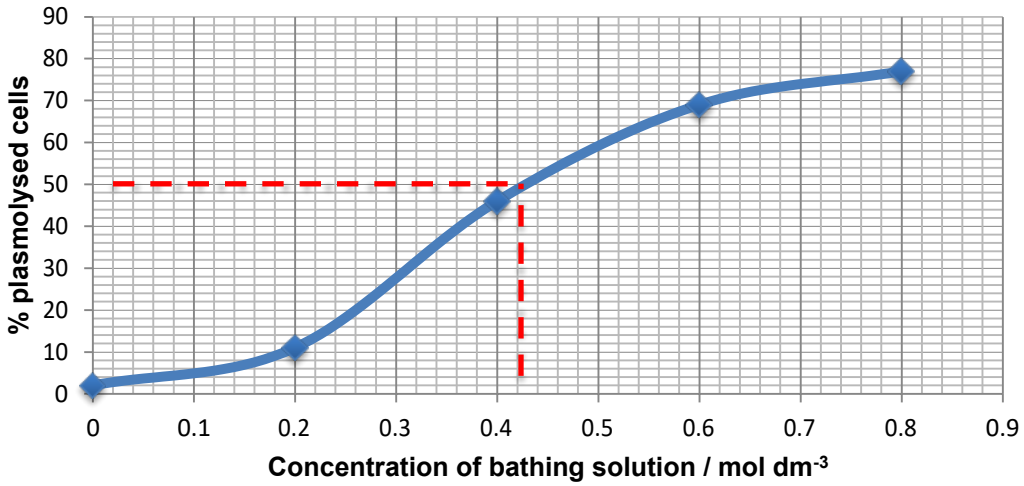
Hazard	Risk	Control measure
Scalpel blades are sharp	May cut skin when cutting cylinders	Cut away from body onto a white tile

Teacher / Technician's notes

- To ensure that the same cell is not counted more than once, a system of tracking across the specimen may be used. The slide is always moved in the same direction across the field of view and successive fields of view are always separated from the previous one by the width of at least one cell.
- Cells occur on the edge of the field of view and are only partially visible. A rule should be made regarding their scoring, for example, a partially visible cell is never counted or a cell is scored if it is at least half visible.
- In biology, it is normal to join data points to construct a line on a graph. In this situation, a curve of best fit may be used. This takes into account all the data, rather than only the two data points either side of that representing 50% plasmolysis.

Sample results

Concentration of bathing solution / mol dm^{-3}	Number of cells in field of view						Total		
	1		2		3				
	plasmolysed	turgid	plasmolysed	turgid	plasmolysed	turgid	plasmolysed	turgid	% cells plasmolysed
0	1	30	0	32	1	36	2	98	2
0.2	4	28	2	32	5	29	11	89	11
0.4	20	15	10	19	16	12	46	54	46
0.6	20	9	25	11	24	11	69	31	69
0.8	19	7	34	8	24	9	77	23	77



The solute potential of the equivalent concentration may be read from the table given in Experiment 4.

Alternatively, the % plasmolysed cells can be plotted against the solute potential, so that the solute potential of the cells may be read directly from the graph.

Further work

Make a scientific drawing of turgid and plasmolysed cells, labelled to show

- Cell wall
- Cytoplasm
- Position of cell membrane (it is too thin to actually see but the boundary of the cytoplasm marks its position)
- Vacuole
- Nucleus
- Position of bathing solution

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).

Investigation into the permeability of cell membranes using beetroot

Specification reference: Core concepts 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 °C.

Apparatus

Beetroot cylinders
White tile
10 test tubes
Scalpel
250 cm^3 beaker
Forceps
Water baths at (25, 35,45,55,65 °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 to measure absorbance. 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.

Risk Assessment

Hazard	Risk	Control measure
Scalpels are sharp	Could cut skin when cutting cylinders	Cut away from body onto white tile

Teacher/ Technician's notes

Beetroot must be raw, not cooked. Use a size 4 cork borer and cut with care using a cutting board. Cut enough cores to make eight 2 cm lengths per working group. Leave the cores overnight in a beaker of distilled water. The pigment from any cells that have been cut by the cork borer will leak into the water. Rinse away any pigmented water in the morning and replace with fresh water.

Each student should be given cylinders of the same diameter (5 – 8 mm. diameter) and enough to be able to cut 5 x 1cm cylinders.

Thermostatically controlled water baths could be set up at the required temperatures or students could be given equipment to enable them to make their own water baths (tripod, gauze, beaker, Bunsen burner and thermometer).

If students are using a colorimeter it would be an advantage to have a simple set of printed instructions by the instrument.

Further details are available from:

http://www.nuffieldfoundation.org/practical-biology/investigating-effect-temperature-plant-cell-membranes

Sample results

Here is a sample of results obtained with a colorimeter – measuring transmission of light at 530 nm (rather than absorbance).

Temperature (°C)	Observation	Colorimeter reading (% transmission of light)			
		Repeat 1	Repeat 2	Repeat 3	Mean
0	clear	100	98.5	99.0	99.2
22	very pale pink	93.9	95.0	96.0	95.0
42	very pale pink	80.1	77.0	76.9	78.0
63	pink	26.3	29.9	31.0	29.1
87	dark pink	0.7	0.7	1.0	0.8
93	red	0.0	0.1	0.0	0.0

Further work

- Students could extend their investigation by finding the effect of alcohol or detergents on membrane permeability.
- The procedure allows for students to identify systematic and random variables. It is a good opportunity to practice graphical treatment of results, including range bars to assess the variation in repeats.

Practical Techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).
- Use appropriate instrumentation to record quantitative measurements, such as a colorimeter or potometer.

COMPONENT: CC4 BIOLOGICAL REACTIONS ARE REGULATED BY ENZYMES EXAM LEVEL: A LEVEL

SPECIFICATION STATEMENT		COMMENT
(a)	metabolism as a series of enzyme controlled reactions	Metabolism is a combination of anabolic and catabolic reactions that are catalysed by enzymes. The principle features of enzyme reactions include: the collision theory of enzyme reactions, the lock and key model and the induced fit model (where changes of shape of both active site and substrate bring reactive groups of enzyme and substrate close to each other, weakening bonds in the substrate so the reaction takes place at a lower activation energy).
(b)	the protein nature of enzymes	
(c)	enzymes acting intra-cellularly or extra-cellularly	
(d)	active sites, interpreted in terms of three dimensional structure	
(e)	the theory of induced fit as illustrated by lysozyme	
(f)	the meaning of catalysis; the lowering of the activation energy	



Select the image (left) for "Investigation into the effect of temperature or pH on enzyme activity" practical work



Select the image (left) for "Investigation into the effect of enzyme or substrate concentration on enzyme activity" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

List of Related External Resources

COMPONENT: CC4 BIOLOGICAL REACTIONS ARE REGULATED BY ENZYMES EXAM LEVEL: A LEVEL

	SPECIFICATION STATEMENT	COMMENT
(g)	the influence of temperature, pH, substrate and enzyme concentration on rate of activity and inactivation and denaturation of enzymes and the importance of buffers for maintaining a constant pH	Environmental conditions such as high temperatures and pH (away from the optimum) alter the three dimensional structure of enzyme molecules. Bonds involved in the tertiary structure may be broken and hence the configuration of the active site is altered, reducing the ability to form enzyme-substrate complexes and hence the reaction rate. High temperatures and extreme changes in pH cause a permanent change in an enzyme's structure, this is called denaturation.
(h)	the principles of competitive and non-competitive inhibition	Candidates should be able to distinguish between competitive and non-competitive inhibition, including explaining the effect of increasing substrate concentration on both. Candidates should understand that inhibition can be reversible or irreversible.
(i)	the importance of immobilised enzymes and that industrial processes use immobilised enzymes, allowing enzyme reuse and improving stability	Candidates should have an appreciation that biosensors are an application of immobilised enzymes, but detailed knowledge of biosensors is not expected.



Select the image (left) for "Investigation into the effect of temperature or pH on enzyme activity" practical work



Select the image (left) for "Investigation into the effect of enzyme or substrate concentration on enzyme activity" practical work

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

List of Related External Resources

Investigation into the effect of temperature or pH on enzyme activity

Specification reference: Core Concepts 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})
5x 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.

Risk Assessment

Hazard	Risk	Control measure
Sodium carbonate is an irritant at high concentrations	May splash or transfer into eye when placing into test tube	Use low concentrations and wear safety glasses
Phenolphthalein indicator contains ethanol which is flammable	If using Bunsen burners for water baths, could ignite the ethanol	Do not use ethanol near Bunsen burners

Teacher/ Technicians notes

Sodium carbonate solution, 0.05 M. Make with 5.2 g of anhydrous solid, or 14.2 g of washing soda per litre of water. See CLEAPSS Hazcard; it is an IRRITANT at concentrations over 1.8 M.

Ethanol (IDA) in the phenolphthalein indicator is described as HIGHLY FLAMMABLE on the CLEAPSS Hazcard (flash point 13 °C) and HARMFUL (because of presence of methanol).

Lipase solution (5%) is best freshly made, but it will keep for a day or two in a refrigerator.

Phenolphthalein is described as low hazard on CLEAPSS Hazcard 32. Refer to Recipe card 33 (acid-base indicators): Dissolve 1 g in 600 cm^3 of IDA then make up to 1 litre with water. Label the bottle highly flammable. Suppliers of phenolphthalein solution may not use IDA; it also may be diluted. Follow any hazard warning on supplier's bottles.

Thermostatically controlled water baths could be set up at the required temperatures or students could be given equipment to enable them to make their own water baths (tripod, gauze, beaker, Bunsen burner and thermometer).

More details available from:

http://www.nuffieldfoundation.org/practical-biology/investigating-effect-temperature-activity-lipase

Sample results

The quantities used should take approximately 4 minutes to change from pink to white at normal laboratory temperature. If this is not the case, change the concentration of enzyme to alter the speed of the reaction (more enzyme will reduce the time or increase the speed). Students will need to use the same volume at each temperature.

Further work

- Use a pH probe or data logger to give quantitative results
- It would be possible to vary the concentration of the lipase and look at the effect of enzyme concentration on the breakdown of fat in milk.
- Different types of milk could be used e.g. Jersey, full cream, semi-skimmed and skimmed in order to explore the effect on the reaction of changing fat concentration.

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).
- Use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions.
- Use ICT such as computer modelling, or data logger to collect data, or use software to process data.

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

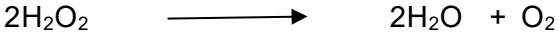
Specification reference: Core concepts 4

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 bi-product of reactions in cells. The reaction of catalase is shown below.



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

Catalase is found in high concentration in raw potatoes.

Apparatus and reagents

Freshly cut potato cylinders
Pestle and mortar
Specimen tubes/ test tubes
Stock solution of hydrogen peroxide 10 vol (labelled stock solution)
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/ testtube.
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 and to float up to the surface again.
5. Remove the disc from the tube using forceps and discard.

Risk assessment

Hazard	Risk	Control measure
Cork borers are sharp	May cut skin when cutting cylinders	The cylinders of tissue must be cut on a white tile with the force directed downwards.
Hydrogen peroxide is an irritant	Hydrogen peroxide may splash onto the skin or into the eyes.	Wear safety glasses

Teacher/ Technician's notes

No details of independent variable have been given in this method and students should be encouraged to plan their own investigation. They should be able to identify all the variables involved as outlined in the guidance notes at the front of the lab book and also an appropriate range for the independent variable. If the variables chosen are not appropriate, then it would be acceptable for the teacher to make some amendments before the plan is carried out.

The hydrogen peroxide solution can be labelled as a stock solution or as a concentration in vol.

If it is impractical for students to make their own enzyme extracts they could be provided with homogenised potato extract. This can be substituted with other sources of catalase, e.g. mung beans.

The filter paper disks can be made using a cork borer or hole punch (5-8mm diameter).

Each student should be supplied with 15 discs of the same size.

Students will need access to safety glasses for this experiment.

Sample results

Concentration hydrogen peroxide. (% stock solution)	**Time taken for disc to rise to surface (seconds)**				
	1	2	3	Mean	Rate 1/time
10	130	134	140	135	0.007
20	60	58	62	60	0.017
40	40	44	42	42	0.024
60	9	10	11	10	0.010
80	8	10	8	9	0.11
100	9	10	8	9	0.11

Further work

- Extension work could include a comparison of catalase activity from a range of sources such as mung bean or liver.
- The technique could be used to study the effect of substrate or enzyme concentration on enzyme activity.
- Students should be encouraged to calculate the rate of reaction (1/time).

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).
- Use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions.

	SPECIFICATION STATEMENT	COMMENT
(a)	the structure of nucleotides (pentose sugar, phosphate, organic base)	Candidates should recognise the differences between DNA, RNA and ATP nucleotides.
(b)	the structure of ATP	
(c)	the importance of chemical energy in biological processes	ATP is formed in an endergonic reaction. The energy required to combine ADP and inorganic phosphate (P_i) to form ATP (and water) comes from exergonic reactions e.g. cell respiration. 30.6kJ mol^{-1} of energy is released when ATP is hydrolysed to ADP and phosphate.
(d)	the central role of ATP as an energy carrier and its use in the liberation of energy for cellular activity	ATP may be called the 'universal energy currency' in organisms because it is a common energy source used in all living organisms. Candidates should be able to explain how the properties, structure and formation of ATP are linked to its role in cells.
(e)	the structure of nucleic acids: DNA bases: purines and pyrimidines; complementary base pair rule; hydrogen bonding and the double helix; antiparallel strands	Candidates should know the structure of the polymers of DNA and RNA. This should include structure, complementary base pairing, hydrogen bonding. They should also recognise similarities and differences between the two molecules. Candidates should be able to differentiate between pyrimidine and purine bases when given structural formulae. In DNA, the two polynucleotide strands are antiparallel ("run" in opposite directions, one from the 5' prime end to the 3' prime end, the other from the 3' prime end to the 5' prime end.) Candidates should know the structure of tRNA and be able to describe differences between mRNA, rRNA and tRNA.
(f)	the similarities and differences in the structure of RNA and DNA	



- Select the image (left) for "Simple extraction of DNA from living material" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(g)	the two major functions of DNA; replication and protein synthesis	In DNA replication, DNA helicase breaks the hydrogen bonds between the bases in the helix, unwinding the DNA, exposing unpaired bases. DNA polymerase then forms bonds between adjacent nucleotides in the new strands of DNA being formed. Candidates should be able to draw a representative diagram of the replication fork (with a small number of nucleotides).
(h)	the semi-conservative replication of DNA including the roles of DNA polymerase and helicase and be able to use evidence from the Meselson and Stahl experiments	
(i)	the term genetic code	The genetic code is a linear, triplet, non-overlapping, degenerate, unambiguous, universal code for the production of polypeptides.
(k)	the triplet code for amino acids	Amino acids are coded for by triplets of bases in the DNA. The DNA is transcribed to produce codons in mRNA and then translated to produce a sequence of amino acids.
(j)	exons as regions of DNA that contain the code for proteins and that between the exons are regions of non-coding DNA called introns	Eukaryotic genes are usually discontinuous genes with coding exons and non-coding introns. Prokaryotic genes are usually continuous genes, lacking non-coding sequences.



- Select the image (left) for "Simple extraction of DNA from living material" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(l)	the transcription of DNA to produce messenger RNA	DNA helicase breaks the hydrogen bonds between the bases in the helix, unwinding the DNA, exposing unpaired bases on the template strand. RNA polymerase links to the template strand of DNA, inserting mRNA nucleotides one at a time, according to the rules of complementary base pairing and forming bonds between them. Beyond the end of the gene there is a stop sequence, where RNA polymerase leaves DNA. The molecule is called pre-mRNA as it contains both exons and introns. Post-transcriptional modification of pre-mRNA then occurs to remove the introns from the molecule to produce functional mRNA.
(m)	the translation of mRNA using ribosomes and the structure and function of transfer RNA, to synthesise proteins	Ribosomes have two attachment sites for tRNA (on the larger sub unit) and one attachment site for mRNA (on the smaller sub unit). Each tRNA molecule carries a specific amino acid. The ribosome binds to the start codon on the mRNA. tRNA molecules bind to the ribosome through codon-anticodon interactions. A peptide bond is formed between the two amino acids. The ribosome moves along the mRNA one codon at a time. This continues until a stop codon is reached.
(n)	the 'one gene - one polypeptide' hypothesis	
(o)	the further modification and combination of some polypeptides	Polypeptides can be further modified by the addition of carbohydrates, lipids or phosphate. Polypeptides can be combined as exemplified by haemoglobin.



- Select the image (left) for "Simple extraction of DNA from living material" practical work

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

Simple extraction of DNA from living material

Specification reference: Core concepts 5

Nucleic acids and their functions

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

1 re-sealable 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 strawberry.
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.

Risk assessment

Hazard	Risk	Control measure
90% ethanol could act as an irritant	Inhalation could cause irritation of nose/ throat/ ethanol could touch skin during experiment	Use in well ventilated area/ wear safety glasses/ gloves when handling ethanol
Acetic orcein contains ethanoic acid which is corrosive	Could be transferred to skin or eyes during experiment	Wear safety glasses when using

Teacher/ Technicians notes

Chill the ethanol by placing in a freezer for at least 2 hours or overnight. Keep on ice throughout the procedure.

Acetic-orcein stain: Grind 1.5 g of solid orcein (described on Hazcard 32 as low hazard) with a pestle and mortar. In a fume cupboard, mix 90 cm^3 of glacial ethanoic acid (Hazcard 38A describes this as flammable and corrosive) with 110 cm^3 of distilled water and bring to the boil. Pour the boiling mixture over the orcein and stir very thoroughly (still in the fume cupboard). Leave overnight, then filter and store in a tightly-stoppered dark bottle.

More information is available from:

http://www.nuffieldfoundation.org/practical-biology/extracting-dna-living-things

Further work

- Comparison of sources of DNA
- Effect of temperature on DNA extraction
- Use of protease with the method

Practical techniques

- Use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions.
- Use qualitative reagents to identify biological molecules.

	SPECIFICATION STATEMENT	COMMENT
(a)	the synthesis of ATP involving a flow of protons through the enzyme ATP synthetase, the process of chemiosmosis and the electrochemical gradient	ATP is produced on the internal membranes of mitochondria and chloroplasts and that synthesis of ATP takes place by means of a flow of protons across these membranes down a concentration gradient through the enzyme ATP synthetase located in the stalked particles. This is called chemiosmosis. In mitochondria the protons flow across the inner membrane from the intermembrane space into the matrix. In chloroplasts the protons flow across the thylakoid membrane from the thylakoid space into the stroma. To maintain the concentration of protons in the inter-membrane space/thylakoid space, proton pumps are needed which are fuelled by energy from electrons. Candidates should be able to draw and interpret diagrams showing the synthesis of ATP. Candidates should be able to give similarities and differences between mitochondria and chloroplasts with reference to their role in chemiosmosis.
(b)	the similarity between mitochondrial and chloroplast membrane function in providing a proton gradient for ATP synthesis	
(c)	the proton gradient; maintained by proton pumps driven by potential energy associated with excited electrons	
(d)	the electron transport chain is formed from an alternate arrangement of pumps and electron carriers (names of proton pumps and electron carriers in the electron transport system are not required)	



Select the image (left) for "Investigation of dehydrogenase activity using artificial hydrogen acceptors, as illustrated by methylene blue, DCPIP or tetrazolium compounds

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

Investigation of dehydrogenase activity in yeast

Specification reference: 1.1

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 (0.1 g/100cm^3)
Yeast suspension (100 g/dm^{-3})
30 ^oC 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^oC.
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.

Risk assessment

Hazard	Risk	Control measure
Redox indicator is harmful	Skin irritation and staining	Avoid skin contact
	Eye irritation if transferred to eye	Use eye protection
Electric shock from waterbath	Electric shock if unplugged with wet hands	Use dry hands to unplug after use

Teacher/ Technician's notes

The concentration of yeast stated is only a guideline, as the activity of yeast can vary greatly. The dried yeast should be mixed to a thin paste with distilled water and then made up to the desired volume. This should be done an hour before the practical starts to allow fermentation to start and then it should be kept in a water bath or incubator at 35°C.

The students should be told to keep the first tube as a reference colour for the end point of the reaction.

This could be used as an alternative to the investigation into factors affecting the rate of respiration in yeast. There is no need for both practicals to be completed as full investigations.

Other indicators can be used as shown below:

Indicator	Concentration / g 100 cm^{-3}	Colour when	
		oxidised	reduced
methylene blue	0.1	dark blue	colourless
DCPIP (dichlorophenol indophenol)	0.1	dark blue	colourless
TTC (triphenyl tetrazolium chloride)	0.5	colourless	red

Sample results

Temperature of water bath (°C)	Time taken for yeast to turn methylene blue colourless (s)			
	Trial 1	Trial 2	Trial 3	Mean
30	641	651	633	642
40	443	441	412	432
50	291	233	306	277
60	136	119	139	131
70	198	208	187	198

Further work

- This could be used as an alternative to the investigation into factors affecting the rate of respiration in yeast. There is no need for both practicals to be completed as full investigations.

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).

- Use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions.

	SPECIFICATION STATEMENT	COMMENT
(a)	the distribution of chloroplasts in relation to light trapping	Including the adaptations of angiosperm leaves and chloroplasts for photosynthesis.
(b)	chloroplasts acting as transducers converting the energy of light photons into the chemical energy of ATP	There are several photosynthetic pigments including chlorophylls *a* and *b*, beta carotene and xanthophylls. The function of these pigments is to absorb light energy and begin its conversion to storable chemical energy. Different pigments absorb photons at different wavelengths of light. Candidates should understand the principles of chromatography as used in the separation of leaf pigments, including the calculation and interpretation of R_f values.
(c)	the process of light harvesting and the absorption of various wavelengths of light by chlorophyll and associated pigments and the energy transfer to reaction centres	Candidates should understand the terms 'absorption spectra' (of chloroplast pigments) and 'action spectra' (of photosynthesis) and describe the relationship of the two. The antenna complex harvests light energy and passes the excitation to the reaction centre (from one pigment molecule to another). At the reaction centre, excited chlorophyll a molecules can each emit one electron.
(d)	the basic features of Photosystems I and II	Photosystem I and Photosystem II each have an antenna complex of all pigment types and a reaction centre of two chlorophyll *a* molecules. They absorb light at different wavelengths.



Select the image (left) for "Investigation into the separation of chloroplast pigments by chromatography" practical work



Select the image (left) for "Investigation into factors affecting the rate of photosynthesis" practical work



Select the image (left) for "Investigation into the role of nitrogen and magnesium in plant growth" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

SPECIFICATION STATEMENT		COMMENT
(e)	cyclic and non-cyclic photophosphorylation as sources of electrons for the electron transport chain	Light dependent reactions occur in the thylakoid membranes and thylakoid cavity and require light energy and water. Light energy is transformed to chemical energy in ATP and NADPH (oxygen as a by-product of photosynthesis). In non-cyclic photophosphorylation (which is also referred to as the Z scheme), • photons of light energy are absorbed by a pigment molecule of the antenna complex of PS II; the excitation is passed to the pair of chlorophyll *a* molecules in the reaction centre; • each has one of their many electrons boosted from their ground state, to an excited state; • the excited electrons pass to electron acceptors, reducing them, leaving the chlorophyll *a* molecules oxidised, they then pass via a series of electron carriers; • electron transfer is linked to proton pumping (via one proton pump) from the stroma into the thylakoid space, raising its proton concentration and lowering the pH; • protons then flow down their gradient, through ATP synthetase, releasing energy to form ATP; • photons of light energy are absorbed by a pigment molecule of the antenna complex of PS I and the energy is passed to the pair of chlorophyll *a* molecules in the reaction centre; • each has one of their many electrons boosted from their ground state, to an excited state; • the excited electrons can each reduce an electron acceptor, which pass an electron to oxidised NADP, reducing it. Each NADP receives two electrons and picks up two hydrogen ions from the stroma to become reduced NADP ($NADPH + H^+$). In cyclic photophosphorylation • excited electrons from PS I can pass from the electron acceptor to the electron carriers and back, via the proton pump, to PS I. Photolysis of water • molecules of water within thylakoid spaces are split into hydrogen ions, electrons, oxygen and reformed water; • the electrons are removed to replace those lost by the chlorophyll *a* of photosystem II; • light is responsible only indirectly for splitting water.
(f)	photolysis as a source of electrons for Photosystem II	
(g)	the reduction of $NADP^+$ by the addition of electrons and hydrogen ions in the stroma maintaining the proton gradient	



Select the image (left) for "Investigation into the separation of chloroplast pigments by chromatography" practical work



Select the image (left) for "Investigation into factors affecting the rate of photosynthesis" practical work



Select the image (left) for "Investigation into the role of nitrogen and magnesium in plant growth" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(h)	reduced NADP as a source of reducing power and ATP as a source of energy for the following reactions: the light- independent stage and the formation of glucose; uptake of carbon dioxide by ribulose bisphosphate to form glycerate-3-phosphate catalysed by Rubisco	The light independent reactions (Calvin cycle) occur in the stroma. They consume CO_2 and energy from ATP and reduced NADP and organic chemicals e.g. carbohydrates are produced. The following steps occur in the light-independent reactions (Calvin Cycle): • uptake of carbon dioxide by 5C ribulose bisphosphate (using the enzyme, Rubisco) to form 2 x 3C glycerate-3-phosphates; • utilisation of ATP and reduced NADP from the light-dependent reactions to reduce glycerate-3-phosphate to the 3C carbohydrate, triose phosphate; • the consequent regeneration of ribulose bisphosphate, via ribulose phosphate, which requires ATP.
(i)	the reduction of glycerate-3-phosphate to produce triose phosphate (carbohydrate) with the regeneration of ribulose bisphosphate	
(j)	the production of other carbohydrates, lipids and amino acids from the triose phosphate (no details of the chemistry of these processes is needed)	These should include that the following substances may be manufactured from triose phosphate (no details of chemistry needed) glucose (actually fructose bisphosphate) • lipids • amino acids {with the addition of nitrogen obtained from nitrates} (no details of chemistry required)



Select the image (left) for "Investigation into the separation of chloroplast pigments by chromatography" practical work



Select the image (left) for "Investigation into factors affecting the rate of photosynthesis" practical work



Select the image (left) for "Investigation into the role of nitrogen and magnesium in plant growth" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > **GCE Biology** > **Specification from 2015**

	SPECIFICATION STATEMENT	COMMENT
(k)	the concept of limiting factors in relation to photosynthesis	The law of limiting factors states that the rate of a physiological process will be limited by the factor which is in shortest supply. Any change in the level of a limiting factor will affect the rate of reaction.
(l)	the role of inorganic nutrients in plant metabolism as illustrated by the use of nitrogen and magnesium	This should include the role of nitrogen and magnesium in plants and that they may become limiting factors to metabolism, if in short supply. Roles should include: • Nitrogen - synthesis of proteins, nucleic acids and chlorophylls. • Magnesium - chlorophyll



Select the image (left) for "Investigation into the separation of chloroplast pigments by chromatography" practical work



Select the image (left) for "Investigation into factors affecting the rate of photosynthesis" practical work



Select the image (left) for "Investigation into the role of nitrogen and magnesium in plant growth" practical work

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

Investigation into the separation of chloroplast pigments by chromatography

Specification reference: 1.2

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	Chromatography paper or silica gel chromatography plates
Scissors	Pencil
Sand	Ruler
Pestle	Capillary tube
Mortar	Hair drier
Propanone	2 x Boiling tube
Distilled water	Petroleum ether
2 x Stopper	Vial
Pipette	

Method

Preparing the pigment solution

1. Chop 2g of the leaf material finely with scissors and place in the mortar.
2. Add a pinch of sand and 5 cm^3 of 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 3cm^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

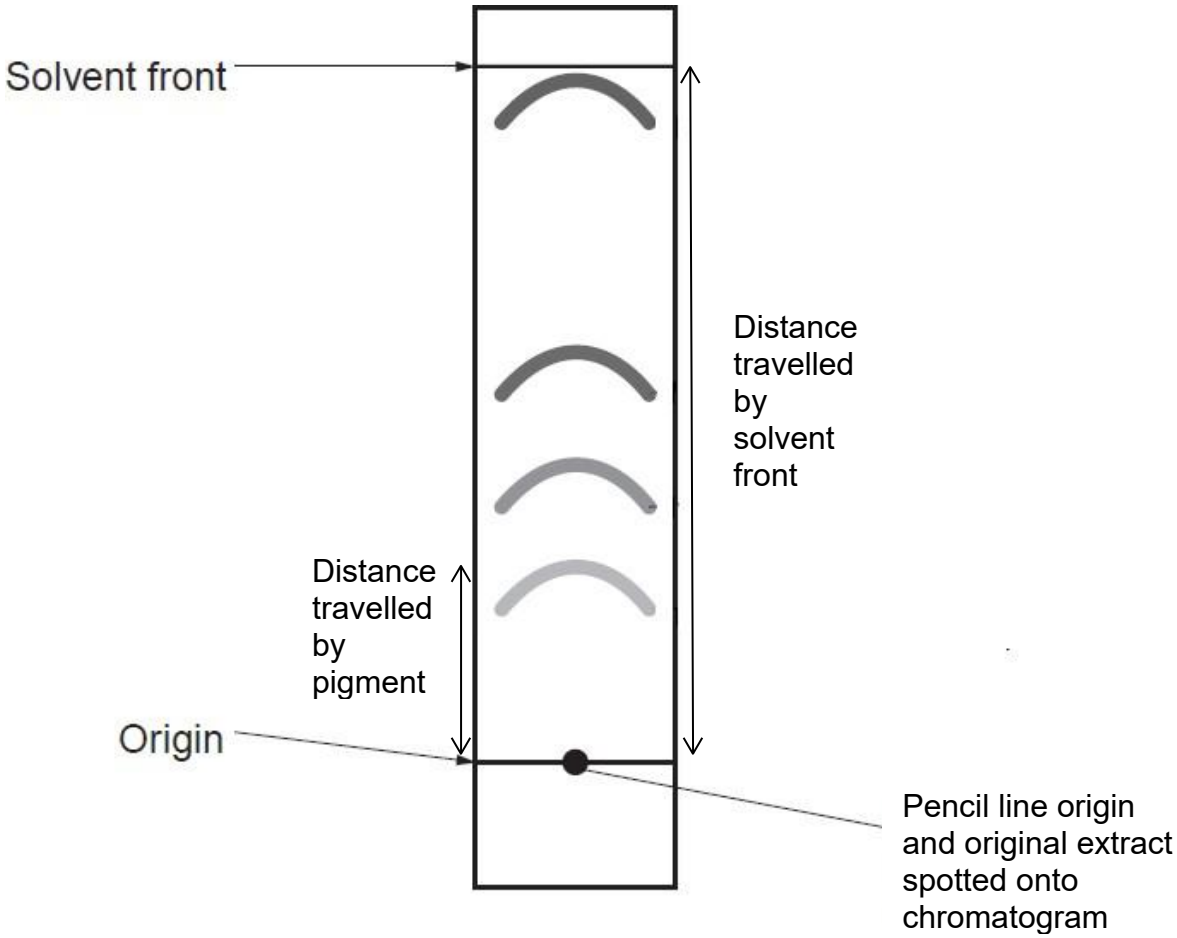
1. Place freshly-made 1:2 propanone : petroleum ether solvent mixture in a boiling tube until it is approximately 5 mm deep.
2. Without touching the sides of the boiling tube, slide the chromatography paper into the boiling tube so that its end is below the surface of the ethanol but the spot is above, 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 $R_f = \frac{\text{distance travelled by pigment}}{\text{distance travelled by solvent front}}$

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)



Risk assessment

Hazard		Risk	Control measure
Propanone Petroleum ether	May cause eye damage	Macerating leaf material; Pouring solvent for chromatography	Eye protection
	May degrease the skin	Macerating leaf material; Pouring solvent for chromatography	Wear gloves
	Inhalation may exacerbate respiratory problems, including asthma	Macerating leaf material; Pouring solvent for chromatography	Work in fume cupboard
	Fire hazards	Accidental ignition	Work in fume cupboard

Teacher / Technician's notes

This method uses chromatography paper. The method in the link below gives simple instructions for the use of TLC. Either is acceptable.

http://www.saps.org.uk/secondary/teaching-resources/189-investigation-of-photosynthetic-pigments-in-green-plants

Further Work

- Use different solvents or solvent proportions.
- Compare pigments in young and senescent deciduous leaves e.g. *Acer* (sycamore, maple).
- Separate pigments in coloured leaves e.g. *Coleus.*
- Compare the pigments in sun and shade leaves of e.g. *Quercus robur* (English oak), *Urtica dioica* (stinging nettle).

Practical techniques

- Separate biological compounds using thin layer/ paper chromatography or electrophoresis.

Investigation into the effect of light on the rate of photosynthesis

Specification reference: 1.2

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:

acid (yellow) → neutral (red) → alkali (purple)

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 photosynthesis

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 and swirl them in distilled water.
7. Repeat steps 5 and 6 twice more.
8. Use immediately or store at 4 °C, 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.

Risk assessment

Hazard	Risk	Control measure
Solid calcium chloride is an irritant to skin and eyes and if inhaled	Making calcium chloride solution	Solid to be weighed in fume cupboard; Students to be given solution.
Gas accumulation in culture vessel could cause the glass to break	During period of algal culture	Ensure cotton wool stopper allows ventilation; Stand culture vessel in deep tray
Excess heat from lamp may cause burns	When decanting from culture vessel	Ensure no contact with skin

Teacher/ Technician's notes

Growing your alga: Prepare a culture of green alga such as unicellular *Scenedesmus quadricauda*. Make up a solution of algal enrichment medium, and subculture the alga into this. Aerate gently and keep at temperatures between 18–22 °C. Constant illumination ensures faster growth of the alga. After 3–4 weeks, the culture should have a green 'pea soup' colour. Subculture the alga again to maintain a healthy culture. You could use other algae, but *Scenedesmus* should produce 2 to 3 litres of dark green 'soup' in about 4 weeks from 50 cm^3 of original culture. (Details from SAPS Sheet 23).

Preparing solutions to make alginate beads (Refer to Recipe card 2):

- Dissolve 3 g of sodium alginate in 100 cm^3 of cold, pure water. Stir with a spatula every half hour or so. Leave overnight and stir in the morning.

- Dissolve 4 g of calcium chloride-6-water in 200 cm^3 of pure water in a 250 cm^3 beaker.

Hydrogencarbonate indicator: Refer to Recipe card 34 and Hazcard 32. Low hazard once made; must be made fresh by qualified staff using fume cupboard. The indicator is very sensitive to changes in pH, so rinse all apparatus with the indicator before use. Avoid exhaling over open containers of the indicator. Make up a 'standard colour scale' of reaction bottles containing buffers from pH 7.6 - pH 9.2 with hydrogencarbonate indicator if students will not have access to a colorimeter.

Lamps: You need a brighter light than a standard 40 W or 60 W bench light. Low energy bulbs produce too limited a spectrum of light for full activity. 150 W tungsten or halogen lamps are best. 150 W portable halogen lamps have a stand and handle separate from the body of the lamp which makes them safer to handle. But they do produce heat, so you will need a heat filter for the investigation.

Heat filter: Use a large flat-sided glass vase or a medical 'flat' filled with water. With a high power lamp, the small volume in a medical 'flat' may get too hot for comfort.

Making alginate beads:

- When making up the alginate or diluting the algal culture it is essential to use pure water; otherwise calcium ions in the water will cause the alginate to 'set' prematurely.

Students can run all distances from the lamp at the same time if they 'fan' the vials out in front of the light source.

It takes at least an hour for colour changes to happen – so students will need to return to the lab at break or after lessons to 'read' the results.

More details are available on the link below:

http://www.nuffieldfoundation.org/practical-biology/investigating-photosynthesis-using-immobilised-algae

Sample results

Distance of vial from lamp / cm	Colour of indicator after 1 hour	Absorbance after 1 hour / a.u.	pH after 1 hour
10	purple	0.92	9.0
30	magenta	0.81	8.8
50	red	0.56	8.4
70	orange	0.39	8.2
90	yellow	0.25	8.0

Plot the pH or the absorbance against the distance from the lamp source.
As the light intensity falls in proportion to $\frac{1}{d^2}$, the pH or absorbance could be plotted against $\frac{1}{d^2}$

Further work

- More than one vial at each distance should be used so that a mean can be calculated from replicate readings.
- Instead of placing vials at different distances, light intensity may be varied by covering the vials with neutral density filters and maintaining the same distance from the lamp. In this way, any potential heating effect has a consistent effect.
- Vials may be covered with coloured filters to expose the algae to different wavelengths. In order that the algae receive the same light intensity, readings must be taken with a light meter with the filters over the probe, to find distance for each wavelength which has the same light intensity, where the vials should be placed.

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).
- Use appropriate instrumentation to record quantitative measurements, such as a colorimeter or photometer.

Investigation into the role of nitrogen and magnesium in plant growth

Specification reference: 1.2

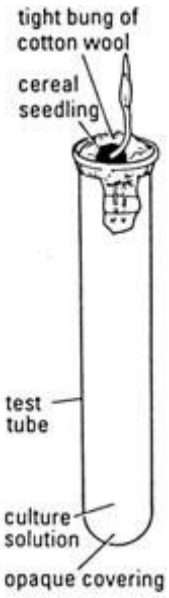
Photosynthesis uses light energy to synthesise organic molecules

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.

Risk Assessment

Hazard	Risk	Control measure
Sach's culture solution can be an irritant	Very low risk of irritation to eye or skin.	Wear goggles. Wash skin thoroughly if solution comes into contact with skin.

Teacher/ Technician's notes

It is advised that this is set up as a class demonstration.

Solid media for Sach's water culture solutions are available from laboratory suppliers. It can be cheaper, and certainly easier to buy the ready-prepared nutrient solutions.

Sach's culture solution

Dissolve the following in 1 dm^3 of distilled water.
0.25 g calcium sulphate(VI)-2-water
0.25 g calcium phosphate(V)-2-water $CaH_4(PO_4)_2.2H_2O$
0.25 g magnesium sulphate(VI)-7-water
0.8 g sodium chloride
0.7 g potassium nitrate
0.005 g iron(III)chloride-6-water.
For Sach's culture solution with nitrogen deficiency replace potassium nitrate(V) with 0.52 g potassium nitrate.
For Sach's culture solution with magnesium deficiency replace magnesium sulphate (VI) with 0.17 g potassium sulphate(VI)

The barley seeds should be germinated about a week before use in a layer of damp vermiculite in a margarine tub.

There are a variety of alternative seedlings which can be used such as tomato. More information is available on the link below

http://www.nuffieldfoundation.org/practical-biology/investigating-effect-minerals-plant-growth

Sample results

Lacking in nitrogen - poor growth, plants short and spindly and chlorosis (yellowing) especially in the older leaves. The young leaves at the tip may be green but small.

Lacking in magnesium - interveinal chlorosis.

Practical techniques

- Safely and ethically use organisms to measure
- Plant or animal responses
- Physiological functions
- Use ICT such as computer modelling, or data logger to collect data, or use software to process data.

	SPECIFICATION STATEMENT	COMMENT
(a)	the need for all living organisms to carry out respiration in order to provide energy in the cell	Respiration is a catabolic process involving a series of enzyme-catalysed reactions in cells, where energy-rich respiratory substrates, e.g. glucose and fatty acids, are broken down to release energy: some is trapped as chemical energy in ATP and some is released as heat energy. During respiration, high energy C-C, C-H and C-OH bonds are broken, lower energy bonds are formed and the difference is released and used to attach iP to ADP to make ATP. Candidates should be able to define the terms aerobic and anaerobic respiration.



Select the image (left) for "Investigation into factors affecting the rate of respiration in yeast" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

SPECIFICATION STATEMENT		COMMENT
(b)	glycolysis as a source of triose phosphate, pyruvate, ATP and reduced NAD and resulting in the formation of acetyl Coenzyme A (the names of intermediates are not required)	The breakdown of a glucose molecule to carbon dioxide and water in aerobic respiration involves: glycolysis; the link reaction; the Krebs cycle; and electron transport chain. **Glycolysis** occurs in the cytosol of cells: • phosphorylation of glucose, the splitting of the 6C hexose phosphate formed into two 3C triose phosphate molecules and the oxidation of each of these to 3C pyruvate with a small yield of ATP and reduced NAD • Glucose + 2 NAD (oxidised) + 2ADP + 2P_i → 2 pyruvates + 2 NAD (reduced) + 2ATP + heat energy The **link reaction** occurs in the mitochondrial matrix: • oxidative decarboxylation of pyruvate to form acetyl coenzyme A • pyruvate + CoA + NAD → acetyl CoA, + CO_2 + reduced NAD **Krebs cycle** (Citric acid cycle) occurs in the mitochondrial matrix: • the acetate from acetyl coenzyme A combines with a 4C compound to form a 6C compound • a series of enzyme-controlled reactions then occurs, including two decarboxylation reactions (removal of carbon from a carboxyl group as CO_2 from intermediates) and four dehydrogenation reactions (removal of pairs of hydrogen atoms from intermediates) • the acetate fragment which entered the Krebs cycle from the glucose molecule is completely broken down to CO_2 and water and the 4C is regenerated via 6C and 5C intermediates. Note that H_2O is fed in at three reactions. • the function of the Krebs cycle is a means of liberating energy from carbon bonds via reduced intermediates to provide ATP and reduced NAD (and reduced FAD), with the release of carbon dioxide. **Electron transport chain** (ETC) occurs in the inner mitochondrial membrane: • reduced NAD and reduced FAD deliver their pairs of hydrogen atoms to the electron transport system chain in the inner mitochondrial membrane. The complete oxidation of one glucose molecule yields 10 reduced NAD and 2 reduced FAD (all finally in the mitochondrial matrix). • reduced NAD and reduced FAD are oxidised in the ETC, with a high yield of ATP, i.e. (traditionally) 3ATPs per oxidised NAD, and 2ATPs per oxidised FAD • role of oxygen as the final electron acceptor of the electron transport chain and the formation of water. • **Anaerobic respiration** • Without oxygen the reduced NAD (and reduced FAD) cannot be reoxidised and therefore made available to pick up more hydrogen; • the link reaction and the Krebs cycle cannot take place; • glycolysis can take place as the reduced NAD formed in glycolysis transfers the hydrogen to pyruvate to form lactic acid in animals and ethanol and carbon dioxide in plants; • there is only a very small yield of ATP (2)
(c)	the Krebs cycle as a means of liberating energy from carbon-carbon bonds to produce ATP and reduced NAD with release of carbon dioxide	
(d)	the role of reduced NAD and FAD as a sources of electrons and protons for the electron transport system	



Select the image (left) for "Investigation into factors affecting the rate of respiration in yeast" practical work

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	SPECIFICATION STATEMENT	COMMENT
(e)	the energy budget of the breakdown of glucose under aerobic and anaerobic conditions	**Aerobic**: 38 ATPs per glucose molecule formed as follows: • net 2 ATPs in glycolysis (substrate-level phosphorylation) • 2 ATPs in the Krebs cycle (substrate-level phosphorylation) • 34 ATPs from oxidative phosphorylation (using chemiosmosis) from 10 reduced NAD (2 from glycolysis, two from the link reaction, six from the Krebs cycle) and 2 reduced FAD (Krebs cycle). However, this maximum yield is never quite reached due to losses (leaky membranes) as well as the cost of moving pyruvate and ADP into the mitochondrial matrix; current estimates range at around 30 to 32 ATP per glucose. **Anaerobic** (mammalian muscle given): • net 2 ATPs in glycolysis (substrate-level phosphorylation) the 2 reduced NAD formed in glycolysis are oxidised when 2 pyruvates are reduced to a lactate. This is because pyruvate is not transferred to the mitochondrion and finally oxidized to carbon dioxide, but is reduced to ethanol or lactic acid in the cytoplasm.
(f)	how lipids and amino acids are used in respiration	Acetyl CoA is an important molecule, which links glucose, fatty acid and amino acid metabolism. • glycerol is converted to a 3-carbon sugar (triose phosphate), an intermediate of glycolysis; • long chain fatty acid chains molecules are split into 2C acetate fragments and are fed into the Krebs cycle as acetyl co-enzyme A; • proteins are hydrolysed into their constituent amino acids, which are deaminated in the liver, forming a keto acid and ammonia; • Some keto acids are fed into glycolysis (e.g. pyruvate) and some others are fed into the Krebs cycle.



Select the image (left) for "Investigation into factors affecting the rate of respiration in yeast" practical work

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Investigation into factors affecting respiration in yeast

Specification reference: 1.3

Respiration releases chemical energy in biological processes

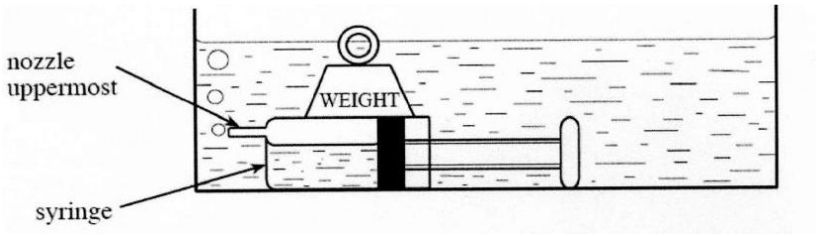
Introduction

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 as necessary to maintain the temperature to $\pm$ 1^{o}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.

Risk assessment

Hazard	Risk	Control measure
Water-bath can cause electric shock	Electric shock if unplugged with wet hands	Use dry hands to unplug after use
Yeast is a potential allergen	Immune response if contact with skin or eyes or if dried yeast inhaled	Manipulate dried yeast in fume cupboard; Students to use suspension only; Keep skin covered; Use eye protection

Teacher/ Technician's notes

Yeast suspension should be made up with dried yeast and kept at 35°C prior to use. Yeast bought from different suppliers will have a huge range of activity and so the yeast should be trialled to ensure activity is at a level that will provide results. If too many bubbles appear, so that counting is difficult please dilute the yeast. It will not damage the students results if a small amount of glucose is added to the stock solution while incubating it.

Please instruct students that if the nozzle of the syringe becomes blocked, they need to start that replicate again.

Sample Results

Temperature / °C	Number of bubbles per minute			
	Trial 1	Trial 2	Trial 3	Mean
35	14	13	12	13
45	8	16	14	16
55	22	22	16	20
65	22	17	15	18
75	16	16	11	14

Graph

The mean number of bubbles is proportional to the rate of reaction. This may be plotted against temperature.

On the part of this curve at temperatures lower than the optimum, draw a line of best fit.
Use the line of best fit to calculate Q_{10} using the equation $Q_{10} = \frac{\text{rate at } (r+10)^oC}{\text{rate at t } ^oC}$. For a normal chemical reaction, $Q_{10} \approx 2$. The closer the experimental value is to 2, the more accurate the readings have been.

Further work

- The effect of substrate concentration may be tested by using different concentrations of sucrose e.g. 0, 0.2, 0.4, 0.8 mol dm^{-3}.

- The effect of pH may be assessed by making all solutions in buffers of different pH values e.g. pH 1, 3,5,7,9 and 11.

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH).

- Use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions.

	SPECIFICATION STATEMENT	COMMENT
(a)	the classification of bacteria according to their shape and by their reaction to the Gram stain as determined by their cell wall structure, including the preparation and examination of bacteria stained using Gram technique	Bacteria can be distinguished from each other by their size, shape, staining characteristics, and their metabolic, antigenic and genetic features. The shape of bacteria is due to their rigid cell wall which has a unique structure: it contains a 3D mesh of peptidoglycan (murein). Gram positive bacteria have cell walls with a thicker layer of peptidoglycan/murein (than Gram-negative bacteria), which retains the crystal violet/iodine complex within their cells when washed with alcohol - staining purple. On treatment with alcohol, the Gram negative cell walls lose their outer lipopolysaccharide membrane, and the thin inner peptidoglycan layer is left exposed, this means that the crystal violet/iodine complexes are washed from the gram-negative cell along with the outer membrane - they stain red with the counterstain safranin.



Select the image (left) for "Investigation into the numbers of bacteria in milk" practical work

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	SPECIFICATION STATEMENT	COMMENT
(b)	the methods by which microorganisms can be cultured in the laboratory	Microorganisms may be grown in the laboratory if supplied with suitable physical conditions, nutrients and water. Different species vary in their requirements and usually grow over a range of temperatures and pH values, with an optimum within the range. Some species are obligate aerobes, requiring oxygen for metabolism, whilst others are obligate anaerobes and can only survive in the absence of oxygen. Many species are facultative anaerobes, i.e. they can respire anaerobically if they need to (if there is little or no oxygen available). Nutrients are supplied in nutrient media and include: carbon compounds, usually organic compounds such as glucose; nitrogen, organic or inorganic; growth factors such as vitamins and mineral salts. Aseptic technique (also called sterile technique) is to prevent: • contamination of the environment by the microbes being handled • contamination of microbial cultures by unwanted microbes from the environment Equipment and media must be sterilised before use by appropriate methods including: • heat - examples being the use of an autoclave at a suitable temperature (121°C) for 15 minutes or heating an inoculating loop in a Bunsen flame • Irradiation - heat labile (stable) plastics
(c)	the conditions necessary for bacterial growth and the principles of aseptic technique	



Select the image (left) for "Investigation into the numbers of bacteria in milk" practical work

General guidance

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	SPECIFICATION STATEMENT	COMMENT
(d)	the methods used to monitor population growth in microorganisms including viable count, using serial dilutions, plating and counting colonies	Candidates should understand: • the differences between total cell count and viable counts (no knowledge of haemocytometers is required) • that for a viable count, a known volume of organisms is added to agar plates, incubated and the colonies counted. It is assumed that one cell gives rise to one colony. This makes no allowance for clumping of cells in the initial inoculum so may lead to an underestimate of the numbers of the original plated cells. In both cases (total viable count and total cell count) the original culture usually requires dilution by e.g. ten-fold steps, serial dilution, in order to provide a final number within a countable range.



Select the image (left) for "Investigation into the numbers of bacteria in milk" practical work

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Investigation into the numbers of bacteria in milk

Specification reference: 1.4

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 10 000 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 microflora, 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

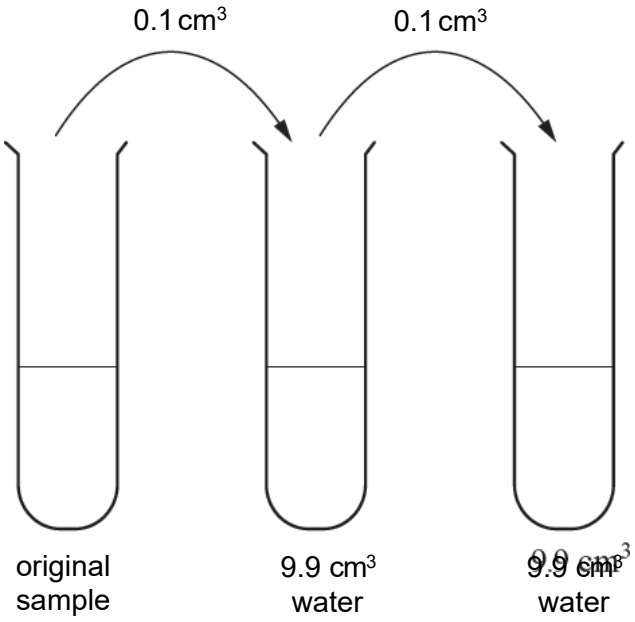
Plating and growing samples
9 cm Petri dishes
Molten MRS agar, maintained at approximately 50°C
Sticky tape
25°C incubator

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.

Risk assessment

Hazard	Risk	Control measure
Pathogenic bacteria	Could be incorporated into culture	Maintain sterile technique throughout the preparation of dilutions and plates; Culture plates at 25°C
	Risk of infection when handling plates after the culture period	Do not re-open plates once taped; Autoclave plates before disposal

Teacher/ Technician's notes

Instead of making 1 in 10 dilutions, to save in time and resources, 1 in 100 dilutions are made instead, mixing 0.1 cm^3 into 9.9 cm^3 water for each dilution.

Dilution plating produces a live cell count, assuming that all species present are equally able to grow under the culture conditions specified. MRS agar is recommended for *Lactobacillus spp* as the sodium acetate it contains suppresses the growth of other species. Thus, *Bifido sp* may grow less well. It is available form standard suppliers.

The incubation temperature of 25°C ensures that no bacteria that are pathogenic to humans will be cultured.

The growth of *Lactobacillus spp* is inhibited by oxygen so pour plates are suitable for culturing them.

Counting colonies

Each viable cell in the plated sample reproduces to make a colony. A plate that is suitable for counting has between 20 and 100 discrete, non-overlapping colonies.

- Colonies can be counted on the plate, each being marked with a board-pen when counted, to prevent counting any colony twice.
- Counting may be easier on a print of a photograph. Colonies can be marked, as above, during counting.
- Imaging software is available for electronic counting.

Sample results

The dilution 10^{-6} gives between 20 and 100 discrete, non-overlapping colonies, so these are suitable for counting.

	Number of colonies per plate	
	With distant use-by date	At use-by date
Mean number per plate	46	96
	68	65
	34	76
	78	44
	56	90
	56.4	74.2
Dilution at which colonies were counted	10^{-6}	10^{-10}
Bacterial concentration	$56.4 \times 10^6 = 5.64 \times 10^7$ bacteria per cm^3 fermented milk.	$74.2 \times 10^{10} = 7.42 \times 10^{11}$ bacteria per cm^3 fermented milk.

Note: the manufacturers state that Yakult has 10^9 bacteria per cm^3. The counts shown here may represent the *Lactobacillus spp*, which grow successfully in MRS agar, but not *Bifido sp,* which grows poorly on it.

Further work

- Compare the growth of fermented milks from different sources.
- Compare the growth of bacteria from one type of fermented milk on different media.
- Investigate the effects of different antibiotics on the growth of bacteria by incorporating e.g. penicillin or streptomycin into the culture medium. A dilution of 10 mg dm^{-3} is suitable.

Practical techniques

- Use microbiological aseptic techniques, including the use of agar plates and broth

	SPECIFICATION STATEMENT	COMMENT
(a)	populations and the way in which they grow - a simple quantitative treatment including immigration, emigration, birth and death rates	Candidates should be able to define the following: ecology, ecosystem, community, habitat, population, environment (biotic and abiotic) and niche. Ecosystems are dynamic (energy flow, biological cycles, succession, species composition and population sizes) and are subject to change. Population numbers will fluctuate and this is dependent on various factors, including birth and death rates, immigration and emigration. Populations will increase in size when births + immigrants are greater than deaths + emigrants. Appropriate terminology should be used depending on the species in question, e.g. births should not be used in relation to yeast.
(b)	graphs showing population growth and factors affecting population growth; competition; carrying capacity	Candidates should • understand that some factors will slow down population growth rate and some will cause a population crash • be able to draw and explain a generalised graph of population growth of an equilibrium species after its initial and successful colonisation of a new habitat, with lag, log/exponential and stationary phases • be able to interpret graphs of changes in population growth rate and know how to calculate population changes when populations are plotted on a log scale • be able to explain how carrying capacity is dependent on the availability of resources and the effect of other species, including how populations might then fluctuate about this set point
(c)	the regulation of populations by density dependent and density independent factors	



Select the image (left) for "Investigation into the abundance and distribution of organisms in a habitat" practical work

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General guidance

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	SPECIFICATION STATEMENT	COMMENT
(d)	the sampling techniques used to assess abundance and distribution of organisms in a habitat	Please see notes in link to practical work below.
(e)	the concept of ecosystems, including that ecosystems range in size from very large to very small	An understanding that an ecosystem is a characteristic community of interdependent species and their habitat.
(f)	the sun is the source of energy for an ecosystem	Light energy, trapped by photosynthesis is the source of energy for most ecosystems (other than those based on chemosynthesis, e.g. hydrothermal vents).
(g)	the concepts of habitat and community	
(h)	the transfer of biomass from plants to animals including trophic levels, efficiency of transfer; gross and net production and pyramids of biomass	Candidates should be able calculate, from appropriate information, the efficiency of energy transfer from one trophic level to the next. Gross primary productivity (GPP) is the rate is the rate of production of chemical energy in organic chemicals by photosynthesis (in kJ m^{-2} $year^{-1}$). Net Primary Production is gross primary production minus the chemical energy generated in respiration and used up by the producers metabolism in the year (NPP = GPP - R). On average GPP is 0.2% of incident global sunlight energy and NPP is 0.1%. NPP represents the potential food/chemical energy available to heterotrophs in ecosystems.
(i)	the principles of succession as illustrated by the colonisation bare rock to form woodland	Succession is the change in structure and species composition of a community over time. The different stages in a succession when particular communities dominate are known as seral stages.



Select the image (left) for "Investigation into the abundance and distribution of organisms in a habitat" practical work

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	SPECIFICATION STATEMENT	COMMENT
(j)	the terms primary and secondary succession, pioneers, sere and climax community	Candidates should understand: • the differences between primary succession and secondary succession • that succession usually involves changes in community structure and progression to a climax community • the roles of immigration, facilitation and interspecific competition in succession • that as succession occurs, species diversity increases, as does the stability of the community.
(k)	the importance of organic breakdown in recycling nutrients	Detritivores and saprotrophic microorganisms play an important role in the process of decay.
(l)	the carbon cycle	Candidates should be able to draw a labelled diagram of the carbon cycle, linking the processes of photosynthesis; respiration in plants, animals and saprotrophs, decomposition, partial decay and the formation of fossil fuels, and potential combustion of organisms and fossil fuels.
(m)	the effects of human activities on the carbon cycle including that climate change affects the distribution of species and is a possible cause of extinction	Candidates should understand • the effects of human activities (deforestation and combustion) on the carbon cycle • why increased atmospheric carbon dioxide leads to an enhanced greenhouse effect (global warming) • that global warming and climate change may affect distribution of species and are a possible cause of extinction • the possible need for changes in farming practices in particular regions • the carbon footprint as the total amount of carbon dioxide attributable to the actions of an individual or a product or service over a period of one year.



Select the image (left) for "Investigation into the abundance and distribution of organisms in a habitat" practical work

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General guidance

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	SPECIFICATION STATEMENT	COMMENT
(n)	the role of bacteria in the nitrogen cycle and the significance of nitrates in producing proteins and nucleic acids	This should include that : • nitrogen is found in all amino acids from which proteins are made; the nitrogenous bases and chlorophyll; • nitrogen is taken up by plant roots only as ammonium and nitrate ions (active transport and facilitated diffusion); • the uptake of these ions is linked with protein synthesis for the synthesis of nucleic acids and chlorophylls; • the nitrogen cycle is the flow of organic and inorganic nitrogen within the abiotic and biotic components of an ecosystem where there is an interchange between certain nitrogenous compounds and atmospheric nitrogen; • the main processes of the nitrogen cycle are assimilation of inorganic nitrogen compounds into plants, decay, involving ammonification, nitrification, nitrogen fixation and denitrification; • the activities of nitrifying, denitrifying, nitrogen-fixing bacteria and decomposers (saprotrophs and detritivores), including generic names *Nitrosomonas, Nitrobacter, Azotobacter* and *Rhizobium.*
(o)	the importance of human activities such as ploughing and drainage in producing the aerobic conditions needed for nitrification and the economic importance of the nitrogen cycle in relation to food production and fertiliser application	These should include: • ploughing and drainage in aeration of the soil; • application of artificial fertilisers (ammonium nitrate) and manure/slurry. • cultivation of legumes



Select the image (left) for "Investigation into the abundance and distribution of organisms in a habitat" practical work

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	SPECIFICATION STATEMENT	COMMENT
(p)	the process of eutrophication and algal blooms and that drainage has adverse effects on habitats	Candidates should understand: • the increased use of nitrogen containing fertilisers has had some harmful effects on both aquatic and terrestrial ecosystems • that on agricultural land there has been a reduction in species diversity in grassland • that nitrate leached into rivers has caused eutrophication and algal blooms and be able to describe this process • that digging drainage ditches has had a detrimental effect on habitats resulting in reduced biodiversity.



Select the image (left) for "Investigation into the abundance and distribution of organisms in a habitat" practical work

General guidance

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EDUQAS > GCE Biology > Specification from 2015

Investigation into the abundance and distribution of plants in a habitat

Specification reference: 1.5

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

0.5 m x 0.5m open frame or gridded quadrat
2 x 10 m tape measures
20 m tape measure
plant identification keys

The nature of this investigation and the method chosen depend upon the habitats available for study. The methods described here assess both abundance and distribution.

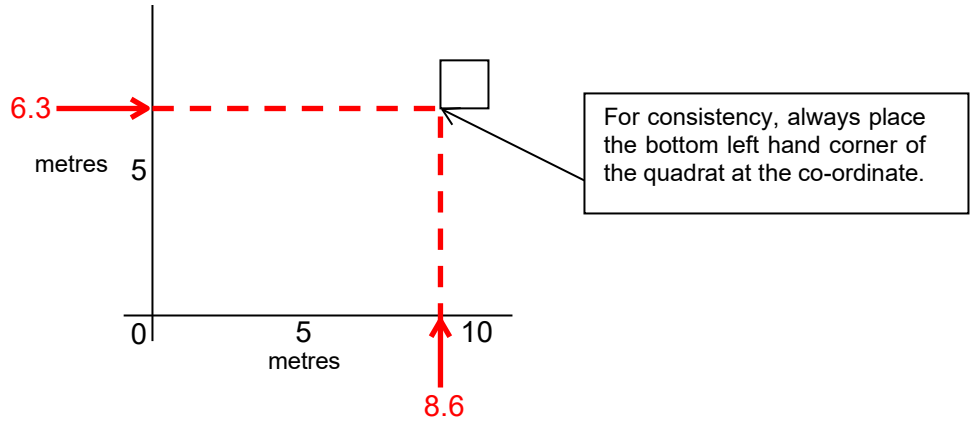
Method

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 or a “gridded quadrat”, a frame the same size, which is divided into a grid of 10 x 10 squares. The plants in the frame are identified and the number of each species is counted or the area each covers is estimated.

Setting up coordinates for sampling

In a uniform grassland, set up at pair of 10 m long axes and use random numbers e.g. use random number 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 8.1m along the axes intersected:



Take readings at 10 pairs of random co-ordinates and calculate a mean for each species.

Risk assessment

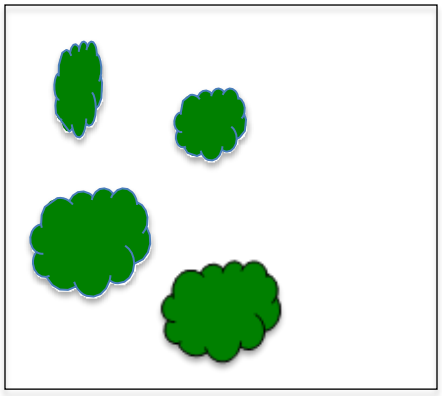
Hazard	Risk	Control measure
Some plants have thorns, sting or are poisonous	Adverse skin response	Cover skin at all times
Biting and stinging insects	Adverse skin response	Cover skin at all times; Use insect repellent
Tripping	Strains and sprains	Care where walking
Weather may be too hot, too cold, too bright	Hypothermia, overheating, sunburn	Wear appropriate clothing; Use sunglasses; Use sun cream

Teacher/ technician's notes

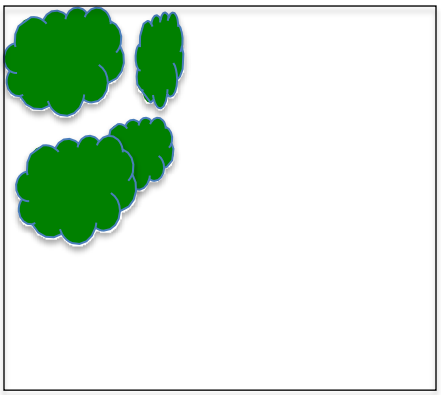
Methods for taking readings depend on the species and its growth habit:

(a) Per cent area cover

If it is difficult to count individual plants, such as grass or moss or ground ivy, estimating the percentage area cover is useful. If the quadrat has patches of your species, imagine the clumps pushed together. Here they occupy about 15 % of the area:

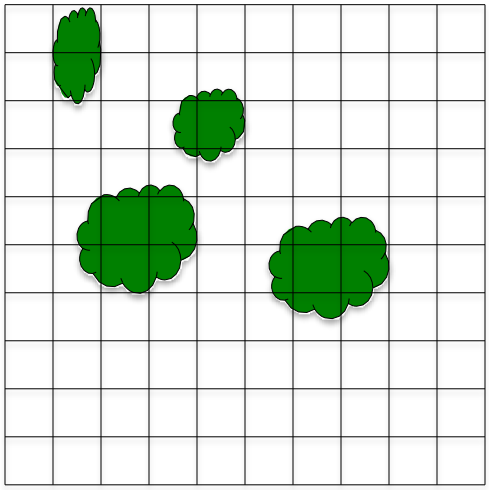


Patches of plant



Patches pushed together

Estimation is more accurate with a gridded quadrat. The quadrat is the same total area but is divided into a grid of 10 x 10 squares, so that each square represents 1% of the area. The diagram below shows the same area of grassland, but this time, with a 10 x 10 gridded quadrat:

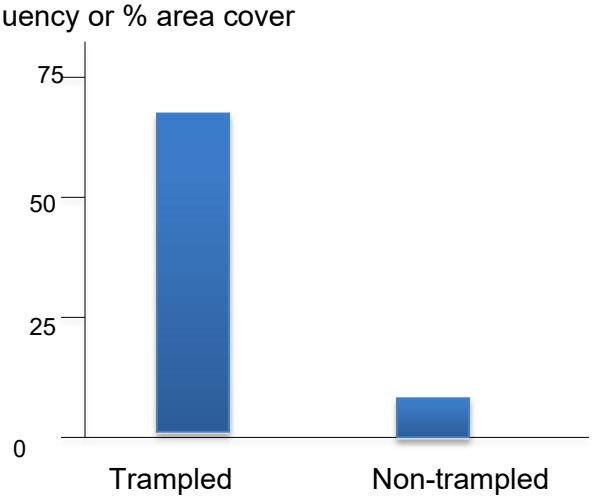


Count each grid square that is covered with the plant with a rule about partly covered squares e.g. the square is only counted if it is more than half covered. Here the area cover is 9%.

(b) % frequency

This is less accurate than assessing percentage cover. Count how many squares the plant appears in. As there are 100 squares, you express it as a percentage. Here 25 squares have some of the plant in so the per cent frequency = 25%.

Data for per cent frequency and per cent area cover may be displayed as bar graphs e.g. of comparing density of moss in a trampled grassland and an non-trampled grassland:



(c) Measuring plant density i.e. number per m^2

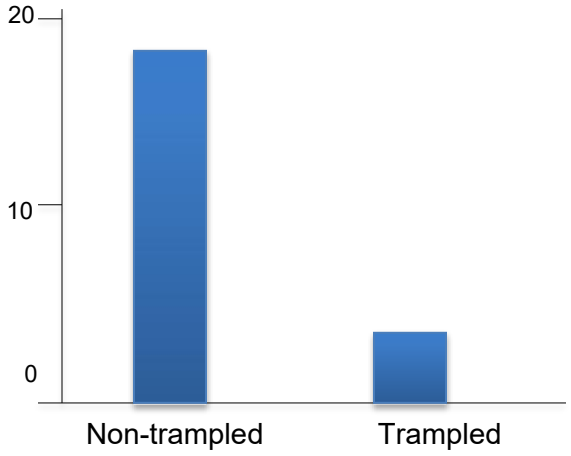
It is easy to count individuals of some species e.g. plantain. If you count the number of individuals in 10 quadrats and calculate the mean, you have the mean number in 0.25 m^2. Convert this to the number per m^2 by multiplying by 4. This is the density:

Quadrat number	Number of plantain plants
1	6
2	8
3	8
4	2
5	0
6	0
7	7
8	4
9	5
10	6
Mean per 0.25 m^2 quadrat	46 ÷ 10 = 4.6
Mean per m^2 = density	4.6 x 4 = 18.4

A density does not have to a whole number, unlike a population count, because it represents the average number over the whole area.

Data may be displayed as bar graphs e.g. of comparing density of plantain in a trampled grassland and a non-trampled grassland:

Density of plantain/ number per m^2

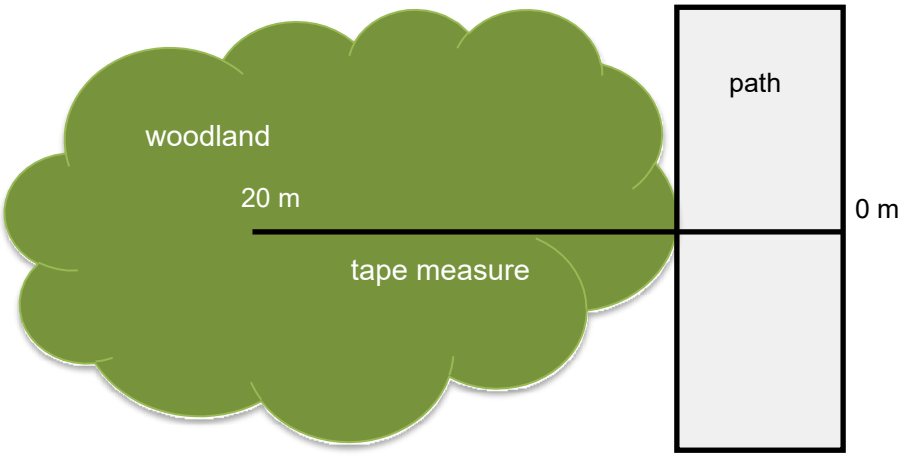


2. In an area where there is a change in an environmental factor

Random quadrats are unsuitable if there is an environmental gradient i.e. a distinct change, such as altitude (e.g. on the side of a hill) or light intensity (e.g. under the shade of a tree or the edge of a woodland). A line along the gradient is a 'transect':

(a) Line transect

To describe the difference in plants as you enter a wood, run a 20 m tape measure into the wood and identify the plant every two metres.



Here is an example of how your results might look with a 20 m transect and a sampling point every 2 m:

Distance along transect (m)	Plant
0	Meadow grass
2	Meadow grass
4	Meadow grass
6	Moss
8	Meadow grass
10	Moss
12	Bracken
14	Bracken
16	Moss
18	Moss
20	Moss

(b) 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.

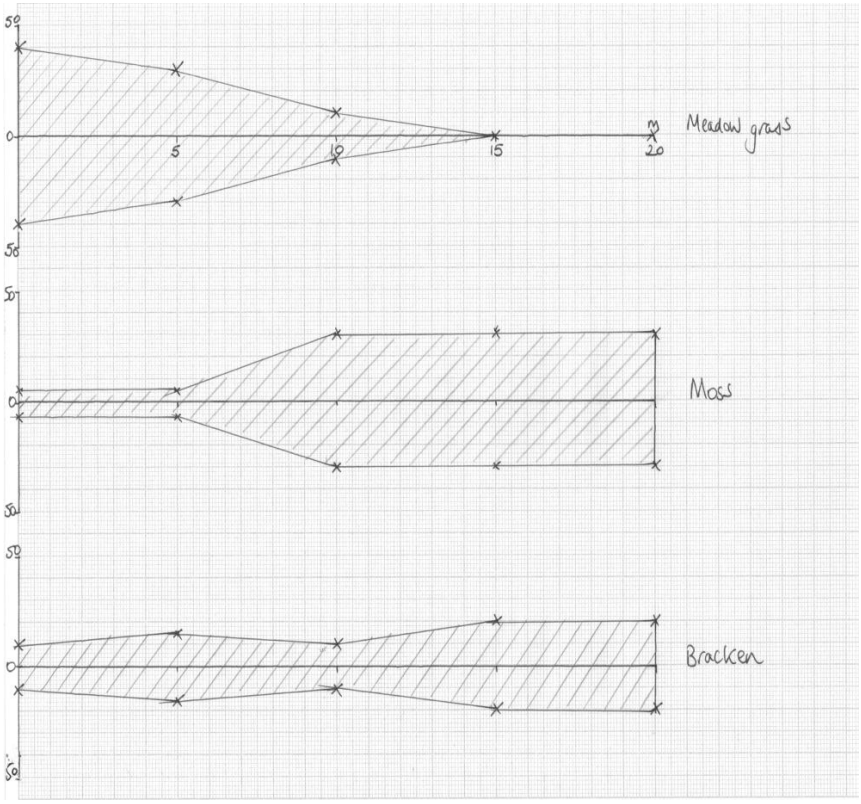
As there is an environmental gradient, it is useful to measure the factor that varies, so in each quadrat, you could measure, for example, the light intensity. You could plot the density, percentage frequency or percentage area cover of your species against the light intensity.

Kite diagrams

Per cent area cover allows you to draw kite diagrams to display your results.
The table shows the percentage area cover of three species along a transect:

Species	% area cover at distance along transect (m)				
	0	5	10	15	20
Meadow grass	80	60	20	0	0
Moss	10	10	60	60	60
Bracken	40	30	10	10	10

- Draw a horizontal axis representing 20m and a vertical axis representing 100 %, with 50 % above and 50 % below the distance axis.
- At each distance, make data points above and below the x-axis, each representing half of the area cover eg for meadow grass, at position 0 m, 40 % above and 40 % below the horizontal axis.
- Join the last two data points with a vertical line.
- Join the data points above the horizontal axis and the data points below



Further work

Suggested strategies for comparing abundance and distribution in terrestrial habitats are given in the table below:

Habitat type	Sampling	Species	Method	Habitats
Uniform	Random quadrats	Moss	% area cover or % frequency	Trampled and non-trampled Mown and unmown Clay soil and sandy soil Grazed and non-grazed
		Bracken		
		Meadow grass		
		Ground ivy		
		Plantain		
		Plantain	Density	
		Buttercup		
		Daisy		
		Celandine		
Environmental gradient	Belt transect	Moss	% area cover or % frequency	From grassland into wood From under tree with dense canopy to open field
		Bracken		
		Bramble		
		Meadow grass		
		Ground ivy		

Practical techniques

- Use sampling techniques in fieldwork

	SPECIFICATION STATEMENT	COMMENT
(a)	the reasons for species becoming endangered and causes of extinction	These should include: natural selection; habitat destruction such as deforestation and loss of hedgerows; pollution such as PCBs and oil; hunting and collecting; competition from domestic animals. An understanding that conservation is the sensible management of the biosphere and enhancement of biodiversity locally. Examples including: habitat protection by nature reserves and SSSI; international cooperation restricting trade; breeding programmes by zoos and botanic gardens plus sperm banks and seed stores and reintroduction programmes.
(b)	how gene pools are conserved in the wild and in captivity	An understanding that conservation of species ensures the conservation of existing gene pools. Candidates should appreciate the importance of conserving existing gene pools in the wild and in captivity: ethical reasons and the loss of potentially useful genes to man and the species
(c)	the issues in agricultural exploitation - conflicts between production and conservation and possible means to resolve such conflicts as illustrated by deforestation and overfishing	Candidates should understand the term 'agricultural exploitation' and the conflicts that exist between the demand for production and the need for conservation. This should be exemplified by the reasons for and consequences of deforestation. The need for managed forests involves sustainable replanting and regeneration, protected areas to preserve species. It is important to preserve natural woodland to enhance biodiversity. It should also be exemplified by the consequences of over-fishing on fish stocks, the methods employed to regulate fishing. The use of fish farming to overcome the problem of overfishing causes other problems.
(d)	the increased human pressures on the environment including the need to achieve sustainability by changes in human attitudes and making informed choices	Environmental monitoring is needed for conservation. Science and technology should enable the effects of human activities to be predicted and taken into account and made part of the overall planning. Countermeasures could be prepared, or the activities planned could be replaced by others with less harmful consequences. Multidimensional thinking and interdisciplinary collaboration are required
(e)	the need for political decision making to be informed by knowledge based on sound scientific principles	

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USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

SPECIFICATION STATEMENT		COMMENT
(f)	the concept of planetary boundaries	Planetary boundaries is the central concept in an Earth system framework proposed by a group of Earth system and environmental scientists. They proposed a framework of “planetary boundaries” designed to define a “safe operating space for humanity” for the international community, including governments at all levels, international organizations, civil society, the scientific community and the private sector, as a precondition for sustainable development. This framework is based on scientific research that indicates that since the Industrial Revolution, human actions have gradually become the main driver of global environmental change. The scientists assert that once human activity has passed certain thresholds or tipping points, defined as “planetary boundaries”, there is a risk of “irreversible and abrupt environmental change”. Nine Earth system processes have been identified which have boundaries that, to the extent that they are not crossed, mark the safe zone for the planet. However, because of human activities some of these dangerous boundaries have already been crossed, while others are in imminent danger of being crossed. The concept of planetary boundaries in relation to human population and its activities. • The biodiversity boundary has been crossed: Extinction in relation to natural selection; the change of habitats such as marine, tundra; coral reefs and coastal plains and its effect on indigenous species; Biodiversity monitoring; the use of gene banks; public awareness • The climate change boundary has been crossed: Greenhouse gases and their sources; the production and use of biofuels; the effect on human populations; the effect on plant and animal populations. • The nitrogen boundary has been crossed: recall the nitrogen cycle and the effects of eutrophication • The land use boundary has been crossed: the conflict over land use and food production; changes to farming practice; political considerations. • The fresh water boundary is avoidable: the need for fresh water; sources of fresh water; desalination. • The chemical pollution boundary is unquantified: air pollution including the effects of SO2 and N oxides. • The aerosol boundary is unquantified: air pollution by particulates • The ocean acidification boundary is avoidable: the effect of fossil fuel burning on oceans; fish farming. • The ozone boundary has been avoided: CFCs and the chemistry of ozone destruction; the effect of the Montreal protocols.

USEFUL INTERACTIVE RESOURCES

EDUQAS > **GCE Biology** > **Specification from 2015**

List of Related External Resources

	SPECIFICATION STATEMENT	COMMENT
(a)	the classification of organisms into groups based on their evolutionary relationships and that classification places organisms into discrete and hierarchical groups with other closely related species	Candidates are required to understand the hierarchy of the taxons including Kingdom, Phylum, Class, Order, Family, Genus, Species and be able to apply to given information.
(b)	the need for classification and its tentative nature	Classification may change as additional information becomes available.
(c)	the three domain classification system as compared with the five Kingdom classification system	Recent biochemical evidence has shown that the kingdom prokaryotae should be split into two separate groups based on some fundamental biochemical differences. All other organisms have eukaryotic cells. This has led to the development of a scheme of classification which suggests all organisms evolved along three distinct lineages, these are called **domains**. The organisms of each domain share a distinctive, unique pattern of ribosomal RNA, which establishes their close evolutionary relationship. The three domains of life are: • **Bacteria** (or Eubacteria) which are the true bacteria. • The **Archaea** (or Archaeabacteria) include the extremophile prokaryotes. • **Eukarya/ Eukaryota** which include all eukaryotic organisms i.e. animals, plants, fungi and protoctista. Extremophiles exist in a wide variety of environmental conditions including extremes of temperature, pH, salinity and pressure.



Select the image (left) for "Investigation into biodiversity in a habitat" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(d)	the characteristic features of Kingdoms: Prokaryotae, Protoctista, Plantae, Fungi, Animalia	Prokaryotae - composed of prokaryotic cells, which lack a nuclear envelope and membrane-bound organelles (the cell wall does not contain cellulose or chitin); Plantae - multicellular eukaryotes, photosynthetic, cellulose cell wall; Animalia - nervous co-ordination; multicellular eukaryotes, no cell wall, heterotrophic; Fungi - heterotrophic eukaryotes, cell walls of chitin, most have filaments called hyphae; reproduce by spores. Protoctista - mainly single cell eukaryotes, no tissue differentiation
(e)	the use of physical features and biochemical methods to assess the relatedness of organisms, including DNA 'genetic fingerprinting' and enzyme studies to show relatedness without the problem of morphological convergence	Only the principle of genetic profiling is required here - biochemical methods measure the proportion of DNA or proteins shared between species to estimate relatedness. DNA fragments or proteins are usually displayed as bands on an electrophoresis gel. Biochemical methods can reduce the mistakes made in classification due to convergent evolution. Homologous features have evolved from the same original structure for different functions, e.g. the limbs of reptiles, birds and mammals (pentadactyl limb), while analogous structures have evolved from different structures to form the same function (wings of birds and insects).
(f)	the concept of species	
(g)	the use of the binomial system in naming organisms	



Select the image (left) for "Investigation into biodiversity in a habitat" practical work

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General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(h)	biodiversity as the number and variety of organisms found within a specified geographic region	Definition of biodiversity: the number of species and the number of individuals of each species in a given environment.
(i)	biodiversity varying spatially and over time and affected by many factors	Genetic, environmental and human factors affect biodiversity.
(j)	biodiversity can be assessed in a habitat e.g. Simpson's Diversity Index	To investigate the biodiversity of a habitat, ecologists need to count the number of species present (species richness) and the number of individuals within each species population (species evenness). It is possible to calculate the diversity of a habitat by using an index of diversity, such as Simpson's diversity index. Any value calculated using Simpson's diversity index ranges between 0 and 1, the greater the value, the greater the sample diversity. The formula to be used is $D = 1 - \frac{\sum n(n-1)}{N(N-1)}$
(k)	biodiversity can be assessed within a species at a genetic level by looking at the variety of alleles in the gene pool of a population, i.e. the proportion of polymorphic loci across the genome	Polymorphism is the word used to describe the presences of several different forms or types of individuals among the members of a single species. Polymorphism results from the presence of polymorphic genes i.e. multiple alleles for the same gene. Genetic biodiversity can be assessed by determining the: • number of alleles at a locus e.g. T/t, $I^A/I^B/I^O$ • proportion of the population that have a particular allele



Select the image (left) for "Investigation into biodiversity in a habitat" practical work

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General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(l)	biodiversity can be assessed at a molecular level using DNA fingerprinting and sequencing	Due to the difficulties involved in counting every single allele in a population researchers collect samples of DNA and analyse the base sequences to look for variations between individuals. The greater the variation in the base sequence, the greater the genetic diversity of the species.
(m)	biodiversity has been generated through natural selection	Candidates need to appreciate the role of selective predation in natural selection but the detailed mechanism of evolution resulting in speciation is not required at this level.
(n)	the different types of adaptations of organisms to their environment including anatomical, physiological and behavioural adaptations	All species are uniquely adapted to the environment they inhabit; these adaptations include anatomical, physiological and behavioural adaptations. For example animals that live in deserts have to cope with extreme temperature fluctuations and a limited availability of water. Different organisms have different adaptations to overcome the same problem.



Select the image (left) for "Investigation into biodiversity in a habitat" practical work

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

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, $D = 1 - \frac{\Sigma\Sigma(\Sigma-1)}{N(N-1)}$

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, $D = 1 - \frac{\Sigma\Sigma(\Sigma-1)}{N(N-1)}$

Risk assessment

A risk assessment should be carried out of the area in which the investigation is being carried out. The table below gives examples of this.

Hazard	Risk	Control measure
Biting and stinging insects	Allergic response	Avoid touching specimens
Stinging plants	Allergic	Wear clothes that cover the body; use rubber gloves
Plant roots; uneven ground	Strains and sprains	Take care when moving around

Teacher/ Technician's notes

The methods given are designed to be a simple approach to introducing fieldwork and measuring biodiversity. This is developed further in the A level fieldwork practical in measuring abundance and distribution. These two practicals can be combined in a fieldwork project if so wished.

Care should be taken in choosing the area to be studied, so that individual organisms can be identified. This could be difficult, for example, in a school playing field, where grass would be the dominant species and it would be difficult to count the individual plants in the quadrat.

Further information available from :

http://www.nuffieldfoundation.org/sites/default/files/19_Ecology.pdf

http://biology-fieldwork.org/woodland/woodland-plants/investigation-comparing-two-areas-of-woodland.aspx

Sample results

Organism	n	n(n-1)
Mayfly nymph	4	12
Stonefly nymph	4	12
Caddis fly larva	3	6
bloodworms	2	2
Freshwater shrimp	10	90
Rat tailed maggot	1	0
	N = 24	Σn(n-1) = 122

Calculating Simpson's index

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

where N = total number of organisms sampled; n = number in a given species.

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

= 1 – $\frac{122}{24\ x\ 23}$

= 1 - $\frac{122}{552}$

= 1 – 0.22

= 0.78 (2dp)

A higher the value of D reflects a higher biodiversity. The maximum theoretical value is 1.

Further work

Animal and plant communities may be compared in contrasting situations, including:

- Different habitats e.g.
  - short grass and long grass
  - oak woodland and birch woodland
  - trampled and non-trampled grassland

- Organisms can be sampled from water habitats using kick sampling and Simpson's index calculated. Comparisons may be made, for example, between
  - areas with different flow rates
  - concentration of dissolved oxygen
  - concentration of nitrate pollution

Practical techniques

- use sampling techniques in fieldwork
- use ICT such as computer modelling, or data logger to collect data, or use software to process data

	SPECIFICATION STATEMENT	COMMENT
(a)	interphase and the main stages of mitosis	Candidates should be able to recognise the stages of mitotic cell division from diagrams and photographs. They should be able to describe and explain the processes occurring at each stage.
(b)	the significance of mitosis as a process in which daughter cells are provided with identical copies of genes and the process of cytokinesis	Description of the process of mitosis in both plant and animal cells.
(c)	the significance of mitosis in terms of damage and disease: repeated cell renewal, damage repair and healing and unrestricted division leading to cancerous growth	No knowledge of oncogenes is required, but may be used as an application of unrestricted cell division.
(d)	the main stages of meiosis (names of subdivisions of prophase 1 not required) and cytokinesis	Candidates should be able to recognise the meiotic stages from diagrams and photographs. They should be able to describe and explain the processes occurring at each stage.
(e)	the differences between mitosis and meiosis, including that meiosis produces non-identical daughter cells	This should include: number of nuclear divisions in the process, number of cells formed; ploidy of parental cells/nuclei; ploidy of daughter cells/nuclei; genetic nature of daughter cells/nuclei; pairing of homologous chromosomes; crossing over; and segregation of homologous chromosomes.



Select the image (left) for "Scientific drawing of cells from slides of root tip to show stages of mitosis" practical work



Select the image (left) for " Scientific drawing of cells from prepared slides of developing anthers to show stages of meiosis" practical work

General guidance | Microscope calibration

USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

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

Specification reference: 2.2

Genetic material is copied and passed on to daughter cells

Introduction

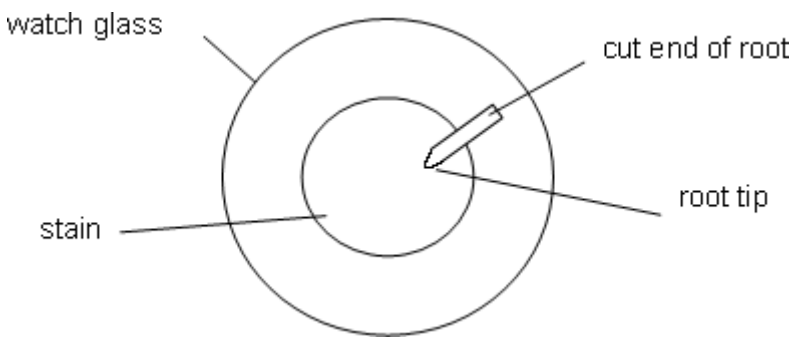
Mitosis is a process of cell replication needed for growth and repair. Onion (*Allium* sp.) is very useful for root tip preparation to study the different stages in mitosis. At the tip of the root there is an apical meristem where cells divide by mitosis. To observe the stages of mitosis the root tissues 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 coverslip
Scalpel	Fine forceps
Watch glass	Mounted needle
propionic-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 propionic-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 propionic-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 x40 objective lens, observe and draw, cells at interphase, prophase, metaphase, anaphase and telophase.

Risk assessment

Hazard	Risk	Control measure
Propionic ethanol is corrosive	Could be transferred to skin or eyes during experiment	Wear gloves/safety glasses
Propionic-orcein stain contains propionic acid and is corrosive	Could be transferred to skin or eyes during experiment	Wear gloves/safety glasses
Hydrochloric acid is corrosive	Could be transferred to skin or eyes during experiment	Wear gloves/safety glasses
Scalpels are sharp	Could cut skin when cutting roots	Cut away from body onto white tile

Teacher/ Technician's notes

Use shallots or small onions. Garlic is often suggested because fresh cloves will sprout very quickly.

Support the onion or garlic clove so that it just touches the surface of water. It can be supported by sticking a cocktail stick through the onion or clove of garlic and supporting on the neck of a wider vessel. Alternatively cut holes in a polystyrene sheet insert the cloves and float on the surface of water.

Roots that have been set to sprout for 2 – 5 days seem to give the highest likelihood of finding actively dividing cells.

Some people find that cutting the root tips close to midday makes a difference to the number of dividing cells so you may want to cut and fix root tips at this time and give these to the students. They can be fixed in ethanoic alcohol and kept in this fluid for several months. Ethanoic alcohol is 3 parts absolute alcohol to 1 part glacial ethanoic acid. Mix just before use by adding the acid to the alcohol.

Propionic orcein stain

Grind 1.5 g of solid orcein with a pestle and mortar.
In a fume chamber mix 90cm^3 of glacial propionic acid with 110 cm^3 of distilled water and bring to the boil.
Pour the boiling mixture over the orcein and stir thoroughly.
Leave overnight and then filter and store in a lightly stoppered dark bottle.
If the chromosomes are overstained dilute the stain with 45% propionic acid.

More information is available from:

http://www.nuffieldfoundation.org/practical-biology/investigating-mitosis-allium-root-tip-squash

It is acceptable to use prepared slides of root tip if you so wish.

Further work

- Alternative to onion or garlic could be used for example seedlings of beans (2n 22) or peas (2n 14).
- Students could calculate the mitotic index:
  Mitotic index = number of actively dividing cells in field of view / Total number of cells in field of view

- The effect of variables such as time, temperature, light on the mitotic index could be studied.

Practical skills

- Use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions
- Use qualitative reagents to identify biological molecules
- Produce scientific drawing from observation with annotations
- Use of light microscope at high power and low power, including use of a graticule.

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

Specification reference: 2.2

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

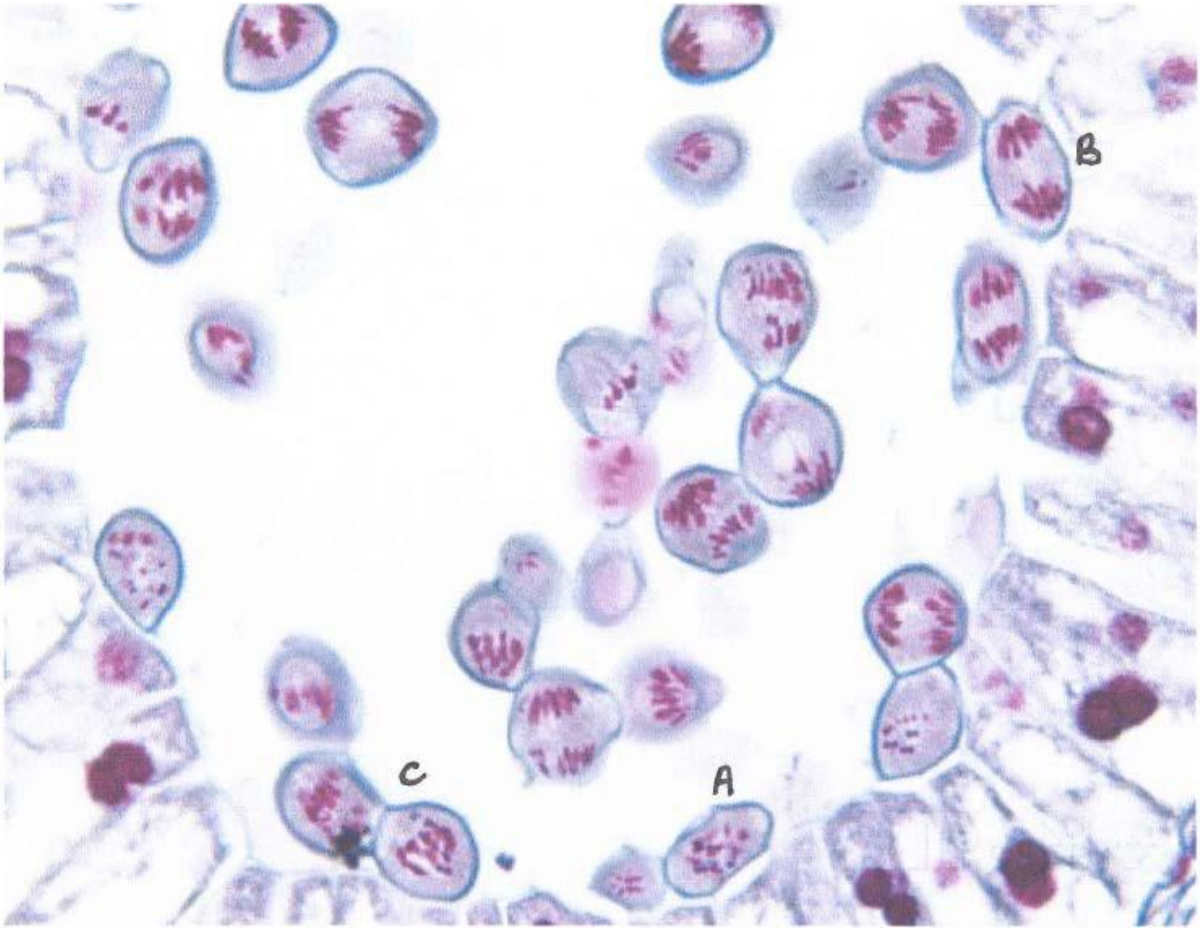
1. Using the x10 objective focus on the cells at the centre of one of the pollen sacs.
2. Use the x40 objective and identify as many stages of meiosis as you can.
3. Repeat this for all four pollen sacs.
4. Make drawings to show all the stages which you have identified.
5. Calculate the size of one cell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

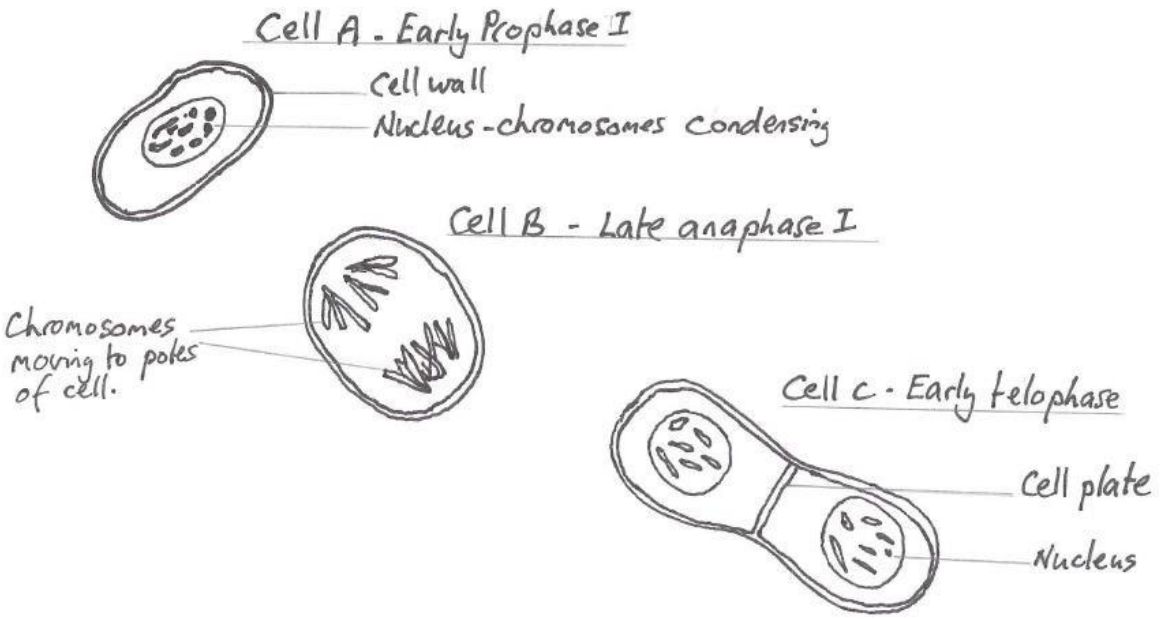
Risk assessment

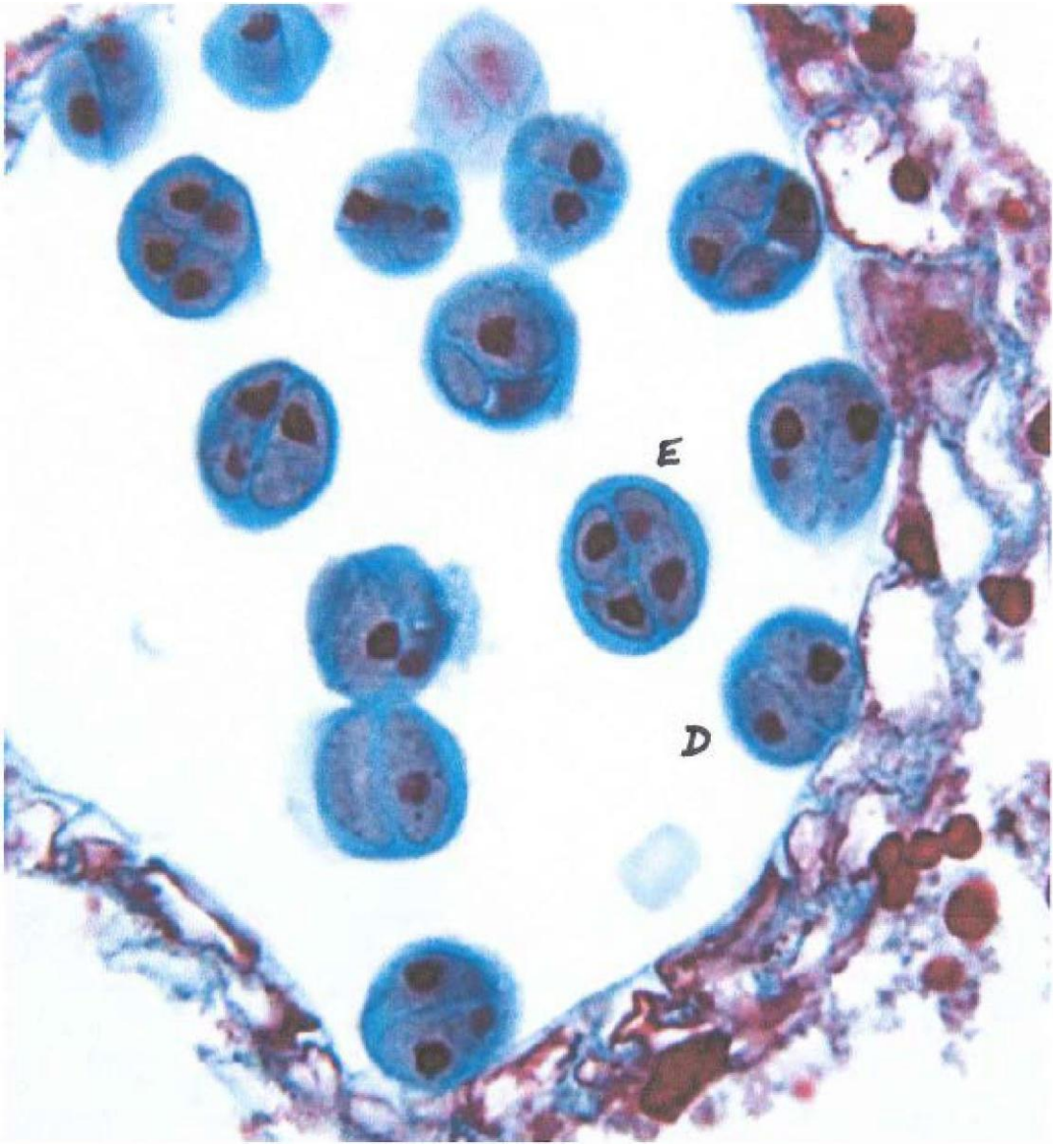
Hazard	Risk	Control measure
Microscope lamp/ bulb is hot	Could burn skin when trying to move lamp/ microscope	Leave lamp to cool before moving

Teacher/ Technician's notes

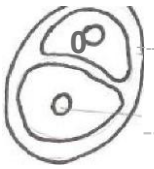
The photographs that follow give an indication of what should be viewed and drawn.







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Further work

- This could be combined with the plan of the anther in the section on plant reproduction.

Practical techniques

- Use of light microscope at high power and low power, including use of a graticule.
- Produce scientific drawing from observation with annotations.

	SPECIFICATION STATEMENT	COMMENT
(a)	the structure and function ofthe reproductive systems in humans, including the examination of histology of ovary and testis	Candidates should be able to label and know the functions of parts of the male and female reproductive systems to include: Male: scrotum, testes, epididymis, vas deferens, seminal vesicle, prostate gland, urethra, penis; Female: ovary, Fallopian tubes (oviducts), uterus, endometrium, cervix, vagina.

Continued on next page

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USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

SPECIFICATION STATEMENT		COMMENT
(b)	the processes of spermatogenesis and oogenesis to produce spermatozoa and secondary oocytes; sexual intercourse; fertilisation and implantation	Spermatogenesis • takes place in the seminiferous tubules • candidates should know the types and stages of cell division which produce mature spermatozoa including: germinal epithelium; spermatogonia; primary and secondary spermatocytes; spermatids; spermatozoa and be able to recognise these cells together with interstitial cells (cells of Leydig) and Sertoli cells on diagrams and sections through a testis • interstitial cells secrete testosterone which is involved in stimulating the process of spermatogenesis and Sertoli cells are involved in providing nourishment and in protecting cells produced in this process • candidates should be able to recognise and know the functions of the structures found in a mature sperm cell. Oogenesis • oogenesis up to the secondary oocyte stage takes place in the ovary • candidates should know the types and stages of cell division which produce secondary oocytes including: germinal epithelium; oogonia; primary and secondary oocytes; first polar body; primary, secondary and Graafian follicles and corpus luteum. They should be able to recognise these structures on a diagram showing stages in the development of a follicle from a primary follicle to a mature Graafian follicle, ovulation and the subsequent development of the corpus luteum • candidates should be able to recognise the structures found in and associated with a secondary oocyte following ovulation to include: the corona radiata; zona pellucida; first polar body; cell membrane; cortical granules and chromosomes / spindle apparatus suspended at metaphase II of meiosis. Fertilization • following sexual intercourse spermatozoa move into the Fallopian tubes • capacitation increases the permeability of the membrane in front of the acrosome • on contact with the zona pellucida the acrosome reaction releases hydrolase enzymes which digest the zona pellucida • the membranes of the sperm and secondary oocyte fuse and the genetic material of the sperm cell enters the secondary oocyte • this triggers the cortical reaction in which cortical granules fuse with the cell membrane and their contents modify the zona pellucida to form the fertilisation membrane and prevents polyspermy • entry of the genetic material also triggers meiosis II to continue forming the ovum and the second polar body • the nuclei of the sperm and ovum fuse to form a zygotic nucleus Implantation • the zygote undergoes repeated mitotic divisions, called cleavage, to form a ball of cells called the blastocyst • the blastocyst is moved into the uterus where it attaches and sinks into the endometrium – implantation • a placenta forms between the tissues of the mother and the foetus.

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USEFUL INTERACTIVE RESOURCES

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SPECIFICATION STATEMENT		COMMENT
(c)	the endocrine control of reproduction in the female: including the menstrual cycle, birth and lactation by reference to follicle stimulating hormone, luteinising hormone, oestrogen, progesterone, oxytocin and prolactin and human chorionic gonadotrophin	Candidates should know and understand the role of hormones in reproduction in the female to include: Menstrual Cycle: • FSH secreted by the anterior pituitary gland stimulates the maturation of a follicle and stimulates the productionof oestrogen; • following menstruation the level of oestrogen, secreted by the developing follicle, increases in the blood which triggers the repair of the endometrium; this inhibits FSH production and stimulates LH production; • a high level of LH, secreted by the anterior pituitary, initiates ovulation; causes the Graafian follicle to develop into a corpus luteum • progesterone, secreted by the corpus luteum, causes further development of the endometrium prior to menstruation • if implantation does not occur, falling FSH and LH levels cause the corpus luteum to degenerate, progesterone levels fall, the endometrium breaks down and is lost during menstruation; FSH secretion is no longer inhibited and another menstrual cycle is initiated. Candidates should be able to interpret a graphical representation of hormonal changes during the menstrual cycle in relation to the development of the endometrium, ovulation and the role of negative feedback in controlling these events.
(d)	the role of the placenta including hormonal control	Pregnancy: • just before and following implantation, the developing embryo secretes HCG which maintains the corpus luteum for the first 16 weeks of pregnancy • the placenta then secretes progesterone and oestrogen which rise to high levels in the plasma • FSH and LH secretion are inhibited • progesterone suppresses the uterine wall's ability to contract • oestrogen stimulates the growth of the uterus to accommodate the growing foetus and stimulates the growth and development of the mammary glands during pregnancy. Birth: • just before birth oestrogen levels increase and progesterone levels decrease – the uterine wall can now contract • oxytocin secreted by the posterior pituitary gland stimulates contraction of the uterine wall which stimulates the secretion of more oxytocin – this is an example of positive feedback • prolactin is also released from the anterior lobe of the pituitary gland during and after birth to stimulate the production of milk by the mammary glands Candidates should be able to recognise and understand the roles of the different structures found in the placenta including: counter-current flow between maternal and foetal blood supplies; chorionic villi; intervillous spaces; transport to and from the foetus via the umbilical arteries and vein. The role of the placenta in terms of: exchange of gases and nutrients; providing a barrier between the maternal and foetal blood; protection from the immune system of the mother and from the difference in maternal and foetal blood pressures; secretion of hormones. The role of the amniotic fluid in acting as a shock absorber and in protecting the foetus during development.

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USEFUL INTERACTIVE RESOURCES

WJEC > GCE Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(a)	the generalised structure of flowers to be able to compare wind and insect pollinated flowers	Candidates should be able to recognise and label a half-flower of a typical regular, diocotyledonous, insect-pollinated flower to include: receptacle, calyx, sepal, corolla, petal, stamen, filament, anther, carpel, ovary, ovule, style and stigma. They should be able to identify differences between an insect and a wind-pollinated flower in terms of function of flower parts and adaptations to different methods of pollination.
(b)	the development of pollen and ovules, including examination of prepared slides of anther and ovary	The role of mitosis and meiosis in the development of pollen grains in an anther to include: • mitosis to produce large numbers of pollen mother cells followed by meiosis to produce a tetrad of four haploid cells; • the role of the tapetum in pollen grain development; • development and structure of a mature pollen grain, including subsequent mitotic divisions of the nucleus; • dehiscence and pollen dispersal. The role of mitosis and meiosis in the development of an ovule in the ovary to include: • meiosis of a megaspore mother cell in the nucellus to produce four haploid megaspores; • the growth and subsequent development of one of the megaspores including three mitotic divisions to produce eight haploid nuclei within the embryo sac. Candidates should be able to recognise structures in a mature ovule to include: funicle, integuments, micropyle, embryo sac, female gamete, two synergids, two polar nuclei, three antipodal cells.



Select the image (left) for " Investigation of the digestion of starch agar using germinating seeds" practical work



Select the image (left) for " Dissection of wind and insect-pollinated flowers" practical work



Select the image (left) for " Scientific drawing of cells from prepared slides of anther" practical work

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USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(c)	cross and self-pollination	Pollination is the transfer of pollen from an anther to a stigma. Candidates should understand the genetic consequences of self-pollination and cross-pollination and appreciate how meiosis and random fertilisation result in increased genetic variation through cross-pollination. There are different adaptations of flowers that promote cross-pollination. These include irregular flower structure and chemical self-incompatibility. Candidates should understand the events following pollination to include: • mitosis of the pollen grain nucleus to produce two male gametes and a pollen tube nucleus • germination of a pollen grain on a compatible stigma; • growth of a pollen tube (under the control of the pollen tube nucleus) through the digestion of the style through the secretion of hydrolase enzymes • entry of the pollen tube into the embryo sac through the micropyle Both male gametes are involved in separate fertilisation events: • one male gamete enters the embryo sac and fuses with the female gamete to produce a diploid zygote • the second male gamete fuses with the two polar nuclei to form a triploid primary endosperm nucleus.
(d)	the process of double fertilisation	



Select the image (left) for " Investigation of the digestion of starch agar using germinating seeds" practical work



Select the image (left) for " Dissection of wind and insect-pollinated flowers" practical work



Select the image (left) for " Scientific drawing of cells from prepared slides of anther" practical work

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USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(e)	the formation and structure of seed and fruit as shown by broad bean and maize	The events that take place following double fertilisation to produce seeds and fruits include: • the ovule developing into a seed; • the diploid zygote divides by mitosis to form the diploid embryo, consisting of plumule, radicle and one or two cotyledons; • the triploid endosperm nucleus divides by mitosis to form endosperm tissue, an important food storage tissue in cereal grains, e.g. wheat; • the integuments develop into the testa; • the micropyle remains as a pore in the testa • the ovary wall develops into a fruit wall enclosing the seeds. Candidates should be able to identify and label diagrams of broad bean and maize seeds to include: hilum (scar of the funicle), micropyle, testa, position of radicle, plumule, cotyledons. Seeds have evolved as a survival strategy for a terrestrial mode of life. Plants have developed different mechanisms to enable the dispersal of seeds. This reduces competition following germination and increases the chance of growth into mature plants.



Select the image (left) for " Investigation of the digestion of starch agar using germinating seeds" practical work



Select the image (left) for " Dissection of wind and insect-pollinated flowers" practical work



Select the image (left) for " Scientific drawing of cells from prepared slides of anther" practical work

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	SPECIFICATION STATEMENT	COMMENT
(f)	the process of germination of *Vicia faba* (broad bean)	Seeds can remain dormant until suitable conditions are present, i.e. availability of water, oxygen and a suitable temperature. Germination involves the rapid onset of biochemical activity and growth of a seedling until the plant can carry out photosynthesis and become independent of the food stores contained in the cotyledons. The stages of germination in a non-endospermic seed, e.g. broad bean include: • water being imbibed through the micropyle; the cotyledons swelling and the testa being split to allow entry of more oxygen for aerobic respiration; • food reserves from the cotyledons, starch and proteins, are mobilised through hydrolysis (and also lipids in some seeds); • providing sources of energy for respiration and growth of the plumule and radical. In endospermic seeds, e.g. maize, gibberellin (a plant hormone) is involved in the process of germination: • following imbibition of water gibberellin is released by the embryo and diffuses to the aleurone layer which contains proteins; • gibberellins induces the production of hydrolytic enzymes, e.g. amylase which break down stored nutrients • glucose and other nutrients diffuse to the embryo where they are used in aerobic respiration and growth.
(g)	the effect of gibberellin	



Select the image (left) for " Investigation of the digestion of starch agar using germinating seeds" practical work



Select the image (left) for " Dissection of wind and insect-pollinated flowers" practical work



Select the image (left) for " Scientific drawing of cells from prepared slides of anther" practical work

General guidance | Microscope calibration | Microscope section labels

USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

Investigation of the digestion of starch agar using germinating seeds

Specification reference: 2.4

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.

Risk assessment

Hazard	Risk	Control measure
Scalpel blades are sharp	May cut fingers when cutting seeds	Cut away from body onto a white tile
Iodine-potassium iodide solution	Risk of damage to eyes or skin	Wear goggles, wash skin immediately under running water

Teacher/ Technician's notes

Starch agar plates.

Starch agar 0.2% soluble starch. 2% agar.

For each pair of plates:

1. Mix 1 g of agar powder with 10 cm^3 of cold water.
2. Boil 0.1 g soluble starch in 50 cm^3 of water.
3. Add the agar to the starch solution.
4. Pour 20 cm^3 into a petri dish.
5. Allow to cool.

Sample results

The area of the clear zone around the seed indicates the degree of amylase activity. The area of the clear zone should be approximately circular and the area can be calculated using the formula:

Area of a circle = πr^2 π = 3.14. r = radius in mm.

If a clear zone of 15mm is produced the area will be:

3.14 x 7.5^2 or 3.14 x 56.25 = 176.63 mm^2

Further work

- The effect of different temperatures on amylase activity.
- Effect of age of seed on amylase activity.
- The effect of gibberellic acid on amylase activity.

Practical Techniques

- Use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions
- Use qualitative reagents to identify biological molecules
- Safely and ethically use organisms to measure
    - plant or animal responses
    - physiological functions

Dissection of wind and insect-pollinated flowers A

level Specification reference: 2.4

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 and 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 3's or multiples of 3. The dicotyledons have leaves with netted veins e.g. geranium, primrose and buttercup.

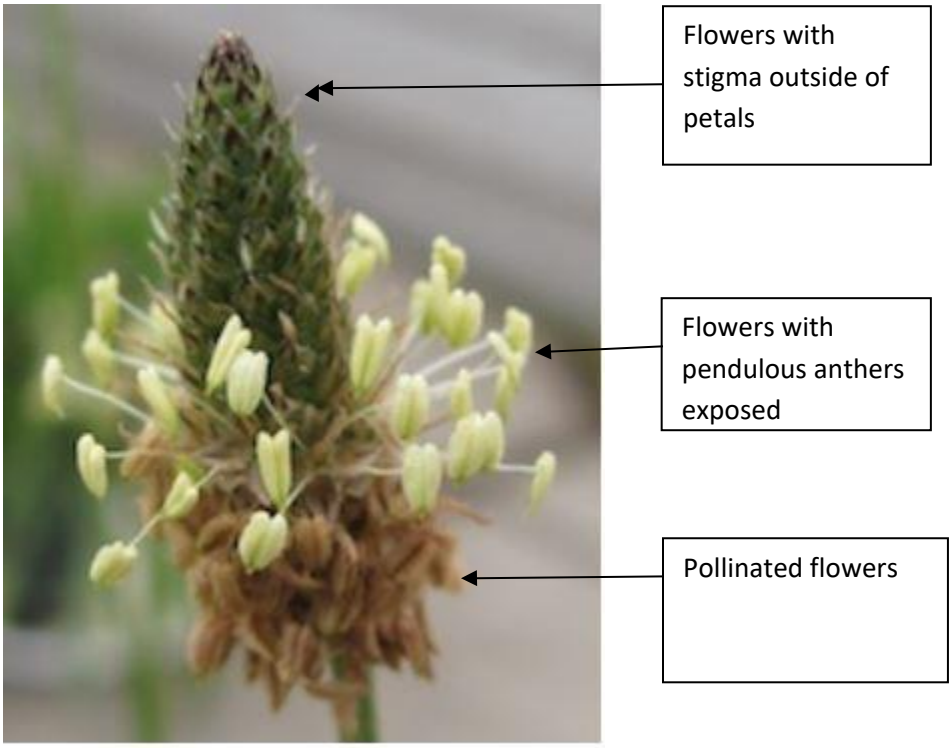
Apparatus

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. One of the petals should be cut in half and the others should be intact.
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



© David Timerman

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.

Risk assessment

Hazard	Risk	Control measure
Scalpel blades are sharp	May cut skin when dissecting flower	Press on stalk of flower and cut away from finger
Flowers can be allergens	Allergic reaction	Avoid species of plants which cause an allergic response e.g. geranium. Make students aware of flower type being used

Teacher / technician's notes

Any flower could be used but it is advised to avoid flower types with indistinguishable sepals and petals (e.g. daffodil) or complex floral structures (e.g. dandelion or daisy)

Half-flower Ranunculus repens [Buttercup]

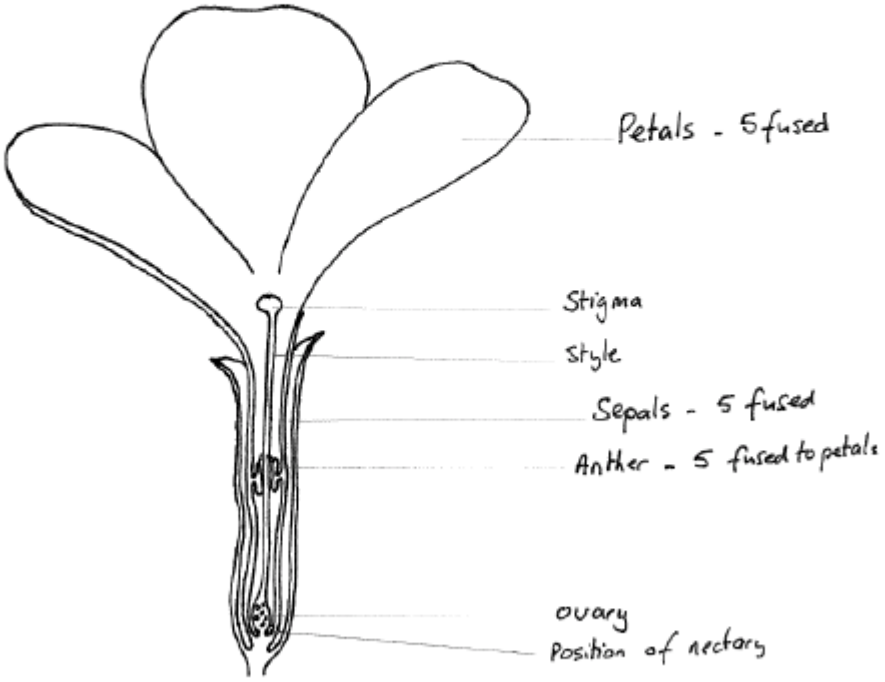


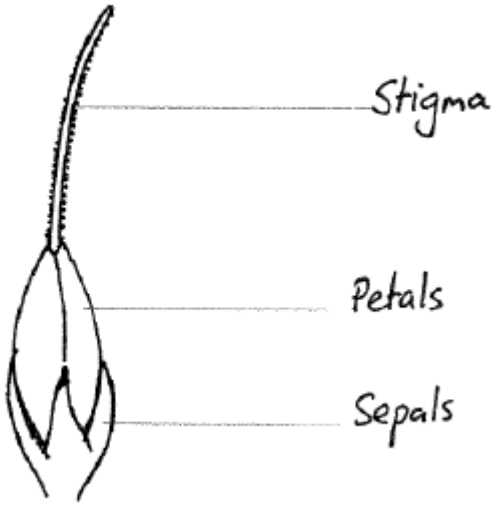
Magnification x 8

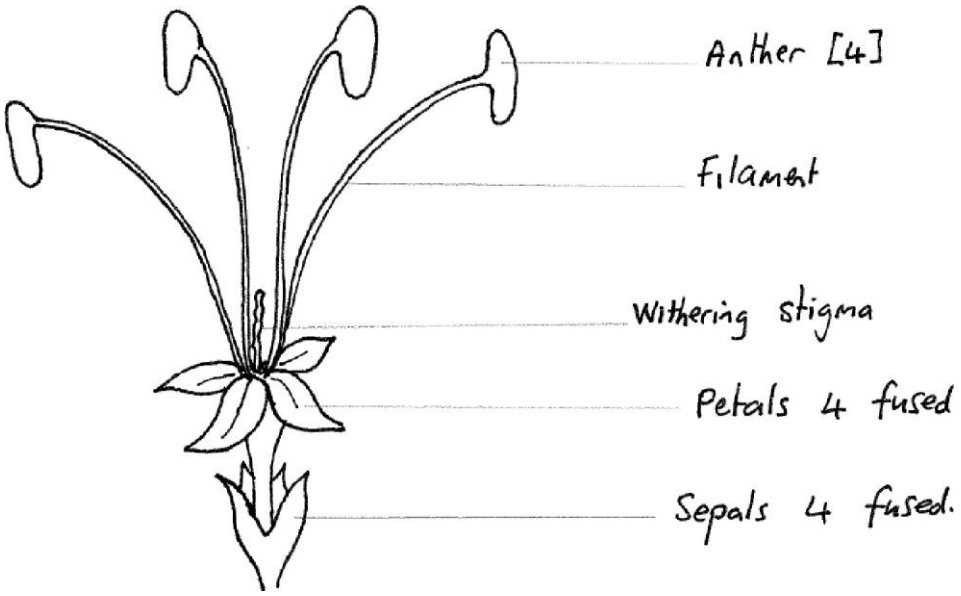
Cut line of sepal and petal indicated by a double line.

sqa part of WJEC

Half-flower Primula Vulgaris [Primrose]







Magnification x 10

Practical Techniques

- Produce scientific drawing from observation with annotations
- Safely use instruments for dissection of an animal organ, or plant organ.

Scientific drawing of cells from prepared slides of anther.

Specification reference: 2.4

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

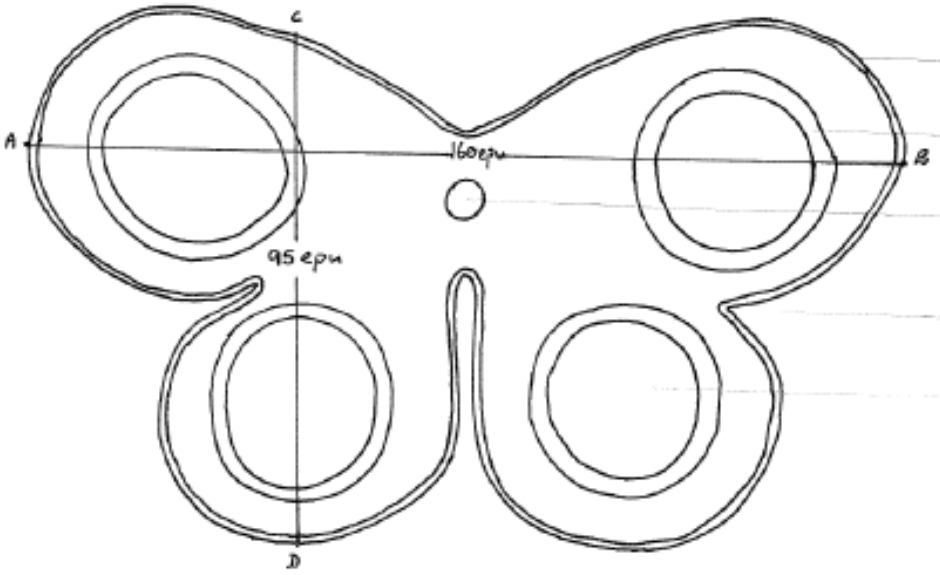
Method

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.

Teacher/ Technician's notes

Anther sections are also used to show stages of meiosis, it may well be possible to do both practicals in the same practical session.

An example of the expected diagram is shown:



epu = eye piece unit.

Actual size A-B = 160 x 10μm = 1600μm
C-D = 95 x 10μm = 950μm

Magnification = $\frac{160000}{1600}$ = x100

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Practical techniques

- Use of light microscope at high power and low power, including use of a graticule
- Produce scientific drawing from observation with annotations.

	SPECIFICATION STATEMENT	COMMENT
(a)	alleles as different forms of the same gene	Candidates should know, understand and use genetic terms to include: gene, locus, alleles, dominant, recessive, codominant, phenotype, genotype, homozygous, heterozygous, F_1, F_2, autosomes and sex chromosomes.
(b)	the principles of monohybrid Mendelian inheritance including simple crosses involving codominance	Candidates should understand • how Mendel used the results of experimental genetic crosses to derive his laws of inheritance and be able to apply these laws when solving genetic problems; • the use of symbols to represent dominant, recessive, codominant and sex-linked alleles and how to represent genetic crosses in diagrammatic form when solving genetics problems; • how and why test crosses may be carried out; • that Mendel's laws only apply if genes are not linked, i.e. on different chromosomes and that if genes show linkage, the results of crosses will not follow the expected Mendelian ratios. Candidates should be able to apply their knowledge and understanding of meiosis to explain the production of recombinants through independent assortment of non-linked genes and how crossing over can produce recombinants in linked genes.
(c)	the principles of dihybrid Mendelian inheritance including simple crosses involving linkage	



Select the image (left) for "Experiment to illustrate gene segregation" practical work

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	SPECIFICATION STATEMENT	COMMENT
(d)	the use of a chi squared test	Candidates should understand under what conditions the Chi^2 test can be used and that the Chi^2 test can be used to determine if the results of a genetic cross are significantly different to expected results or whether the differences are due to chance alone. They should know how to carry out and interpret the results of this test as follows: • formulate a null hypothesis; • calculate expected numbers from Mendelian ratios; • calculate degrees of freedom; • choose a suitable probability level; • identify a Chi^2 value from a Chi^2 distribution table; • accept or reject the null hypothesis.
(e)	sex linkage as illustrated by haemophilia and Duchenne muscular dystrophy	Candidates should understand sex-linkage in organisms with X and Y sex chromosomes, as the inheritance of a gene present on the X chromosome only. They should understand the significance of the lack of a corresponding allele on the Y chromosome in terms of expression of recessive alleles.
(f)	gene mutation as illustrated by sickle cell anaemia and chromosome mutation as illustrated by Down's syndrome	Mutations are spontaneous random events and that mutation rates are normally very low, but in organisms with short life cycles and more frequent cell division, the rate of mutation is higher. It is the source of genetic variation which can result in evolution through natural selection. Most mutations occur during crossing over in prophase-I and non-disjunction in anaphase-I and anaphase-II. Mutations can affect protein synthesis and can change the phenotype of an organism, but some mutations have no effect on the phenotype. Gene (point) mutations affect single bases in a gene and chromosomal mutations affect many genes. The rate of mutation may be increased by mutagens, including ionising radiations (gamma radiation, UV and X-rays), and certain chemicals, such as polycyclic hydrocarbons in cigarette smoke. A mutagen which causes cancer is called a carcinogen. Some genes called proto-oncogenes can mutate to become oncogenes which are involved causing uncontrolled cell division to form a cancer.
(g)	the effect of mutagens, carcinogens and oncogenes	



Select the image (left) for "Experiment to illustrate gene segregation" practical work

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List of Related External Resources

	SPECIFICATION STATEMENT	COMMENT
(h)	the control of gene expression by factors other than changes in the DNA sequence; the study of this is called epigenetics	Epigenetics: • DNA can be modified post-replication • this does not change the DNA base sequence but changes the ability of a gene to be transcribed during protein synthesis • addition of methyl groups to bases prevents those bases being recognised and reduces the ability of that gene to be expressed • the histone proteins used to organise the DNA in a chromosome can also be modified – if the histone coils more tightly this can prevent gene expression or if it coils more loosely it can increase gene expression Different epigenetic modifications can occur in cells of the same tissue and in different tissues resulting in different expression of the same gene in different parts of the same organism.



Select the image (left) for "Experiment to illustrate gene segregation" practical work

General guidance

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Experiment to illustrate gene segregation

Specification reference: 2.5

Inheritance

Introduction

Mendelian genetics explains how different ratios 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 chi^2 test (χ^2), 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.



© biologycorner.com

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

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

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

Risk assessment

Hazard	Risk	Control measure
Soil may infect cuts on skin or be ingested	Sowing seeds	Wear rubber gloves; cover skin

Teacher/ Technician's notes

Genetic corn is the example used here to obtain the data for the statistical test. This comprises ears of corn in which the grains are, variously, yellow, white or purple and sweet (shrivelled) or starchy. The ears have been produced by crossing parents of known genotype. It is useful to wrap the ears of corn permanently in cling film to prevent the loss of individual kernels.

If you wish, genetic seeds can be bought from standard biological suppliers, such as Blades Biological Ltd. and Philip Harris. These companies provide seeds of pure breeding parents and their F_1 and F_2 hybrids. Suitable material includes

- maize seeds producing seedlings which can be scored as are either green or albino, tall or dwarf
- tomato seeds producing seedlings which can be scored as hairy or hairless stems, smooth or dissected leaf margins
- seeds of green and albino rapid cycling *Brassica* and their hybrids.

Seeds can be sown following the instructions accompanying the packs and the seedlings will be ready to score within about three weeks, depending on the lab conditions.

Using any of these examples, the kernels on each ear of corn or the individual plants are scored for their phenotype and a theoretical ratio can by hypothesised based on Mendelian genetics. The chi^2 (χ^2) test can be applied to test if this ratio is consistent with the observations.

The example given here is testing a dihybrid backcross in maize, in which the parents are tall, green with genotype TtGg, and dwarf albino, with genotype ttgg

Phenotype	Number of individuals	Possible ratio
tall green	31	1
tall albino	24	1
dwarf green	29	1
dwarf albino	16	1

Worked examples of statistical analysis

The chi^2 test is suitable because there is a large sample and there are predicted values based on a null hypothesis, proposed based on Mendelian genetics. The correct use of this test also requires that at least 80% of the cells have values not less than 5.

1. Formulate the null hypothesis:

   The phenotypic ratio is 1 tall green : 1 tall albino : 1 dwarf green : 1 dwarf albino.

2. Construct the table:
   The expected values be calculated on the basis of the expected 1:1:1:1 ratio. The difference between the observed (O) and expected (E) values is calculated (O-E) and squared [(O-E)^2]. This value is divided by the expected number (E) and the sum of these values gives the value χ^2.

Phenotype	Observed	Expected	(O – E)	$(O - E)^2$	$\frac{(O-E)^2}{E}$
tall green	31	25	6	36	1.44
tall albino	24	25	-1	1	0.04
dwarf green	29	25	4	16	0.64
dwarf albino	16	25	-9	81	3.24
				$\chi^2 = \Sigma\frac{(O-E)^2}{E}$	5.36

3. Statistical parameters:

   (i) The number of degrees of freedom (df) is one less than the number of phenotypes, so df = 4 – 1 = 3.

   (ii) The level of significance or probability level (p) is 0.05, corresponding with 95% confidence limits.

   (iii) Using chi^2 tables, for df = 3 and p = 0.05, the critical value for χ^2 = 7.81.

4. Interpreting the result:

   the calculated value is less than the critical value

   ∴ the null hypothesis is accepted at the 0.05 level of significance

   ∴ the ratio is 1:1:1:1 and any deviation from the ideal ratio is due to chance.

5. Conclusion:

   The phenotype of the sample is determined by two alleles each of two unlinked genes, where “tall” is dominant over “dwarf” and “green” is dominant over “albino”. The sample is an F_2 generation, produced by crossing a parent that is heterozygous for both genes with a parent that is homozygous recessive at both genes. Inheritance is Mendelian.

Further work

- Use data which does not match the Mendelian ratios to look at linkage

Practical Techniques

- Safely and ethically use organisms to measure
  - plant or animal responses
  - physiological functions
- Use ICT such as computer modelling, or data logger to collect data, or use software to process data.

	SPECIFICATION STATEMENT	COMMENT
(a)	genetic and environmental factors producing variation between individuals	Candidates should be able to explain the difference between continuous and discontinuous variation in terms of: • number of genes controlling a particular phenotype • the effect of environmental factors
(b)	variation as continuous and discontinuous; heritable and non- heritable	
(c)	the effect of inter- and intra-specific competition on breeding success and survival	Competition, environmental and human factors place selective pressures on the survival of different phenotypes and hence breeding success.
(d)	the impact of selective agencies (e.g. supply of food, breeding sites, climate, human impact) on the survival of organisms	
(e)	the concept of gene pool and genetic drift	The gene pool is the total of all alleles for all of the genes in a population. Selection pressures can change the allele frequencies of the alleles present at a particular gene locus in a population and that allele frequency can be expressed either as a proportion or a percentage of the total number of copies of all alleles for that gene.
(f)	the effect of selection changing the frequency of alleles in a population	



Select the image (left) for " Investigation of continuous variation in a species" practical work

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General guidance

USEFUL INTERACTIVE RESOURCES

WJEC > GCE Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(g)	the use of the Hardy-Weinberg principle and equation	The Hardy-Weinberg principle states that the frequencies of dominant and recessive alleles and genotypes will remain constant from one generation to the next, if certain conditions remain true. These conditions include: • a large population (100+ individuals); • no selection for or against any phenotype; • random mating throughout the population; • no mutations; • the population is isolated, i.e. no immigration or emigration. Candidates should know to apply the Hardy-Weinberg principle to estimate frequencies of dominant or recessive alleles or of different genotypes of a characteristic in a population using: $p^2 + 2pq + q^2 = 1$ where p = frequency of the dominant allele (A) q = frequency of the recessive allele (a) $p + q = 1.0$ The three terms of this binomial expansion indicate the frequencies of the three genotypes: p^2 = frequency of AA (homozygous dominant) $2pq$ = frequency of Aa (heterozygous) q^2 = frequency of aa (homozygous recessive)
(h)	the conditions under which the Hardy-Weinberg principle applies	



Select the image (left) for " Investigation of continuous variation in a species" practical work

Continued on next page

General guidance

USEFUL INTERACTIVE RESOURCES

EDUQAS > GCE Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(i)	the concepts of isolation and speciation	Evolution, in terms of speciation, will not take place if the conditions under which the Hardy-Weinberg principle applies do not change. Speciation can occur due to: • genetic drift in isolated population • the founder effect of disproportionate allele frequencies in small populations • natural selection Isolation can be allopatric or sympatric and can be effected under these situations. Candidates should be able to apply their knowledge and understanding of meiosis to explain hybrid sterility e.g. in the mule, and hybrid fertility e.g. in wheat. Selection pressures can affect the survival of different phenotypes in a population e.g. selective predation, camouflage, mimicry. Only individuals which survive to reproductive age can pass on selected alleles to their offspring, thus changing the allele frequencies over time.
(j)	the separation of populations by geographical, behavioural, morphological, seasonal and other isolation mechanisms including hybrid sterility	
(k)	Darwin's theory of evolution that existing species have arisen through modification of ancestral species by natural selection	



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General guidance

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Investigation of continuous variation in a species

Specification reference: 2.6

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	1747	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

Risk assessment

Hazard	Risk	Control measure
Ivy leaves may be allergenic and generate contact dermatitis	Skin contact on handling may occur	Avoid skin contact
Berries are toxic	If leaves are collected in autumn, berries may be present	Avoid ingesting berries

Teacher / Technician's notes

Make sure the maximum width of each leaf is measured. ICT packages can be used to manipulate the data, but students must understand each step in the statistical process.

Sample data

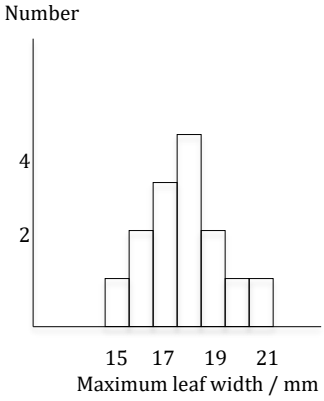
Maximum width of ivy leaf (mm) grown in	
shade	sun
17	7
16	11
18	8
21	8
19	9
20	10
17	9
19	10
18	9
17	10
18	10
18	11
16	12
15	18
18	10
mean = 17.8	mean = 10.1

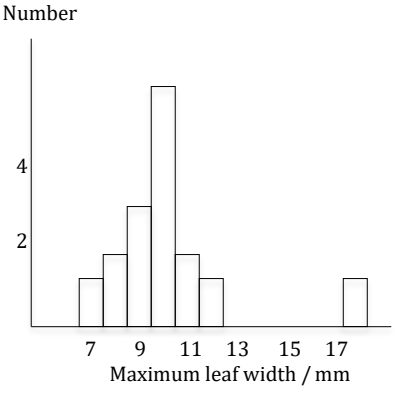
The data can be placed in size classes and frequency histograms plotted. In this example, a size class of 1 mm is suitable.

Maximum width of ground ivy leaf (mm) growing in shade	
15	1
16	2
17	3
18	4
19	2
20	1
21	1

Maximum width of ground ivy leaf (mm) growing in sun	
7	1
8	2
9	3
10	5
11	2
12	1
18	1

The frequency histograms show distributions that are approximately normal and so that data can be analysed with a Student t test.





The Student t test

This test can be used as the sample sizes are suitable (15-30) and the data are approximately normally distributed.

1. Formulate a null hypothesis: there is no significant difference between the mean maximum widths of the two populations of ground ivy leaves growing in the sun and shade.
2. Calculate the standard deviation, s.
   (i) First calculate the differences from the mean. As a check on your arithmetic, remember that, allowing for rounding errors, the sum of these deviations from the mean, $(\bar{x} - x) = 0$.
   (ii) Then square those values and add them.
   (iii) Then divide by the number in the sample -1.
   (iv) Then find the square root of this figure.

Ivy leaves growing in shade			Ivy leaves growing in the sun		
Maximum width / mm	Deviation from mean $(\bar{x} - x)$	Deviation from mean^2 $(\bar{x} - x)^2$	Maximum width / mm	Deviation from mean $(\bar{x} - x)$	Deviation from mean^2 $(\bar{x} - x)^2$
17	0.8	0.64	7	3.1	9.61
16	1.8	3.24	11	-0.9	0.81
18	-0.2	0.04	8	2.1	4.41
21	-3.2	10.24	8	2.1	4.41
19	-1.2	1.44	9	1.1	1.21
20	-2.2	4.84	10	0.1	0.01
17	0.8	0.64	9	1.1	1.21
19	-1.2	1.44	10	0.1	0.01
18	-0.2	0.04	9	1.1	1.21
17	0.8	0.64	10	0.1	0.01
18	-0.2	0.04	10	0.1	0.01
18	-0.2	0.04	11	-0.9	0.81
16	1.8	3.24	12	-1.9	3.61
15	2.8	7.84	18	-7.9	62.41
18	-0.2	0.04	10	0.1	0.01
mean = 17.8		sum = 34.4	mean = 10.1		sum = 89.75
		s_{shade} = 1.57			s_{sun} = 2.53

3. Calculate the test statistic, t, using the standard deviation and the mean:

   In this example, t is calculated using the equation $t = \frac{|\bar{x}_{shade} - \bar{x}_{sun}|}{\sqrt{\frac{s^2_{shade}}{n} + \frac{s^2_{sun}}{n}}}$

   where $\bar{x}_{shade}$ = mean reading for ivy growing in the shade
   $\bar{x}_{sun}$ = mean reading for ivy growing in the sun
   s^2_{shade} = for ivy growing in the shade
   s^2_{sun} = for ivy growing in the sun
   n = number in sample

   Substituting into the equation $t = \frac{|17.8 - 10.1|}{\sqrt{\frac{6.41}{15} + \frac{2.46}{15}}} = \frac{7.7}{0.77} = 10.0$

4. For n = 15 in each sample, the number of degrees of freedom (df) = (15-1) + (15-1) = 28.
5. For df = 28 and level of significance, p = 0.05, the critical value of t = 2.048
6. Comparing the critical value with the calculated value: the calculated value is greater than the critical value so the null hypothesis is rejected at the 5% level of significance, or p = 0.05.

7. Formulating the conclusion: the maximum mean widths of the two populations are significantly different at the 5% level of probability. The maximum width of ivy leaves is greater growing in the shade than growing in the sun.

Further work

The table below shows other suitable examples of continuous variation, of which the means may be tested with the t test.

Location	Example of character	Example of comparison to be tested	Notes
In school	length of beans	lengths of beans can be compared e.g. broad beans with kidney beans	
	student height	heights of Year 7 and Y13 students	use either all girls or all boys
		heights of Year 13 students	compare mean height of girls with boys
Coast	length of periwinkle	lengths of periwinkles growing at high water mark compared with those at low water mark	slipping is a hazard
	length of marram grass leaves	compare leaf length in grey dunes with yellow dunes	
Woodland	height of woodsage or soft rush	height of plants growing in high light intensity compared with low light intensity	

Practical Techniques

- Use sampling techniques in fieldwork
- Use ICT such as computer modelling, or data logger to collect data, or use software to process data.

	SPECIFICATION STATEMENT	COMMENT
(a)	the Human Genome Project and its extension to the 100K Genome Project	The intended purpose of the Human Genome and 100K Projects is to improve knowledge and understanding of genetic disorders and improve their diagnosis and treatment. The Human Genome Project used 'Sanger Sequencing' which sequences relatively small sections of DNA at a time (usually <1,000 bps). This process took a long time. New techniques e.g. Next Generation Sequencers (NGS) can sequence an entire genome in just a few hours. NGS is enabling scientists to study variation within the human genome amongst 100 000 people in the U.K. This is known as the 100K genome project.
(b)	the ethical issues surrounding the use of this knowledge and its application to the screening of embryos for genetic disorders e.g. cystic fibrosis, Huntington's disease, thalassaemia	Candidates should understand: • the ethical issues in terms of ownership of genetic information, potential discrimination, social stigmatisation and misuse of the data. • the identification of allele sequences has enabled scientists to scan a patient's DNA sample for mutated sequences and also to compare the sequence of DNA bases in a patient's gene to a normal version of the gene. • that the screening of embryos has been performed to detect the presence of disorders such as cystic fibrosis, Huntington's disease and thalassaemia. • that there are a number of concerns regarding the possibility of routine screening for adult onset disorders such as Alzheimer's disease and some cancers. • that screening of embryos has led to concerns over choosing alleles to ensure specific characteristics. • that there are concerns that the risks of discrimination and social stigmatization that could outweigh the benefits of testing. • the use of genetic screening and the value of genetic counselling. • the concerns regarding the ownership of genetic information and its misuse.

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List of Related External Resources

	SPECIFICATION STATEMENT	COMMENT
(c)	how the genomes from other organisms have also been sequenced including the mosquito, *Anopheles gambiae* and the *Plasmodium* parasite that it transmits and that better methods to control malaria may be developed as a result	Genome projects have also been completed for a number of other species including chimpanzees and other primates allowing scientists to look at evolutionary relationships and to conserve species in the future. • Malaria is transmitted by the mosquito *Anopheles gambiae*. Rapid evolution of insecticide resistance in the species is hampering attempts to eradicate the disease which is responsible for over a million deaths per year. • The malarial parasite, *Plasmodium sp*. has also developed multi-drug resistance. Details of the life cycle of the mosquito or the parasite are not required. • Sequencing of the *Anopheles gambiae* genome is allowing scientists to develop chemicals, which could render the mosquito susceptible again to insecticides. • Sequencing of the *Plasmodium sp*. *genome* is allowing for the development of more effective drugs.

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	SPECIFICATION STATEMENT	COMMENT
(d)	the use of PCR and electrophoresis to produce a genetic fingerprint; the forensic use of genetic fingerprinting	Polymerase Chain Reaction allows, the quantity of DNA to amplified for analysis. Gel electrophoresis can then be used in the analysis of the DNA by producing a DNA profile. These techniques can be used to identify the DNA of an individual: • An individual's DNA profile is different from that of other individuals. • Exons are regions of DNA that code for proteins. Between exons are regions of non-coding DNA called introns which contain blocks of repeated nucleotides. It is the number of times that these blocks (Short Tandem Repeats or STRs) are repeated that produces the variation in individuals. • A number of STRs are used to build up a unique fingerprint in UK. • D7S280 is an example of a STR where 'GATA' bases repeat on human chromosome 7. Different alleles of this locus have from 6 to 15 tandem repeats of this sequence. The more times it repeats, the larger the fragment of DNA will be. • The polymerase chain reaction (PCR) is used to amplify small sections of DNA rapidly. • PCR, is used to amplify the STRs by using a primer (single stranded DNA typically 6-25bp in length) which is complimentary to the start of the sequence. • PCR involves heating the DNA to 95°C to separate the two strands. • The sample is then cooled to 50-60°C to allow the primers to bind to the DNA strands (annealing). • Heating to 70°C allows a thermally stable DNA polymerase (*Taq*) to add complimentary nucleotides (extension) by forming the phosphodiester bonds in the sugar-phosphate backbone. • This cycle is repeated. After 40 cycles over a billion copies of the target sequence can be produced from just one piece of DNA. • Gel electrophoresis is a method of separating DNA fragments according to size. The gel is made from agarose (similar to agar), which contains pores in its matrix. • DNA samples are loaded into wells at one end and a voltage is applied across the gel. DNA is attracted to the positive electrode due to its negative charge on the phosphate group. Smaller fragments find it easier to migrate through the pores in the gel and so travel further than large fragments in the same time. • Fragment size can be estimated by running a DNA ladder (which contains fragments of known size) alongside. Candidates should appreciate the limitations of these techniques and the ethical issues raised in their use.

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	SPECIFICATION STATEMENT	COMMENT
(e)	the formation of recombinant DNA by insertion of foreign DNA into bacterial plasmids and the cloning of the bacteria to produce useful molecules as illustrated by insulin	The processes by which recombinant plasmids can be engineered include: • the role of restriction endonuclease enzymes and DNA ligase; • the significance of sticky ends; • the use of antibiotic resistance genes in the selection of recombinant bacteria. Candidates should be able to describe how a fragment of DNA containing a human gene can be prepared: • by using restriction endonuclease enzymes to cut out the gene from a human chromosome or • by extracting mRNA from a cell actively synthesising the required protein/ polypeptide and using reverse transcriptase and DNA polymerase to produce a double stranded cDNA fragment. Candidates should understand that producing cDNA overcomes the following problems: • locating the gene; • restriction enzymes cutting the gene into non-functional fragments; • the presence of introns; • the need for post-transcriptional processing to produce functional mRNA. Candidates should be aware of the reasons why there are concerns over the genetic engineering of bacteria to include: • the use of antibiotic resistance genes in plasmids and the ready exchange of genetic material between bacteria; • the possible transfer of antibiotic resistance genes to pathogenic bacteria; • the possible transfer/ activation of oncogenes by using fragments of human DNA.

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	SPECIFICATION STATEMENT	COMMENT
(f)	issues surrounding the use of gene technologies to produce genetically modified crops by inserting a gene from one organism into another to convey disease resistance e.g. in GM tomatoes or a desired characteristic e.g. in GM soya	Candidates should be able to evaluate the possible benefits of GM crop production against the concerns associated with the use of this technology including: Benefits: • superior keeping qualities; • higher yield; • a substantial reduction in pesticide use on crops engineered for resistance to fungal pathogens and insect attack. Concerns: • dispersal of pollen from crops engineered for herbicide resistance to wild relatives; • unknown effects of eating new protein produced in the crop; • a reduction in biodiversity.

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	SPECIFICATION STATEMENT	COMMENT
(g)	the advantages and disadvantages of using gene therapy for the treatment of disease as illustrated by muscular dystrophy	Gene therapy can be used to treat genetic disorders by inserting functional DNA sequences into cells to counteract the effect of a defective gene. • Genetic disease can be treated by replacing genes or replicating the function of genes using drugs. • There are two possible methods of replacing defective genes: somatic cell therapy and germ line therapy. Somatic cell therapy will not prevent the condition being passed on and germ line therapy is very rare. • The aim of gene therapy is to treat a genetic disease by replacing defective alleles in a patient with copies of a new DNA sequence. • Duchenne Muscular Dystrophy (DMD) is a recessive, sex linked form of Muscular Dystrophy affecting up to one in 3 500 live male births. • It is caused by a mutation in the dystrophin gene resulting in the failure to produce dystrophin, which is an important structural component of muscle tissue. The result is severe wasting of the muscles and sufferers are often wheelchair bound by the time they reach teenage years. • A drug called drisapersen has been developed which aims to treat DMD by introducing a 'molecular patch' over the exon with the mutation making the gene readable again. A shorter from of dystrophin is produced, but one thought to be more functional than the untreated version. This type of treatment is called exon skipping.

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	SPECIFICATION STATEMENT	COMMENT
(h)	the use of genomics and its possible impact on healthcare of the future	Genomics is the study of the structure, function, evolution and mapping of genomes as exemplified by the Human Genome and 100K Projects. This should enable healthcare to be improved by more accurate diagnosis, better prediction of the effect of drugs and improved design of drugs; new and improved treatments for disease. With the introduction of NGS technology it may be possible to look at tailoring therapies to individual patients where an individual could have a unique treatment for a common disease.
(i)	the issues surrounding the use of stem cells for replacing damaged tissues and organs	Candidates should be able to explain the term tissue engineering including the role of stem cells. There are ethical issues associated with obtaining stem cells from embryos and the cloning of human tissues and organs.

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	SPECIFICATION STATEMENT	COMMENT
(a)	the adaptations for gas exchange which allow an increase in body size and metabolic rate	The total oxygen requirement of an organism is proportional to its total volume. The rate of absorption of oxygen is proportional to the organism's surface area. The surface area to volume ratio of organisms decreases as size increases. The surface area to volume ratio affects the level of activity of an organism. As organisms have increased in size they have developed different adaptations to overcome this problem. *Amoeba* is a single celled organism which has a large surface area to volume ratio so that gaseous diffusion through the cell membrane is fast enough to meet its oxygen demands. Flatworms are multicellular organisms with a smaller surface area to volume ratio, but are flattened to reduce diffusion distance and so can rely on their external surface for gas exchange. Earthworms also rely on their external surface but have a circulatory system to deliver oxygen to the tissues.
(b)	gas exchange in small animals across their general body surface	
(c)	the comparison of gas exchange mechanisms in Amoeba, flatworm and earthworm	
(d)	the common features of the specialised respiratory surfaces of larger animals and the adaptation of respiratory surfaces to environmental conditions - fish have gills for aquatic environments and mammals have lungs for terrestrial environments	A comparison of the adaptations of the gas exchange systems in larger animals to their habitat is required. This includes fish, amphibians and mammals.
(e)	the need for large active animals with high metabolic rates to have ventilating mechanisms to maintain gradients across respiratory surfaces	



Select the image (left) for "Investigation into stomatal numbers in leaves" practical work



Select the image (left) for "Dissection of fish head to show the gas exchange system" practical work



Select the image (left) for "Scientific drawing of a low power plan of a prepared slide of T.S. dicotyledon leaf e.g. *Ligustrum* (privet), including calculation of actual size and magnification of drawing" practical work

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EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(f)	ventilation in bony fish and comparison of counter current flow with parallel flow	Candidates should understand the mechanism of ventilation in bony fish, the advantages of counter current flow compared to parallel flow and how the diffusion gradient is maintained across the whole gas exchange surface.
(g)	the structure and function of the human breathing system, including examination of microscope slides of T.S. lung and trachea	The human respiratory system includes larynx, trachea, bronchi, bronchioles, alveoli, pleural membranes, pleural cavity, ribs, external and internal intercostal muscles, diaphragm.
(h)	ventilation in humans and how gases are exchanged	Candidates should understand that humans ventilate their lungs by negative pressure breathing. When the external intercostal muscles contract they raise the ribcage. The outer pleural membrane is pulled out. This reduces pressure in the pleural cavity and the inner pleural membrane moves outward. This pulls on the surface of the lungs and causes the alveoli to expand. As a result alveolar pressure decreases to below atmospheric pressure and air is drawn into the lungs. Surfactant in the alveoli reduces surface tension and prevents the alveoli collapsing during exhalation. The role of internal intercostal muscles in forced exhalation is not required.



Select the image (left) for "Investigation into stomatal numbers in leaves" practical work



Select the image (left) for "Dissection of fish head to show the gas exchange system" practical work



Select the image (left) for "Scientific drawing of a low power plan of a prepared slide of T.S. dicotyledon leaf e.g. *Ligustrum* (privet), including calculation of actual size and magnification of drawing" practical work

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	SPECIFICATION STATEMENT	COMMENT
(i)	the adaptations of the insect tracheal system to life in a terrestrial environment	Insects have an impermeable cuticle to reduce water loss by evaporation. Adult insects have pairs of spiracles on segments of the thorax and abdomen. They allow air to enter the tracheae and tracheoles. Tracheoles enter cells and are the site of gas exchange. Whole body contractions aid ventilation by speeding up the movement of air through the spiracles. To conserve water, spiracles may close and open.
(j)	the structure of the angiosperm leaf	Candidates should be able to label the following structures of a dicotyledonous leaf: cuticle, upper epidermis, palisade and spongy mesophyll (with chloroplasts), vascular bundle (xylem, phloem and bundle sheath parenchyma), air spaces, lower epidermis, stomata, guard cells.
(k)	the role of leaf structures in allowing the plant to photosynthesise effectively	This should include adaptations for harvesting light energy, inward diffusion of CO_2, provision of water and removal of products of photosynthesis.
(l)	the role of the leaf as an organ of gas exchange, including stomatal opening and closing	This should include the 'malate' theory, by which stomata open and close. In the light, chloroplasts in the guard cells produce ATP through photosynthesis. ATP is used for active transport of K^+ into the guard cells. Starch is converted to malate ions. K^+ and malate ions lower water potential to below that of surrounding cells, so water moves in by osmosis. Due to the uneven thickening of the inner walls of the guard cells, as the cell increases in size, the stomatal pore opens. The reverse occurs in dark conditions.



Select the image (left) for "Investigation into stomatal numbers in leaves" practical work



Select the image (left) for "Dissection of fish head to show the gas exchange system" practical work



Select the image (left) for "Scientific drawing of a low power plan of a prepared slide of T.S. dicotyledon leaf e.g. *Ligustrum* (privet), including calculation of actual size and magnification of drawing" practical work

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EDUQAS > A LEVEL Biology > Specification from 2015

Investigation into stomatal numbers in leaves

Specification reference: 3.1

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 = $\frac{mmmm\ mnmnmn\ oo\ ssommsm\ pmn\ ofmff\ oo\ vfmv}{mmmm\ oo\ ofmff\ oo\ vfmv\ fm\ mm^2}$

Risk assessment

Hazard	Risk	Control method
Leaves, depending on species can be toxic	Could be transferred from hand to mouth	Avoid ingesting
Dissecting instruments can be sharp	Could pierce or cut skin when cutting sample	Handle with care

Teacher/ Technician's notes

- Fully-expanded leaves are used. Leaves grow by cell expansion, rather than by cell division, so the number of stomata generally remains constant throughout the leaf's development. Their separation, and therefore, their density, is not fixed until the leaf is fully expanded.
- A stoma may be only partially visible. Consequently, it is useful to make a rule concerning stomata that are only partially within the field of view e.g. they will be counted if more than half the area of the stoma is visible, or they if appear in the top half of the field of view, but not the lower half.
- When making successive counts on a single slide, care must be taken to count a stoma only once. It is useful to move the slide on the stage in an organised fashion e.g. always to the right.

Sample results

Field of view	Number of stomata	Mean number of stomata
1	5	$\frac{37}{10}$ = 3.7
2	4	
3	2	
4	2	
5	5	
6	3	
7	4	
8	3	
9	4	
10	5	

Using a x10 objective, the field of view area is likely to be 0.4 mm^2.

Stomatal density = mean number of stomata per mm^2

$$= \frac{mmmm\ mnmnmn\ oo\ ssommsm\ pmn\ ofmf\!f\ oo\ vfm\boldsymbol{v}}{mnmm\ oo\ ofmf\!f\ oo\ vfm\boldsymbol{v}\ fm\ \mathrm{mm}^2}$$

$$= \frac{3.7}{0.1} = 37.0\ \mathrm{mm}^{-2}.$$

Further work

- Repeat the procedure with different leaves, such as a xerophyte e.g. *Kalanchoë*, a hydrophyte e.g. *Nymphaea* and a plant that evolved in the presence of plenty of water, such as *Ficus*.
- Compare the distributions on the lower epidermises of these plants and account for the differences, in view of the characteristics of the habitats in which they evolved.
- Compare distributions on the upper and lower epidermis of these plants and account for the differences, considering the arrangement of the leaves on the plant.
- Compare with the distribution on the two epidermises of cereal leaves, such as maize or barley and relate them to the growth habit of the leaves.

Practical techniques

- Use of light microscope at high power and low power, including use of a graticule.

Dissection of fish head to show the gas exchange system

Specification reference: 3.1

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

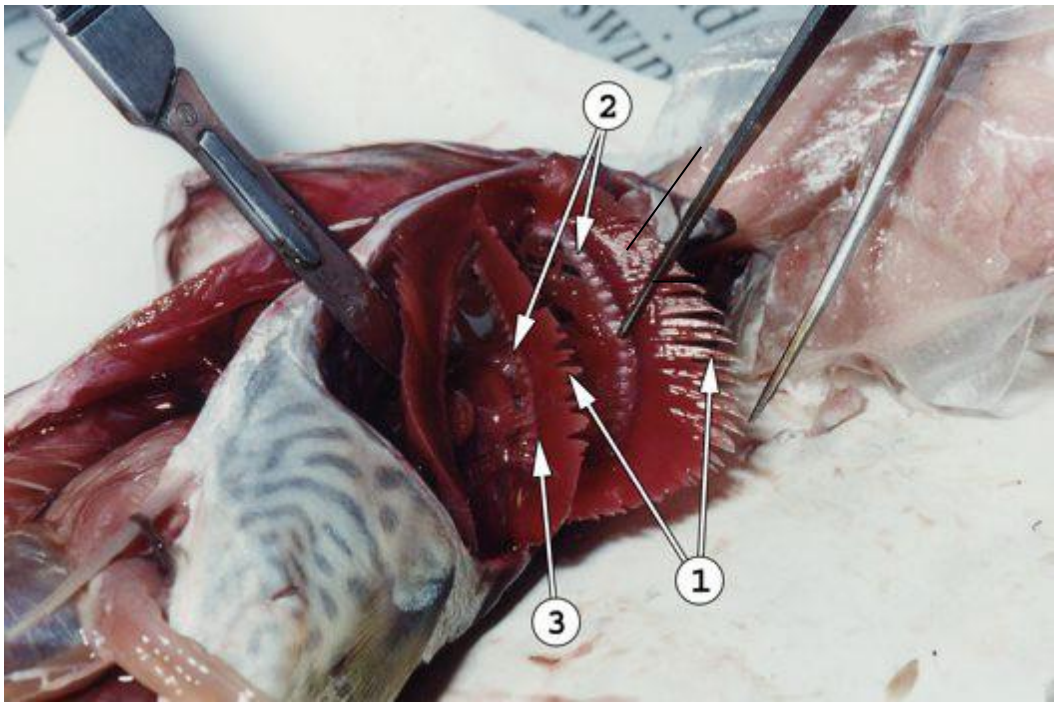
* e.g. salmon

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.



© Australian Museum

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

Risk assessment

Hazard	Risk	Control measure
Dissecting instruments are sharp	Can pierce or cut the skin while dissecting fish	Take care with instruments and cut away from body

Teacher/ Technician's notes

Any large fish head would be suitable. Students may also need disposable aprons or lab coats to protect their clothing.

Students should be encouraged to make records of each stage of their dissection either by drawings or photographs which can then be annotated.

More information is available below:

http://australianmuseum.net.au/image/fish-dissection-blue-mackerel

Further work

- If whole fish are used, then students could be encouraged to dissect the whole fish and examine other organs such as the heart, kidneys, swim bladder and reproductive organs. A useful guide can be found on the link below http://www.dec.ny.gov/docs/administration_pdf/ifnyfdlp.pdf

Practical techniques

- Safely use instruments for dissection of an animal organ, or plant organs
- Produce scientific drawing from observation with annotations.

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: 3.1

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)

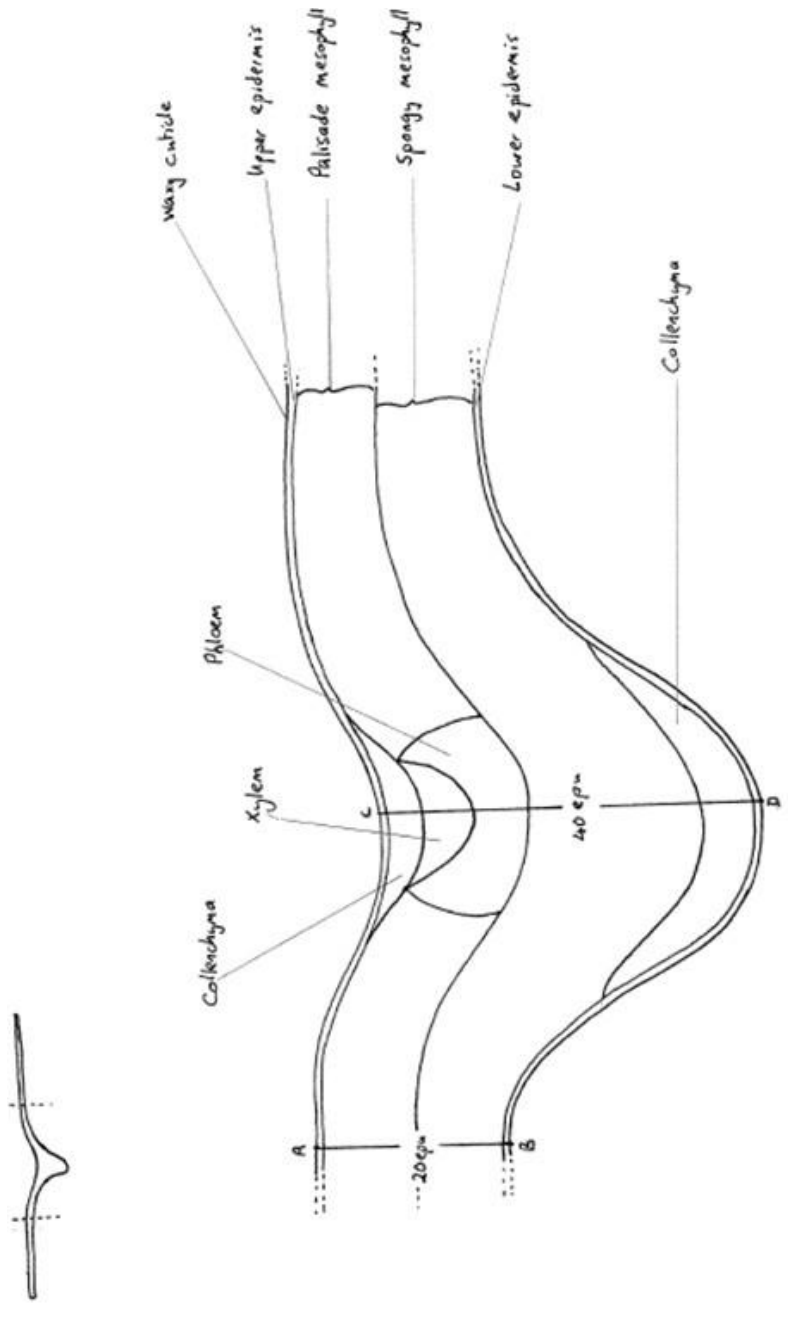
Method

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.

Teacher/ technician notes

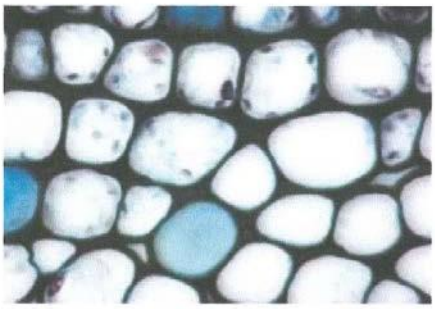
An example of the expected diagram is shown

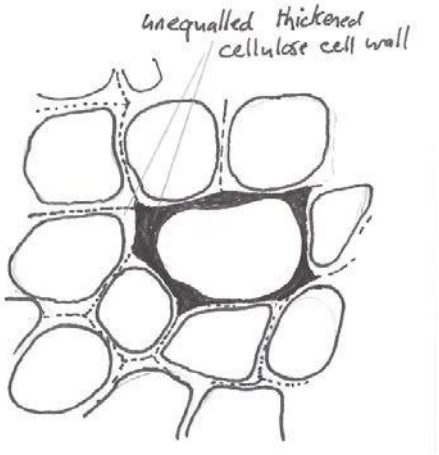


In a T.S. of a leaf students may observe a leaf vein seen in L.S. - the spiral lignin thickening of the xylem vessels gives it away!

The most difficulty students will find is in the identification of collenchyma and sclerenchyma. Collenchyma is a tissue often associated with leaves, it gives support to short lived structures. Collenchyma cells have an unequal thickening of cellulose in their walls, commonly at the corners. Collenchyma is best identified by looking for ‘star shaped’ structures between adjacent cells (the cells look rather like the polystyrene boxes used for the transport of bottles and electrical goods).The thinner areas of the cell walls in collenchyma tissue often breaks as the cells are dehydrated in the process leading to preparation for sectioning. The thickening material is made entirely of cellulose and contains no lignin. Lignin needs nitrate ions for its production, and so in metabolic terms, it is expensive to make.

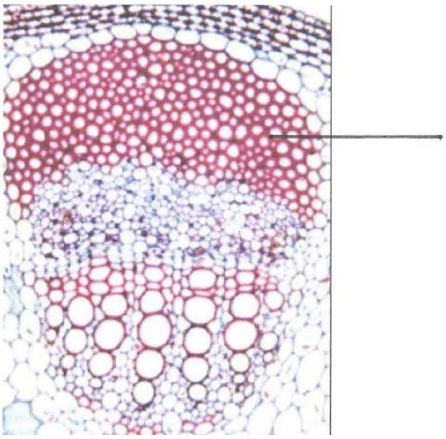
Collenchyma

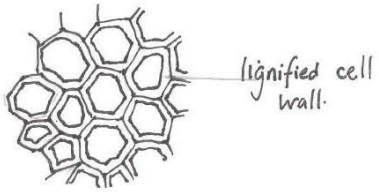




Sclerenchyma is a tissue which is used for long term support and protection. Sclerenchyma cells have lignin added to the walls and in most stains used for biological material will stain red.

Sclerenchyma. Fibres transverse section





Further work

- Drawing of some of the individual cells in the section
- Comparison with monocotyledon leaf or leaf showing adaptations to habitat, e.g. nymphaea or marram grass

Practical techniques

- Use of light microscope at high power and low power, including use of a graticule
- Produce scientific drawing from observation with annotations.

	SPECIFICATION STATEMENT	COMMENT
(a)	the similarities and differences in the vascular systems of animal groups: • Earthworm - vascularisation, closed circulatory system and pumps, carriage of respiratory gases in blood • Insects - open circulatory system, dorsal tube-shaped heart, lack of respiratory gases in blood • Fish - single circulatory system Mammal – double circulatory system	A comparison of the adaptations of the transport systems in larger animals to their habitat is required. This should include comparison of earthworms, insects, fish and mammals. Candidates should understand the advantages and disadvantages of different transport systems.
(b)	the mammalian circulatory system including the structure and function of heart and blood vessels and the names of the main blood vessels associated with the human heart	Candidates should be familiar with the internal and external structures of the mammalian heart. Comparison of the structure and function of arteries, arterioles, capillaries, venules and veins is required.



Select the image (left) for "Investigation into transpiration using a simple potometer" practical work



Select the image (left) for "Scientific drawing of a low power plan of a prepared slide of T.S artery and vein, including calculation of actual size and magnification of drawing" practical work



Select the image (left) for "Dissection of mammalian heart" practical work

Continued on next page

General guidance | Microscope calibration | Microscope section labels

USEFUL INTERACTIVE RESOURCES

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	SPECIFICATION STATEMENT	COMMENT
(c)	the cardiac cycle and the maintenance of circulation to include graphical analysis of pressure changes, the role of sino-atrial node and Purkyne/ Purkinje fibres and the analysis of electrocardiogram traces to show electrical activity	Candidates should understand the myogenic nature of cardiac muscle and the transfer of electrical impulses through the heart during a heartbeat. The electrical activity that spreads through the heart during the cardiac cycle can be detected using electrodes placed on the skin. The electrical signals can then be shown on a cathode ray oscilloscope or a chart recorder. The record produced by this procedure is called an electrocardiogram (ECG). A typical ECG consists of characteristic patterns, or waves, which correspond to particular events in the cardiac cycle. • The P wave shows the depolarisation of the atria during atrial systole. • The QRS wave shows the spread of depolarisation through the ventricles resulting in ventricular systole. • The T wave shows the repolarisation of the ventricles during ventricular diastole. No direct knowledge of abnormal ECG traces is required, but candidates may be required to apply their knowledge of ECG traces in unfamiliar contexts. No knowledge of nervous stimulation or hormonal control of heart rate required. Candidates should be able to describe and explain the pressure changes in the heart and blood vessels during the circulation of the blood. They should understand how the structure of the blood vessels results in the delivery of blood to the tissues and its return to the heart, including the role of valves in maintaining unidirectional flow of blood in the heart and veins.



Select the image (left) for "Investigation into transpiration using a simple potometer" practical work



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Select the image (left) for "Dissection of mammalian heart" practical work

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General guidance | Microscope calibration | Microscope section labels

USEFUL INTERACTIVE RESOURCES

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	SPECIFICATION STATEMENT	COMMENT
(d)	the function of red blood cells and plasma in relation to transport of respiratory gases, dissociation curves of haemoglobin of mammal (adult and foetus), including examination of microscope slides of erythrocytes	Candidates should be able to explain how CO_2 is transported from respiring cells to the lungs. Most CO_2 is transported in the plasma as hydrogen carbonate ions. The understanding of this should include: • the role of carbonic anhydrase; • the dissociation of carbonic acid to hydrogen and hydrogen carbonate ions; • the chloride shift; • the diffusion of hydrogen carbonate ions out of RBCs and into the blood plasma in a one to one exchange for chloride ions (Cl^-), via facilitated diffusion; • the reverse process occuring in the blood capillaries surrounding the alveoli. Some CO_2 is carried as carbaminohaemoglobin. Candidates should understand that the conversion of carbon dioxide to hydrogen carbonate ions results in the Bohr effect. This is explained as: • the increased H^+ from carbonic acid decreasing the affinity of haemoglobin for oxygen. • oxyhaemoglobin binds H^+ and releases oxygen which diffuses out of the red blood cells into the tissues. Candidates should be able to draw and interpret the relative position of oxygen dissociation curves.
(e)	the dissociation curves of some animals adapted to low oxygen level habitats e.g. llama, lugworm	
(f)	the Bohr effect and chloride shift	



Select the image (left) for "Investigation into transpiration using a simple potometer" practical work



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Select the image (left) for "Dissection of mammalian heart" practical work

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	SPECIFICATION STATEMENT	COMMENT
(g)	the transport of nutrients, hormones, excretory products and heat in the blood	Candidates should understand that the plasma transports nutrients, hormones, excretory products and distributes heat.
(h)	the formation of tissue fluid and its importance as a link between blood and cells	Candidates should be able to explain that at the arterial end of the capillary bed, hydrostatic pressure is higher than osmotic pressure and so water and small soluble molecules are forced through the capillary walls, forming tissue fluid between the cells. Proteins and cells in the plasma are too large to be forced out. Blood pressure falls along the capillary because of friction/ resistance of the walls and reduced volume of blood. At the venous end of the capillary bed, osmotic pressure of the blood is higher than the hydrostatic pressure and so most of the water from tissue fluid moves back into blood capillaries (down its water potential gradient). The remainder of the tissue fluid is returned to the blood, via lymph vessels.
(i)	structure of the dicotyledon root, including examination of microscope slides of T.S. dicotyledon root	Candidates should be able to draw a section of a dicotyledon root and show the position of the vascular tissue.
(j)	the absorption of water by the root	Candidates should understand the importance of root hairs in the absorption of water.
(k)	the movement of water through the root: apoplast, symplast and vacuolar pathways	
(l)	the structure and the role of the endodermis	An understanding of the role of the Casparian strip and the endodermis in the control of transport of mineral ions is required.



Select the image (left) for "Investigation into transpiration using a simple potometer" practical work



Select the image (left) for "Scientific drawing of a low power plan of a prepared slide of T.S artery and vein, including calculation of actual size and magnification of drawing" practical work



Select the image (left) for "Dissection of mammalian heart" practical work

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General guidance | Microscope calibration | Microscope section labels

USEFUL INTERACTIVE RESOURCES

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	SPECIFICATION STATEMENT	COMMENT
(m)	the detailed structure of xylem as seen by the light and electron microscope, including examination of microscope slides of T.S. dicotyledon primary stem	Candidates should be able to draw a section of a dicotyledon stem and show the position of the vascular tissue.
(n)	the movement of water from root to leaf including the transpiration stream and cohesion-tension theory	This should include the importance of the uptake of ions in producing root pressure, the cohesion tension theory and capillarity in the movement of water molecules through the xylem. Transpiration is the loss of water, as water vapour, from the leaves and shoots of plants. This gives rise to the transpiration stream.
(o)	the effect of environmental factors affecting transpiration	These should include temperature, humidity, air movement and light.
(p)	the adaptations shown by some angiosperms: hydrophytes, xerophytes, including examination of microscope slide of T.S. leaves of marram grass and water lily	Candidates should understand the term mesophyte and how mesophytes survive at unfavourable times of the year, e.g. by shedding leaves in winter. The adaptations of hydrophytes as exemplified by water lily. The adaptations of xerophytes as exemplified by marram grass.



Select the image (left) for "Investigation into transpiration using a simple potometer" practical work



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Select the image (left) for "Dissection of mammalian heart" practical work

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	SPECIFICATION STATEMENT	COMMENT
(q)	the detailed structure of phloem as seen by the light and electron microscope	
(r)	the translocation of organic materials from source to sink, including the ideas surrounding phloem transport: diffusion; cytoplasmic strands; mass flow models; experimental evidence that solutes e.g. sucrose, are carried in the phloem; use of aphids and autoradiographs	Candidates should be able to explain the mass flow model and describe some aspects of translocation which the mass flow hypothesis cannot account for. Candidates should be able to interpret and explain results obtained from investigations into phloem transport.



Select the image (left) for "Investigation into transpiration using a simple potometer" practical work



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Select the image (left) for "Dissection of mammalian heart" practical work

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Investigation into transpiration using a simple potometer

Specification reference: 3.2

Adaptations for transport

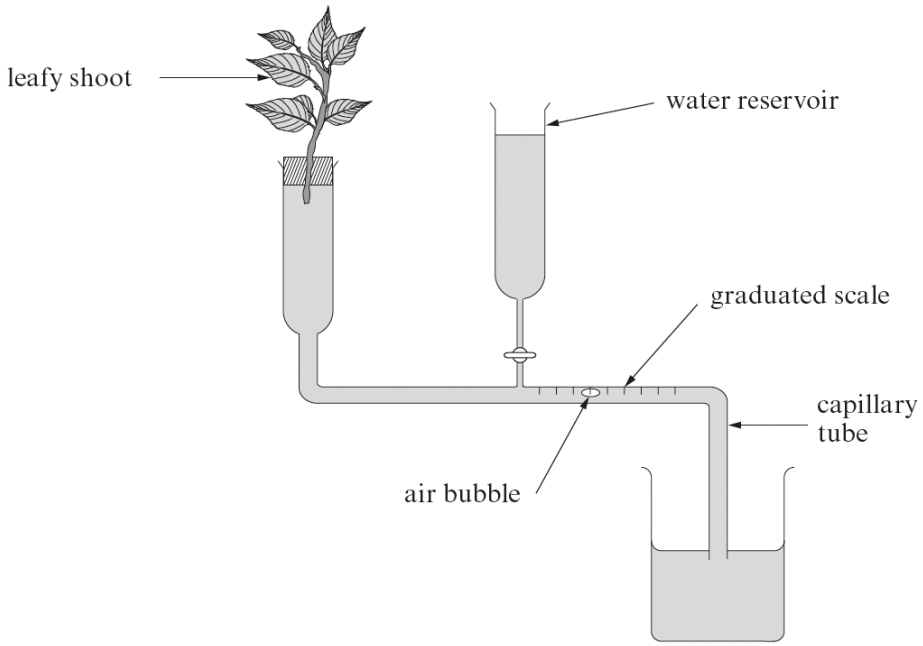
Introduction

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
Petroleum jelly
Bowl of water
Small beaker of water
Clamp stand
Stop clock
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 petroleum jelly 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.

Risk assessment

Hazard	Risk	Control measure
Scissors/ knife are sharp	Cutting hand when cutting shoot	Take care and cut away from hand

Teacher/ Technician's notes

There are various forms of simple potometers available, all of which would be suitable for this investigation. This could be carried out in large groups or as a demonstration as it is often difficult to set up. Further information is available on the link below.

http://www.nuffieldfoundation.org/practical-biology/measuring-rate-water-uptake-plant-shoot-using-potometer

There are numerous simulations available which could be used to generate results. The link for one is given below:

http://www.reading.ac.uk/virtualexperiments/ves/preloader-transpiration.html

An alternative method for determining the surface area of the leaves is:

1. On a white tile cut a 1 cm^2 square from the lamina of a leaf.
2. Weigh this 1 cm^2 of leaf.
3. Remove all the leaves from the shoot used in the experiment and weigh them.
4. Divide the weight of the leaves by the weight of 1 cm^2 of leaf. The value will be the surface area of the leaves in cm^2.

Expected results

The transpiration rate of bean plants was measured in the following conditions:

Conditions	Water loss by transpiration / m^2 / cm^3 min^{-1}			
	1	2	3	Mean
Laboratory conditions at 20 °C.	4.4	4.0	4.3	4.2
Laboratory conditions at 30 °C.	10.8	10.6	10.4	10.6
Laboratory conditions at 20 °C in moving air.	9.8	9.4	9.2	9.5
Laboratory conditions at 20 °C but plant misted to give high humidity.	1.9	2.0	2.1	2.0

Further work

- Investigate the effect on transpiration rate of temperature, light, wind or humidity.
- Compare the transpiration rates of different plant species.

Practical techniques

- Use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH)

- Use appropriate instrumentation to record quantitative measurements, such as a colorimeter or photometer

- Safely and ethically use organisms to measure
    - plant or animal responses
    - physiological functions

- Use ICT such as computer modelling, or data logger to collect data, or use software to process data.

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: 3.2

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

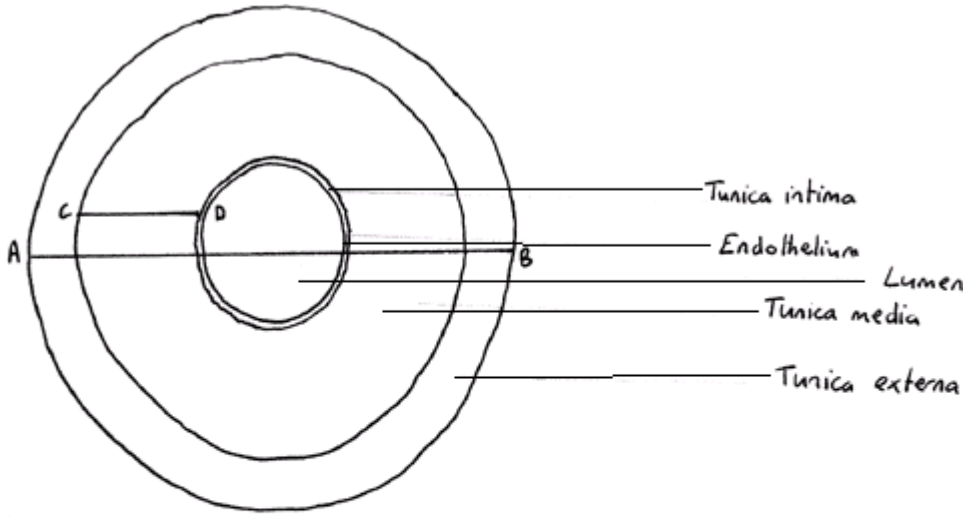
Microscope
Slide of T.S. artery/vein

Method

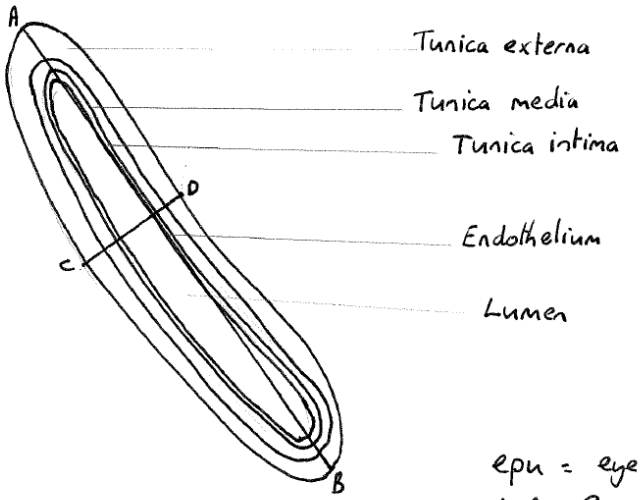
1. Examine a T.S of an artery using the x10 objective lens.
2. Draw a plan to show the distribution of tissues in the correct proportion.
3. You may need to use the x40 objective to identify some of the tissue layers.
4. Identify and label: endothelium; tunica intima/interna; tunica media; tunica externa/ 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.

Teacher/ Technician notes

Examples of the expected diagrams are shown below:



epu = eyepiece unit
A-B = 80 epu
C-D = 20 epu
Actual size A-B = 80 x 10μm = 800μm
Drawing A-B = 80 mm = 80000 μm
Magnification = $\frac{80000}{800}$ = x 100



epu = eye piece unit
A-B = 90 epu
C-D = 20 epu
Actual size A-B 90 x 10μm = 900μm
Actual size C-D 20 x 10μm = 200μm
Drawing A-B 90 mm = 90000 μm
Magnification = $\frac{90000}{900}$ = x 100

Practical techniques

- Use of light microscope at high power and low power, including use of a graticule
- Produce scientific drawing from observation with annotation.

Dissection of a mammalian heart

Specification reference: 3.2

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.

Risk assessment

Hazard	Risk	Control measure
Dissecting instruments are sharp	Can pierce or cut the skin while dissecting heart	Take care with instruments and cut away from body

Teacher/ Technicians notes

It is common for butchers to cut off the blood vessels and some of the atria from the top of the heart. If hearts are ordered from them in advance, they can try and limit this. If you order a ‘pluck’ – heart and lungs together – the heart should then be complete.

Safety of handling butcher material: Anything that a butcher sells must have been passed as ‘fit for human consumption’, but may be carrying food-poisoning bacteria. It is not necessary to wear gloves during the dissection (although the students will probably be happier doing so), but it is necessary to wash hands thoroughly after handling the material and before leaving the laboratory.

Cleaning equipment and work surfaces: Dissecting instruments are best autoclaved after use because most disinfectants attack metal instruments. Clean contaminated equipment such as dissecting boards with hot water and detergent and soak for at least 10 minutes in a freshly-prepared 1% solution of Virkon disinfectant. Wipe down working surfaces with 1% Virkon, and leave wet for 10 minutes before drying off. If you perform dissections on layers of newspaper you may protect the bench surface enough for it not to need to be disinfected.

More information is available on the link below.

http://www.nuffieldfoundation.org/practical-biology/looking-heart4

A virtual heart dissection is available on the link below:

http://www.bristol.ac.uk/anatomy/media/elearning/internet/letsdissect/letsdissectheart/index.html

Students should be encouraged to make records of each stage of their dissection either by drawings or photographs which can then be annotated.

Further work

- If a pluck is used, you can cut sections of the veins and arteries for studies of vascular structure

  http://www.nuffieldfoundation.org/practical-biology/elastic-recoil-arteries-and-veins#node-2766

  and also dissection of lung tissue

  http://www.nuffieldfoundation.org/practical-biology/dissecting-lungs

Practical techniques

- Safely use instruments for dissection of an animal organ, or plant organs
- Produce scientific drawing from observation with annotations.

	SPECIFICATION STATEMENT	COMMENT
(a)	the terms autotrophic and heterotrophic and that autotrophic organisms can be photoautotrophic or chemoautotrophic	
(b)	the terms saprotrophic/ saprobiotic, holozoic, parasitic in relation to heterotrophic organisms	
(c)	saprotrophic nutrition involves the secretion of enzymes, external digestion of food substances followed by absorption of the products of digestion into the organism, e.g. fungi	
(d)	holozoic nutrition is the internal digestion of food substances	Holozoic nutrition involves the processes of ingestion, digestion, absorption, assimilation and egestion.
(e)	nutrition in unicellular organisms, e.g. Amoeba, food particles are absorbed and digestion is carried out intracellularly	This should include the transport processes by which food particles are ingested, digested and the waste egested.
(f)	the adaptation of multicellular organisms for nutrition showing increasing levels of adaptation from a simple, undifferentiated, sac-like gut with a single opening, e.g. Hydra, to a tube gut with different openings for ingestion and egestion and specialised regions for the digestion of different food substance	Many multicellular organisms have evolved a specialised gut for the digestion of food. In simple organisms, the gut is undifferentiated and is often a sac-like structure with a single opening, e.g. *Hydra.* More advanced organisms, with a varied diet, have evolved a tube gut that is divided into various parts along its length and each part is specialised to carry out particular functions.
(g)	the adaptations of the human gut to a mixed, omnivorous diet that includes both plant and animal material, including examination of microscope slides of duodenum and ileum	The structure, function and relative proportions of the layers of the gut wall (epithelium, mucosa, submucosa, muscle layers, serosa) in the different parts of the gut. Details of the roles of the various parts of the human gut and associated glands for digestion and absorption are required. This would include: buccal cavity, teeth, tongue, salivary glands, oesophagus, stomach, liver (secretes bile via the gall bladder and bile duct), duodenum, pancreas, ileum, colon, rectum and anus.

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	SPECIFICATION STATEMENT	COMMENT
(h)	the efficient digestion of different food substances requiring different enzymes and different conditions	Animals with a varied diet require more than one type of enzyme to carry out the digestion of the different food substrates and usually more than one type of enzyme is needed for the complete digestion of a particular food type. The enzymes and other secretions produced in the various parts of the gut and their roles. These should include • The roles of saliva and mucus. • The chemical digestion of starch and glycogen by salivary amylase and pancreatic amylase to maltose, which is hydrolysed further by maltase to alpha-glucose. • Chemical digestion of lactose (by lactase) and sucrose (by sucrase). • Sites of production of the carbohydrases; gut regions where they function, and usual pH levels. • Proteases called endopeptidases hydrolyse (non-terminal) peptide bonds within the protein/polypeptide molecule, adjacent to specific amino acids, and form peptides. Then exopeptidases hydrolyse the terminal peptide bonds of peptides, from the free amino end or the free carboxyl end. • Pepsin is an endopeptidase secreted by gastric glands as inactive pepsinogen. Trypsin is an endopeptidase secreted by the pancreas as inactive trypsinogen. • These proteases are secreted as inactive enzymes and are subsequently activated (including the role of enterokinase and hydrochloric acid). • The sites of production of these peptidases; gut regions where they function, and usual pH levels. • The role of bile salts in emulsifying lipid globules. • Action of pancreatic lipase in the small intestine: hydrolysis of triglycerides to monoglycerides/glycerol and fatty acids. Candidates need to understand the site and mechanism of final digestion and absorption of dipeptides, disaccharides, fatty acids and glycerol. This should include: • Digestion on the membranes of epithelial cells covering the villi • Absorption of amino acids by active transport into the epithelial cells and facilitated diffusion into the capillaries • Absorption of glucose and other monosaccharides into epithelial cells by co-transport (with sodium ions) and then into capillaries by facilitated diffusion. • Glucose and amino acids are transported via the hepatic portal vein to the liver. • Fatty acids and glycerol diffuse into the epithelial cells, where they are reassembled into triglycerides. These then pass into the lacteal and are carried via the lymphatic system to the blood. No detail of the fates of absorbed nutrients is required.

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	SPECIFICATION STATEMENT	COMMENT
(i)	the adaptations of herbivore guts and dentition, in particular ruminants to a high cellulose diet and the adaptations of carnivore guts and dentition to a high protein diet, including examination of skulls and dentitiion of a herbivore and a carnivore	The adaptations of herbivores and carnivores include the following: Herbivore (high cellulose diet): functions of the different teeth; the horny pad on the upper jaw; the diastema; loose articulation of the lower jaw; the rumen; cellulose digesting bacteria; chewing the cud; a relatively long gut. Carnivore (high lipid and protein diet, with a high energy content): functions of the different teeth including carnassials; relatively short gut.
(j)	parasites; highly specialised organisms that obtain their nutrition at the expense of a host organism e.g. *Taenia* and *Pediculus*, including examination of specimens and slides of tapeworm e.g. *Taenia*	Parasites are organisms that live on (ectoparasites) or in (endoparasites) another organism, called the host, and obtain nourishment at the expense of the host. This usually causes damage to the host organism. The head louse (*Pediculus*) is an example of an ectoparasite which feeds by sucking blood from the scalp of the host. It has claws to hold onto the hairs and lays eggs which are glued to the base of hairs. Transfer between hosts is by direct contact. The pork tapeworm, *Taenia solium* is an example of an endoparasite. The adult tapeworm lives in the gut of humans - the primary host; a larval form develops in pigs – the secondary host. Infection of humans occurs when a person eats undercooked pork containing live larval forms. The gut is a hostile environment due to the presence of various secretions and peristalsis. The tapeworm has adapted to living in the gut by having a thick cuticle, produces anti-enzymes, and a scolex to attach to the gut wall. *Taenia solium* has a reduced gut and feeds by absorbing pre-digested nutrients through its cuticle. To increase the chances of infecting a secondary host it produces large numbers of eggs that pass out in the faeces.

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	SPECIFICATION STATEMENT	COMMENT
(a)	the concept of homeostasis and its importance in maintaining the body in a state of dynamic equilibrium	Homeostasis describes the mechanisms by which a constant internal environment is achieved, e.g. core body temperature, glucose levels, and solute potential. Candidates should appreciate the importance of homeostasis i.e. that cells of the body can function efficiently, independently of fluctuations in the conditions of the external environment; and they are provided with constant conditions, even during different levels of activity of the organism.
(b)	the role of negative feedback in restoring conditions to their original levels and the role of positive feedback in enhancing the size of the stimulus	There is a set point/norm/normal level for each condition determined by a control centre and that deviations from the set point are corrected by negative feedback so the set point is restored. Negative feedback uses a detector/receptor, which monitors the condition and provides input to the control centre/ coordinator, which evaluates the information and provides output to an effector, which makes a response designed to take away the deviation, i.e. restore the set point/norm.
(c)	the structure of the mammalian kidney and the nephron, including examination of microscope slides and electron micrographs of kidney	Candidates should recognise the main regions of a mammalian kidney and its blood supply and know the position of a nephron. Candidates should be able to label a diagram of a nephron and recognise these structures on microscope slides and electron micrographs: glomerulus, Bowman's capsule, proximal and distal convoluted tubules; loop of Henlé, vasa recta, collecting duct, afferent and efferent arterioles. The glomerulus, Bowman's capsule (including podocytes) and the afferent and efferent arterioles are involved in ultrafiltration of the blood and produce glomerular filtrate in the first stage in the production of urine.



Select the image (left) for "Dissection of kidney" practical work

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	SPECIFICATION STATEMENT	COMMENT
(d)	the functions of the mammalian kidney including nitrogenous excretion and water regulation	Osmoregulation is the control of the water content and solute composition of body fluids e.g. blood, tissue fluid and lymph and excretion is the elimination of waste products of metabolism from an organism. Amino acids cannot be stored and surplus amino acids, not used for the synthesis of proteins and other nitrogenous compounds are deaminated in the mammalian liver. The amino group forms ammonia which is converted to less toxic urea and is then transported into the blood plasma.



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	SPECIFICATION STATEMENT	COMMENT
(e)	the adaptations of the cells of the proximal tubule for reabsorption	The adaptations of the structure of the proximal convoluted tubule (PCT) in relation to selective reabsorption include: large surface area (due to its length and large number per kidney), cuboidal epithelial cells with microvilli and basal channels; numerous mitochondria; tight junctions preventing seepage of reabsorbed materials back into the filtrate, and close association with peritubular capillaries. The process of selective reabsorption from the filtrate in the PCT to the peritubular capillaries of the following: • all of the filtered glucose (below the kidney threshold) and amino acids by secondary active transport • using a co-transport mechanism with Na^+; • most of the mineral ions by active transport; • most of the water by osmosis; • some filtered proteins (and some urea) by diffusion. The filtrate at the end of the PCT is isotonic with blood plasma. The loops of Henlé concentrate salts in the tissue fluid of the medulla and that causes an osmotic flow of water out of the collecting ducts and distal convoluted tubules. This is achieved by: • actively pumping Na^+ and Cl^- out of the filtrate in the ascending limb into the tissue fluid creating a low water potential • the ascending limb being impermeable to water while the descending limb is permeable; • the low water potential of the tissue fluid means that water leaves the descending limb by osmosis and is carried away by the vasa recta; • the contents of the descending limb becoming more concentrated as they reach the tip of the loop of Henlé due to the loss of water; • as the filtrate passes up the ascending limb it becomes more dilute due to loss of ions; • an osmotic gradient is maintained down to the tip of the loop of Henlé • this is called a hair-pin counter-current multiplier



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	SPECIFICATION STATEMENT	COMMENT
(f)	the contribution of the endocrine glands to homeostatic balance as illustrated by the role of the posterior pituitary gland in the secretion of antidiuretic hormone	ADH secretion has a role in negative feedback restoring the normal osmotic concentration in the blood. Osmoreceptors in the hypothalamus detect the concentration of the blood plasma. The hypothalamus secretes ADH which is stored in the posterior lobe of the pituitary. If the blood plasma concentration is too high, ADH is secreted and increases the permeability of the distal convoluted tributes and collecting ducts of kidneys to water. ADH enables more concentrated urine to be formed: • ADH makes the plasma membranes of the distal convoluted tubule cells and collecting duct cells more permeable to water; • ADH causes aquaporins to become incorporated in the plasma membranes, from within the cytosol; • water is reabsorbed, by osmosis, from the filtrate into the surrounding, hypertonic, tissue fluid (and hence blood capillaries) around the DCTs and collecting ducts; • the urine reaching the bottom of the collecting ducts has a concentration close to the concentration of the tissue fluid near the bottom of the loop, that is, hypertonic to the general body fluids.
(g)	the role of antidiuretic hormone	
(h)	the effects of kidney failure and its potential treatments	Treatments can be used to balance the fluids in blood in the case of kidney failure. These include: medication to control blood potassium and calcium levels; a lower protein diet; drugs to reduce blood pressure; dialysis and kidney transplant.



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	SPECIFICATION STATEMENT	COMMENT
(i)	the need for different excretory products and adaptations of the loop of Henle in different environments	The mode of life and the environment in which an animal lives plays a part in the nitrogenous waste produced and different animals deal with its disposal in different ways, e.g. many freshwater animal species produce ammonia, birds, reptiles and insects produce uric acid and mammals produce urea. The relative length of the loop of Henlé is adapted to the typical availability of water in the environment of species of mammal, e.g. beavers, humans, and the kangaroo rat.



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Dissection of a mammalian kidney

Specification reference: 3.4

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.

Risk assessment

Hazard	Risk	Control measure
Dissecting instruments are sharp	Can pierce or cut the skin	Care with use

Teacher/ Technician's notes

Safety of handling butcher material: Anything that a butcher sells must have been passed as 'fit for human consumption', but may be carrying food-poisoning bacteria. It is not necessary to wear gloves during the dissection (although the students will probably be happier doing so), but it is necessary to wash hands thoroughly after handling the material and before leaving the laboratory.

Cleaning equipment and work surfaces: Dissecting instruments are best autoclaved after use because most disinfectants attack metal instruments. Clean contaminated equipment such as dissecting boards with hot water and detergent and soak for at least 10 minutes in a freshly-prepared 1% solution of Virkon disinfectant. Wipe down working surfaces with 1% Virkon, and leave wet for 10 minutes before drying off. If you perform dissections on layers of newspaper you may protect the bench surface enough for it not to need to be disinfected.

A virtual kidney dissection is available on the link below:

http://www.bristol.ac.uk/anatomy/media/elearning/internet/letsdissect/letsdissectkidneytutor/index.html

Students should be encouraged to make records of each stage of their dissection either by drawings or photographs which can then be annotated.

Practical techniques

- Safely use instruments for dissection of an animal organ, or plant organs
- Produce scientific drawing from observation with annotations.

	SPECIFICATION STATEMENT	COMMENT
(a)	components of a nervous response; from the detection of internal and external stimuli by receptors to the response by the effector	In mammals, responses to many external and internal stimuli involve the reception of information and its transfer from a receptor to an effector via the nervous system or as hormones via the blood. The gross structure of the human nervous system includes: the central nervous system, composed of the brain and spinal cord and the peripheral nervous system. Candidates should be able to label a diagram of a transverse section through the spinal cord, showing: central canal, grey matter, white matter, dorsal root, ventral root, sensory neurone, dorsal root ganglion, connector neurone, motor neurone, meninges. A simple reflex is an inborn response to a stimulus and is rapid, automatic and beneficial. The transmission of a nerve impulse along a three-neurone reflex arc involves the following: stimulus → receptor → **sensory neurone** → **relay neurone (in CNS)** → **motor neurone** → effector → response. In simple organisms, such as *Hydra*, the sense receptors respond to a limited number of stimuli and so the number of effectors is small. Their nerve net system consists of simple nerve cells with short extensions joined to each other and branching in a number of different directions.
(b)	the main areas of the spinal cord, including examination of T.S. spinal cord	
(c)	the basic pattern of spinal nerves in relation to the spinal cord including the dorsal root and ventral root	
(d)	the simple reflex arc as the basis for rapid, protective, involuntary actions	
(e)	the structure of a nerve net in Cnidaria and be able to draw comparisons with the nervous systems in more complex organisms	

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	SPECIFICATION STATEMENT	COMMENT
(f)	the structure of a motor neurone including drawing and labelling of diagram	In mammals, there are three functional types of neurones: sensory, motor and relay (connector or association). Candidates should be able to draw a labelled diagram of a mammalian motor neurone; dendrites, cell body/ centron, nucleus, axon, myelin sheath of Schwann cells, nodes of Ranvier, axon endings/terminals, synaptic end bulbs. They should also be able to describe the functions of; dendrites; cell body/ centron, nucleus, axon, myelin sheath, Schwann cell, nodes of Ranvier, axon endings/ terminals, synaptic end bulbs.

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	SPECIFICATION STATEMENT	COMMENT
(g)	the nature and transmission of the nerve impulse	Candidates should be able to understand how a nerve impulse is transmitted along an axon as shown by the passage of an action potential by an oscilloscope trace. They should be able to describe the propagation of the action potential to include: • to maintain the resting potential, sodium-potassium pumps actively transport sodium ions out of the neurone and potassium ions into the neurone ($3Na^+$ out for each $2K^+$ in, per ATP hydrolysed); • voltage-gated Na^+ channels are closed but some K^+ channels allow K^+ to 'leak' out of the action; • large protein anions and organic phosphates (e.g. ATP^{4-}) remain in the cytoplasm thus producing a negative potential difference across the membrane at around -70mV relative to the exterior of the axon; • a change in the voltage across the axon membrane (a stimulus) opens the Na^+ channels so that Na^+ flood in and depolarises the axon to about +40mV – this is the action potential; • repolarisation occurs as the Na^+ channels close and K^+ channels open resulting in K^+ flooding out of the axon and reducing the potential difference across the axon membrane – an overshoot results in the membrane being hyperpolarised; • during the refractory period the concentrations of K^+ and Na^+ ions are restored to that of the resting potential – during this time the axon cannot transmit another action potential and that this ensures that transmission is in one direction only. The size of the action potential is independent of the size of the stimulus, as long as the stimulus exceeds the value of the threshold potential an action potential is generated; this is called the 'all or nothing' law. Candidates should be able to explain how saltatory propagation increases the rate of transmission of a nervous impulse.
(h)	how to analyse oscilloscope traces showing the passage of an action potential	

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	SPECIFICATION STATEMENT	COMMENT
(i)	factors affecting speed of conduction of a nervous impulse in other organisms	Different factors affect the speed of conduction. These include: • temperature - the higher the temperature, the faster the speed; • axon diameter - the larger the diameter, the faster the speed; • myelin sheath - only vertebrates have a myelin sheath surrounding their neurones. The voltage-gated ion channels are found only at the nodes of Ranvier, and between the nodes the myelin sheath acts as a good electrical insulator. The action potential can therefore jump large distances from node to node (1 mm), a process that is called saltatory propagation. This increases the speed of propagation dramatically, so while nerve impulses in unmyelinated neurones have a maximum speed of around 1 m/s, in myelinated neurones they travel at 100 m/s.
(j)	the structure and role of a synapse	Candidates should be able to label a diagram of a synapse and be able to explain the role of the following in synaptic transmission: pre and post-synaptic membranes; synaptic vesicles, neurotransmitters (e.g. acetylcholine), synaptic cleft, Ca^{2+} channels; receptors on post-synaptic membrane. They should be able to explain how the merging of impulses is prevented including: the effect of cholinesterase, active transport of Ca^{2+} out of the synaptic knob and reabsorption of neurotransmitter molecules. Candidates should be able to describe the effects of chemicals on synaptic transmission as illustrated by agonists such as organophosphorus insecticides acting as cholinesterase inhibitors.
(k)	the process of synaptic transmission	
(l)	the effect of chemicals e.g. organophosphates and psychoactive drugs on the transmission of impulses	

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	SPECIFICATION STATEMENT	COMMENT
(a)	the meaning of the following terms: pathogenic, infectious, carrier, disease reservoir, endemic, epidemic, pandemic, vaccine, antibiotic, antigen, antibody, resistance, vector, toxin, antigenic types	Candidates should know the meanings of these terms and be able to use them correctly in explaining their answers: • pathogenic: an organism that causes damage to its host • infectious: a disease that may be passed or transmitted from one individual to another. • carrier: a person who shows no symptoms when infected by a disease organism but can pass the disease on to another individual • disease reservoir: where a pathogen is normally found; this may be in humans or another animal and may be a source of infection. • endemic: a disease, which is always present at low levels in an area • epidemic: where there is a significant increase in the usual number of cases of a disease often associated with a rapid spread. • pandemic: an epidemic occurring worldwide, or over a very wide area, crossing international boundaries and usually affecting a large number of people • vaccine: uses non-pathogenic forms, products or antigens of micro-organisms to stimulate an immune response which confers protection against subsequent infection. • antibiotics: substances produced by microorganisms which affect the growth of other microorganisms. • antibiotic resistance: where a microorganism, which should be affected by an antibiotic, is no longer susceptible to it. • vector: a living organism which transfers a disease from one individual to another. • toxin: is a chemical produced by a microorganism which causes damage to its host. • antigenic types: organisms with the same or very similar antigens on the surface. Such types are sub groups or strains of a microbial species which may be used to trace infections. They are usually identified by using antibodies from serum.
(b)	the human body acting as a host to other living organisms	Many organisms live in or on the human body in symbiotic or parasitic relationships. These may help to defend us against disease or cause disease.

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	SPECIFICATION STATEMENT	COMMENT
(c)	the following diseases in terms of: the types of organisms; source of infection; tissue affected; mode of transmission; prevention; control methods and treatment, including vaccines: Bacterial infections cholera; tuberculosis Viral infections: smallpox; influenza Protoctistan infections: malaria	Candidates should know and understand how different types of disease are transmitted, how they affect us and how they are treated: • Cholera is caused by a Gram negative bacterium which is endemic in some areas of the world. Its toxins affect the gut lining causing watery diarrhoea leading to severe dehydration and frequently death. Humans act as reservoirs or carriers and contaminate water supplies in which the organism is transmitted, although it only multiplies in the human host. Cholera prevention is by the treatment of water, good hygiene and the provision of clean drinking water. Antibiotic treatment is possible but treatment is largely by rehydration; vaccine (killed organism or possibly genetically engineered) may provide temporary protection. • Tuberculosis is a bacterial disease that is again on the increase, partly due to the link with the HIV epidemic. It can be spread rapidly in overcrowded conditions and is transmitted in airborne droplets when infected people cough and sneeze. The most common form of TB attacks the lungs and neck lymph nodes. Symptoms include coughing, chest pain and coughing up blood. Tuberculosis is prevented by a BCG vaccination programme for children. Treatment involves a long course of antibiotics. • Smallpox is the only organism that humans have intentionally made extinct (outside specialist laboratories). It is caused by the virus *Variola major* and can have a 30 to 60% fatality rate. A successful immunisation program was based on its low rate of antigenic variation/ mutation and the highly immunogenic nature of its component antigens. This meant that the vaccine was highly effective. In addition, there was no animal reservoir and people were keen to be immunised because of the devastating effects of the disease. • Influenza is caused by a virus of which there are three main sub-groups. Within each sub-group there are many different antigenic types. It infects cells lining the upper respiratory tract causing sore throat, coughing and fever. Sufferers spread the disease by droplet infection. Prevention includes quarantine and hygiene but influenza's mode of spread is difficult to control. Antibiotics are ineffective against influenza and are only used to treat the symptoms of secondary bacterial infection. Annual vaccination programmes are available but due to the number of types, together with the emergence of new types, they are not always effective. • Malaria is caused by *Plasmodium spp*., a protoctistan parasite, endemic in some sub-tropical regions. The disease is caused mainly by two species within which are many antigenic types. The organism initially invades liver cells and then multiplies in red blood cells which burst, releasing more parasites and causing severe bouts of fever. Female mosquitoes, feeding on blood taken, act as vectors to transmit the parasite to new victims. The prevention of malaria relies on knowledge of the life cycle of both the vector and the parasite in order to exploit their weak points. A variety of methods are used either to prevent transmission (prevent biting by use of nets, clothing, insect repellent) or to destroy populations of the vector. The mosquito larvae are aquatic and can be eaten by introduced fish, or killed by drainage of breeding sites or the spraying of oil on the water surface. The adults are killed with insecticides, with bacterial infections or by sterilisation. Each of these control measures has advantages and disadvantages. Drug treatment is available but mainly to reduce the chances of infection. Vaccines have proved difficult to develop because the malarial parasite mutates and there are different antigenic types. *Plasmodium* is affected by drugs when outside the cells in the blood but these have limited effectiveness and have side effects; resistance is an increasing problem. Antibodies also are only effective against the parasite when outside body cells so limiting the target stages for a vaccine.

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	SPECIFICATION STATEMENT	COMMENT
(d)	the relationship between the pathogenicity of viruses and their mode of reproduction	Candidates should understand that viruses are intracellular parasites that use a cell's metabolic pathways to produce more virus particles. They cause pathogenic effects in several ways including: • cell lysis when they escape from cells to infect other cells/ organisms (shedding) • production of toxic substances • cell transformation where they can trigger cells to become cancerous • suppress the immune system (e.g. HIV).

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	SPECIFICATION STATEMENT	COMMENT
(a)	the control of bacterial infections by antibiotics which can be bacteriostatic or bactericidal in their mode of action and that antibiotics can be broad or narrow spectrum	Different types of antibiotics can be used to treat bacterial diseases. Antibiotics either prevent growth (bacteriostatic) or kill bacteria (bactericidal), depending on which aspect of bacterial metabolism is affected. Antibiotics used medically affect bacterial metabolism but do not interfere with the host cell metabolism.
(b)	the modes of action of penicillin and tetracycline and how the structure of the bacterial cell wall in Gram negative bacteria affords protection against many antibiotics and immune defences	The cell wall of bacteria has a unique structure: • it contains peptidoglycan (murein) consisting of molecules of polysaccharide cross linked by amino acid side chains. The cross linking provides strength and the wall protects against osmotic lysis. • it is surrounded by an outer layer of lipoprotein and lipopolysaccharide. • the Gram reaction reflects the more complex structure of Gram negative cell walls • the presence of the extra layers protects the cells from the action of some antibacterial agents such as lysozyme and penicillin. Candidates should know and understand how penicillin and tetracycline affect bacteria: • Penicillin affects the formation of cross linkages in the cell wall during the growth and division of bacterial cells. It does this by binding to and inhibiting the enzyme responsible for the formation of cross-links between molecules of peptidoglycan. The wall is weakened so when osmotic changes occur, the cells lyse. Consequently, penicillin is more effective against Gram positive organisms than Gram negative due to the difference in the structure of the cell wall. • Tetracycline is an antibiotic that affects protein synthesis, a metabolic process common to all bacteria, and is effective against a broader range of bacteria. It acts by acting as a competitive inhibitor of the second anticodon-binding site on the 30S subunit of bacterial ribosomes and prevents the binding of a tRNA molecule to its complementary codon. In this way tetracycline inhibits the translation stage of protein synthesis. Viruses are not affected due to the absence of metabolic pathways.
(c)	how the overuse of antibiotics has resulted in the spread of antibiotic resistance amongst pathogenic bacteria	The overuse of antibiotics has resulted in the spread of antibiotic resistant strains of bacteria: • bacteria divide rapidly under optimum conditions and have a high mutation rate; • naturally occurring mutations that confer resistance to antibiotics have given these bacteria a selective advantage in the presence of antibiotics; • overuse of antibiotics has resulted in the accidental selection of bacterial strains that are completely unaffected by some antibiotics; • in the absence of antibiotics they no longer have an advantage over non-mutated forms but if they cause an infection are becoming increasingly difficult to control.

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	SPECIFICATION STATEMENT	COMMENT
(a)	the natural barriers in the body which reduce the risk of infection, including natural skin flora, connective tissue, localised inflammation, phagocytosis, clotting, tears, mucus and ciliated epithelium	Natural barriers reduce the risk of infection: • the skin is a tough barrier and vitamin C is needed to maintain strong connective tissue • skin flora offer protection by competing with pathogenic bacteria and unlike these bacteria, the flora is not easily removed by washing • blood clotting to seal wounds • inflammation to localise breaks in the barrier • phagocytosis to destroy invading microbes • ciliated mucous membranes that trap microbes in inhaled air • lysozyme in tears, saliva and stomach acid that kills bacteria.

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SPECIFICATION STATEMENT		COMMENT
(b)	how specific immune responses are developed as a result of exposure to foreign antigens	A specific immune response develops as a result of antigens being recognised as foreign to the body: Humoral response involves: • B lymphocytes, which originate from stem cells in the bone marrow, and mature in the spleen and lymph nodes; • each B lymphocyte has receptors for the detection of its specific antigen; • activation stimulates the proliferation of antibody producing cells, plasma cells, and memory cells; • memory cells remain in the circulation ready to divide if the same antigen is encountered again; • antibodies are proteins (globulins) which are specific to the antigen with which they bind to form an antigen-antibody complex; • antibodies are Y- shaped, formed from four polypeptide chains and have two binding sites; • an antigen-antibody complex renders the antigen inactive in some way, such as through agglutination, which increases the rate of engulfment by phagocytes. Cell-mediated immune response involves: • T lymphocytes, which also originate from stem cells in the bone marrow, but are activated in the thymus gland; • detection of the corresponding specific antigen causes the proliferation of T lymphocytes; • there are many subpopulations of T cells including: effector cells (T killer or cytotoxic T lymphocytes) which cause lysis of the target cells; helper T cells which cooperate with B lymphocytes to initiate an antibody response; memory cells which remain dormant until the host is next exposed to the antigens; • cell-mediated defences include the activation of phagocytes, antigen-specific killer / cytotoxic T-lymphocytes; • activation of B cells involves the release of various chemicals called cytokines in response to an antigen. Candidates should understand the differences between the primary and secondary immune responses to include: Primary immune response • following first exposure to a foreign antigen there is a latent period during which antigen presenting cells (including macrophages) carry out phagocytosis and incorporate foreign antigen into their cell membranes; • T helper cells detect these antigens and secrete cytokines which stimulate B cells and macrophages; • B cells are activated and undergo clonal expansion – some then differentiate to become antibody secreting plasma cells with short lives and others to become long lived memory cells that retain the ability to undergo mitosis; • a low level of antibody is secreted which over a period of 2 – 3 weeks clears the infection and symptoms disappear. Secondary immune response • following re-exposure to the same antigen there is a very short latent period due to the presence of memory cells; • only a very small amount of antigen is required to stimulate rapid production of plasma cells; • antibody levels increase to between 10 and 100 times greater than the initial response and in a very short time; • antibody levels stay high for longer and no symptoms develop.
(c)	humoral immune responses, involving the production and secretion of antigen-specific antibodies	
(d)	cell -mediated immunity, by direct cell contact involving the destruction of pathogens, infected cells and cancerous cells	
(e)	the role of T lymphocytes and B lymphocytes in cell-mediated and humoral immune responses	

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	SPECIFICATION STATEMENT	COMMENT
(f)	specific immune responses induced naturally or artificially to produce an active, long-lived response or acquired passively resulting in short-term protection	Immunity, whether humoral or cell mediated, can be acquired either actively or passively: • Active immunity is where the individual produces antibodies and may be: - natural if it follows natural infection - artificial when it follows vaccination e.g. against *Rubella*. - protection is long-lasting due to the production of antigen-specific memory cells. • Passive immunity is where the individual receives antibodies produced by another individual and may be: - natural when antibodies are transferred to the foetus via the placenta, or to the baby in breast milk. - artificial when pre-synthesised antibody is injected into an individual e.g. tetanus antitoxin. - protection is short lived because the antibodies are recognised as non-self and are destroyed and no memory cells are produced.
(g)	the principles of the active immune response can be used medically to immunise against disease, e.g. rubella, without infection by the pathogen	Candidates should understand the sequence of events in the development of a protective immune response and that this can be used to develop artificial immunity through exposure to a vaccine.
(h)	the use of injection of antibodies to provide passive, emergency treatment against an infection, e.g. the treatment of rabies	In an emergency, antibodies can be injected to provide rapid protection against a pathogen, e.g. in cases of rabies, and that this allows time for the person's immune system to develop an active immune response. Injections of antibodies are also used with people who do not develop a strong immune response to vaccination or who have a weakened immune system.

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	SPECIFICATION STATEMENT	COMMENT
(i)	the different levels of effectiveness of immunisation programmes against different diseases	Pathogens that exhibit no or low levels of antigenic variation/ mutation (e.g. *Rubella*) are more likely to be protected by a single round of immunisations than an organism that has many antigenic types and mutates frequently (e.g. *Influenza*). Protection against these organisms requires repeated immunisation against the most common antigens and even then is not 100% effective. The antigens used in a vaccine must be highly immunogenic and stimulate a protective immune response that is specific to the pathogen or antigen. Different types and programmes of vaccination are used to increase the chance of a person developing protective, long-lasting immunity.
(j)	the ethics which must be taken into consideration when designing vaccination programmes	Immunisation has been shown to be the only way to protect people against some diseases. When designing and implementing vaccination programmes consideration must be made of a number of factors, including: • cost vs effectiveness of the vaccine • protection of the individual compared to protection of the community • the rights of the individual when considering mandatory compared with voluntary programmes • side effects, whether real or perceived

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	SPECIFICATION STATEMENT	COMMENT
(a)	the structure of cartilage including hyaline cartilage, yellow elastic cartilage, white fibrous cartilage	Cartilage is a hard but flexible, compressible and elastic connective tissue consisting of cells (chondrocytes) embedded in a matrix. There are no blood vessels within cartilage and so nutrients and oxygen must diffuse through the matrix to the cells. There are three main types of cartilage: • hyaline cartilage is located at the ends of bones, the nose and in the trachea; • yellow elastic cartilage, contains yellow elastic fibres located in the external ear; • white fibrous cartilage contains many bundles of the protein collagen giving a much greater tensile strength than hyaline cartilage and is found in the discs between vertebrae.
(b)	the components of compact bone, a matrix which is 30% organic (mainly the protein collagen) and 70% inorganic (the main component being hydroxy-apatite containing calcium and phosphate)	The inorganic components of bone help prevent compression while the organic components help to resist fracture.
(c)	the functions of the organic and inorganic components of compact bone	
(d)	the functions of osteoblasts and osteoclasts; bone is constantly being broken down and reformed by cells called osteoblasts embedded in the matrix which lay down the inorganic component of the matrix and osteoclasts which break it down	Candidates should know the structure of bone, how it is formed and how this relates to its function. They should understand the limitations to transport of materials to the bone tissue and the role of the Haversian system in providing a transport system in bone.
(e)	the structure and function of the Haversian systems	
(f)	rickets and osteomalacia as disorders of bone caused by a calcium or vitamin D deficiency	Rickets is caused by defective calcification of bone as a result of a Vitamin D deficiency or a lack of calcium in the diet. Vitamin D is required for the absorption of calcium from the gut. Vitamin D is a fat soluble vitamin found in foods such as butter, eggs and fish liver oils. It can also be synthesised in the skin by the action of UV on a precursor in the skin.

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	SPECIFICATION STATEMENT	COMMENT
(g)	the causes, symptoms and treatment of osteoporosis and brittle bone disease	Brittle bone disease (*osteogenesis imperfecta*) is an inherited condition causing an imbalance between the organic and inorganic components of the matrix in bone. It is caused by a mutation in the gene responsible for making collagen. Treatments include the use of drugs to increase the mineral density in bone and surgery to place metal rods in the long bones.
(h)	the structure and ultra-structure of skeletal muscle	Skeletal muscle is made up of a bundle of fibres, which have a striated appearance, and that each fibre contains numerous myofibrils. Candidates should be able to draw and label diagrams showing the arrangement of: thin actin and thick myosin filaments in a myofibril, a sarcomere showing the Z line, A band (entire length of myosin filaments including overlap with actin), I band (actin only), H zone (myosin only), and M line.

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	SPECIFICATION STATEMENT	COMMENT
(i)	the sliding filament theory to include structure of the thin filaments (actin with two accessory proteins, tropomyosin and troponin) and thick filaments (myosin)	Candidates should be able to describe the structure of the thick and thin filaments found in striated muscle: Thick filaments: • made from many myosin molecules • each myosin molecule has a tail and a head which projects from the surface of the thick filament Thin filaments: • consist of two strands of globular actin molecules which twist round each other • the protein tropomyosin is wrapped around the actin fibre • the protein troponin is found at positions along the actin /tropomyosin fibre During the contraction of striated muscle, the sarcomeres shorten due to increased overlap, the H and I bands become smaller, A band stays the same and that this provides evidence for the sliding filament theory of muscle contraction. Candidates should be able to explain the sliding filament theory as: • a nervous impulse causes Ca^{2+} ions to be released from the sarcoplasmic reticulum; • Ca^{2+} binds to troponin and changes the shape of the troponin molecule; • this causes tropomyosin to change position exposing the myosin binding sites on the actin; • myosin heads can then form cross bridges with the myosin binding sites on the actin ; • the myosin head bends pulling the actin past the myosin, the power stroke; • ATP at the end of the myosin head is hydrolysed into ADP and Pi which are released; • the cross-bridge is broken when ATP attaches to the myosin head which returns to its original position; • more ATP is hydrolysed to ADP and Pi and a cross-bridge forms with the thin filament further along; • the process continues until the Ca^{2+} is actively pumped back into the sarcoplasmic reticulum.

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	SPECIFICATION STATEMENT	COMMENT
(j)	the differences between 'fast twitch' and 'slow twitch' muscles	Candidates should be able to compare slow twitch fibres with fast twitch fibres. There are two different types of fibre in striated muscle: • slow twitch fibres help athletes run marathons and: - carry out mainly aerobic respiration - fire slowly - contract for a longer time - fatigue slowly - have a rich blood supply - high numbers of mitochondria - high myoglobin levels - low resistance to lactic acid - a low density of myofibrils. • fast twitch fibres are an asset to a sprinter and: - use anaerobic metabolism to create ATP - generate short bursts of strength or speed
(k)	the effects of anaerobic conditions including the role of creatine phosphate and the build-up of lactic acid in muscles	Candidates should understand the different types of anaerobic respiration that can take place in muscle when oxygen levels become too low during strenuous exercise. • Creatine phosphate is made under aerobic conditions to act as a store of phosphate. • As oxygen levels fall creatine phosphate enables the rapid conversion of ADP to ATP but only lasts until the creatine phosphate runs out. • Glycolysis leading to lactic acid production then supplies a limited amount of energy. Candidates should understand that a build-up of lactic acid in the muscles causes fatigue and cramp.
(l)	the use of glycogen and protein as the main sources of energy during muscle contraction	Candidates should know that: • glycogen is stored in muscles and is the main energy source rather than fat • carbohydrate loading is a technique practised by athletes to increase muscle glycogen stores • protein is also used as an energy source in preference to fat.

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	SPECIFICATION STATEMENT	COMMENT
(a)	the structure of the appendicular and axial skeleton (pectoral and pelvic girdles, forelimb and hind limb)	Candidates should be able to recall the names of the bones forming the axial skeleton (to include the skull, vertebral column, sternum and ribcage) and the appendicular skeleton.
(b)	the types of fractures that can occur in the skeleton and their causes	Fractures can be caused by high impact/ stress or can be related to a medical condition that results in weaker bones such as *osteoporosis*, bone cancer or *osteogenesis imperfecta*. Fractures are caused when the physical force on a bone is greater than the strength of the bone. Different types of fractures include: • • displaced; • non-displaced; • comminuted; • simple; • compound. Treatment involves the realignment of the bone and subsequent immobilisation using a splint or cast to allow the bone to heal. Osteoblasts produce new bony tissue to support the broken bone and osteoclasts then remodel the bone. Surgery can be used to insert screws or metal plates to support the bones. This can speed up recovery, which is important in cases such as a fracture of the hip, where lack of mobility during the healing process could lead to complications such as pressure sores, deep vein thrombosis and pulmonary embolism.
(c)	the structure and function of the vertebral column, general structure of a vertebra and the differences between cervical, thoracic and lumbar vertebrae and be able to relate them to their function	Candidates should be able to label a generalised vertebra and relate the differences in structure of cervical, thoracic and lumbar vertebrae to function.

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	SPECIFICATION STATEMENT	COMMENT
(d)	medical conditions which affect posture, their causes and treatment	Scoliosis is a postural condition which affects the spine. It is caused by a gene mutation. Minor cases can be treated using physiotherapy but more severe cases may need bracing or surgery. The condition can also be caused by muscle problems, which can be treated using surgery. Other postural conditions include: • flat foot where there is no arch formation in the foot or the arches collapse; this can be treated using specialised footwear or surgery in severe cases; • knock knees, which may be a result of a lack of vitamin D or calcium but can also be caused by a congenital or developmental condition or arise after an infection or a traumatic knee injury; treatment includes bracing or surgical realignment.
(e)	the functions of the skeleton, including support, muscle attachment, protection, production of red blood cells and as a store of calcium	

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List of Related External Resources

	SPECIFICATION STATEMENT	COMMENT
(a)	the different types of joint in the human; immovable/fused; gliding joints; hinge; ball and socket	Different types of joint to include: • gliding joints where the bones glide over each other collectively providing a wide range of movement, e.g. between the vertebrae, wrist and ankle bones • hinge joints allow movement in one plane, e.g. knee and elbow • ball and socket joints allow movement in more than one plane, e.g. hip and shoulder • immovable or fused joints, e.g. cranium
(b)	the cause and treatment of osteoarthritis	Osteoarthritis is a degenerative disease which results in a breakdown of the articular cartilage in a joint. The cartilage is broken down faster than it is replaced as a result of changes in the collagen and glycoprotein. It is not an autoimmune disease but there may be a genetic link. Repeated vigorous bending of a joint e.g. dancing or sporting activity, damage to a joint and being overweight all increase the risk of developing osteoarthritis. Treatment includes the use of non-steroid anti-inflammatory drugs such as aspirin. In severe cases, the joint may be replaced. Osteoarthritis is a degenerative disease affecting the cartilage and bone in joints while rheumatoid arthritis is an auto-immune disease involving an inflammatory response in joints leading to tissue damage and swelling. Candidates should appreciate the advantages and disadvantages of hip or knee replacement surgery. Advantages are • relief from long term pain; • reduced drug intake; • increased mobility; • restore normal activity and quality of life. Disadvantages are: • surgical risks such as increased risk of a blood clot and infection; • long recovery period; • increased risk of hip dislocation; • the replacement joint could fail after 15-20 years and there are increased risks with a second replacement.

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	SPECIFICATION STATEMENT	COMMENT
(c)	the cause and treatment of rheumatoid arthritis	This is a result of genetic and environmental factors. The immune system recognises proteins in the tissues of the joint as being antigenic. This results in severe inflammation of the joint together with an increased blood flow. The affected joint becomes swollen, painful and movement is severely restricted. Environmental factors such as the weather (cold and damp), smoking, high levels of red meat in the diet and high intake of coffee are all linked to the likelihood of suffering from this disease. Treatment includes: • the injection of steroidal anti-inflammatory drugs into the joint; • physiotherapy; • surgery.
(d)	the concept of joints acting as levers including examples of 1st order, 2nd order and 3rd order levers	Joints as lever systems act as force or distance magnifiers and this depends on the position of the fulcrum, load and effort: • first order levers have the fulcrum between the load and the effort, e.g. neck muscles balancing the load of the skull using the neck vertebrae as the fulcrum • second order levers have the load between the fulcrum and the effort, e.g. calf muscles lifting the load of the body using your toes as the fulcrum • third order levers where the effort is between the load and the fulcrum, e.g. the biceps lifting your hand using the elbow as the fulcrum.
(e)	the structure of a typical synovial joint including the roles of cartilage, synovial membrane, synovial fluid, ligaments	Candidates should be able to label a typical synovial joint as demonstrated by the elbow and appreciate the function of each structure.
(f)	the antagonistic muscle action in the human forelimb including the role of tendons	Candidates should be able to label a human forearm showing the origins and insertions of the biceps and triceps muscles and understand that these muscles act as an antagonistic pair.

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	SPECIFICATION STATEMENT	COMMENT
(a)	the structure of the human brain – the position of the cerebrum, hypothalamus, hippocampus, cerebellum, and medulla oblongata	Candidates should understand the structure and function of the following parts of the human brain. • brain - surrounded by three membranes known as the meninges - inflammation of these membranes is called meningitis; • four ventricles (spaces) inside the brain - filled with cerebrospinal fluid, which supply the neurones of the brain with oxygen and nutrients such as glucose; • three main regions of the brain: the hindbrain, midbrain and forebrain; • hindbrain includes the medulla oblongata and cerebellum; • medulla oblongata - involved with the control of heart rate, ventilation and blood pressure and contains many important centres of the autonomic nervous system; • cerebellum - involved with the maintenance of posture and the co-ordination of voluntary muscular activity e.g. writing; • midbrain contains nerve fibres that link the forebrain to the hindbrain; • forebrain is made up of the hypothalamus, thalamus and cerebrum; • cerebrum controls the body's voluntary behaviour, learning, reasoning, personality and memory; • hypothalamus - is involved in regulating body temperature, blood solute concentration, thirst, hunger and sleep. It is the main controlling region of the autonomic nervous system and provides a link between the brain and the endocrine systems via the pituitary gland. • thalamus is an important relay centre, sending and receiving information to and from the cerebral cortex; • hypothalamus and thalamus interconnect with different areas of the brain, including the hippocampus, to form the limbic system, which is involved in emotion, learning and memory; • hippocampus interacts with other areas of the cortex. It is involved in learning, reasoning and personality and also consolidates memories into a permanent store.
(b)	the main functions of the cerebrum, hypothalamus, cerebellum and medulla oblongata	

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	SPECIFICATION STATEMENT	COMMENT
(c)	the role of the sympathetic and parasympathetic nervous systems	The autonomic nervous system (ANS) is the part of the nervous system controlling "automatic" processes; these include heart rate, ventilation rate, blood pressure, digestion and temperature regulation. The ANS is divided into the sympathetic nervous system and the parasympathetic nervous system. • The sympathetic nervous system generally has excitatory effects on the body e.g. increasing heart rate and ventilation rate. Most sympathetic neurones release noradrenaline as a neurotransmitter which has similar effects on target cells as the hormone adrenaline. • The parasympathetic nervous system generally has an inhibitory effect on the body e.g. decreasing heart rate and ventilation rate. Most parasympathetic neurones release acetylcholine as a neurotransmitter. A mammal is rarely fully relaxed or fully active and the two divisions work antagonistically - opposing signals from the two types of neurones adjust an organ's activity to an appropriate level. As exemplified by the control of heart rate.
(d)	the hypothalamus as the link between nervous and endocrine regulation	

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	SPECIFICATION STATEMENT	COMMENT
(e)	the role of the sensory areas and motor areas of the cortex	Candidates should understand the structure and function of the regions of the cerebral hemispheres: • cerebrum consists of two hemispheres which are connected to each other by a bundle of nerves known as the corpus callosum; • the cortex forms the outer layer (2-3mm deep) and its surface is highly folded to increase the amount of cortical area available for processing information; • it contains thousands of millions of neurones each with many synaptic connections and is responsible for most conscious thoughts and actions; • the cortex is composed of grey matter and contains many cell bodies, the inner area of the cerebrum is composed of white matter and contains myelinated axons. • each cerebral hemisphere can be subdivided into four structural regions: the frontal lobe, temporal lobe, parietal lobe and occipital lobe; • the frontal lobe - reasoning, planning, part of speech and movement (the motor cortex) emotions and problem solving; • the temporal lobe - language, learning and memory; • the parietal lobe - somatosensory functions and taste; • the occipital lobe – vision; • the cerebral cortex can be subdivided into three discrete functional areas: the sensory areas, motor areas and association area; • sensory areas receive nerve impulses from receptors in the body; • motor areas send nerve impulses to appropriate effectors via motor neurones; • motor neurones, from the motor area, of one cerebral hemisphere cross over in the medulla oblongata and innervate the effectors on the opposite side of the body; • association areas make up most of the cerebral cortex and receive impulses from sensory areas and associate this information with previously stored information (from memory) which allows the information to be interpreted and given meaning; • the association area is also responsible for initiating appropriate responses which are passed to the relevant motor areas.

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(f)	the relationship between the sizes of the relevant parts of the cerebrum and the complexity of innervation of the different parts of the body as illustrated by the sensory homunculus and the motor homunculus	The tongue, lips and finger tips are highly sensitive. This is due to the large number of receptors and sensory neurons originating from these parts of the body. As a result, a large proportion of the sensory areas of the brain are involved with receiving impulses from these parts of the body (represented by the sensory homunculus). The muscles of the hands and face can bring about intricate movements as there are many motor neurones controlling these muscles. A large proportion of the motor area is involved with the innervation of these muscles (represented by the motor homunculus).
(g)	role of the areas of the cerebrum involved in language comprehension and speech	There are 2 main areas for speech in the brain: Wernicke's area (association area) and Broca's area (motor area); both are located in the left hemisphere. The sensory areas involved are either the auditory area or visual area. • Wernicke's area is responsible for interpreting both written and spoken language. • Motor neurones arising from Broca's area innervate the muscles of the mouth, larynx and intercostal muscles and diaphragm in order to produce vocal sounds. • A bundle of nerve fibres, the arcuate fisciculus, link the two areas.

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	SPECIFICATION STATEMENT	COMMENT
(a)	the different techniques used for studying the brain without invasive neurosurgery including functional magnetic resonance imaging (fMRI), computerised tomography (CT), positron emission tomography (PET) and electroencephalography (EEG)	Brain imaging techniques are non-invasive and can reveal aspects of brain structure and function. • Electroencephalography (EEG) involves the placing of electrodes on the scalp which record general changes in the electrical activity of regions of the brain over time. • A computerised tomography (CT) scan combines special X-ray equipment with sophisticated computers to produce multiple high resolution, cross-sectional images of internal organs (including the brain). • Magnetic resonance imaging (MRI) uses a powerful magnetic field, radio frequency pulses and a computer to produce detailed 3D images of organs and other soft tissues. MR images often provide more detailed scans than ultrasound or CT scans. • Functional magnetic resonance imaging (fMRI) is a technique for examining activity of brain tissue in real time (whereas MRI is a technique for examining anatomy). fMRI can produce detailed images over a period of time which can show changes in brain activity making it the most frequently used scanning technique to study brain function. • Positron emission tomography (PET) is a neuroimaging technique which involves the injection of a small amount of radioactive isotope (with a short half-life).

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SPECIFICATION STATEMENT		COMMENT
(b)	how the brain develops and that there are critical periods for certain aspects of human learning and language acquisition	Neuroplasticity refers to the brain's ability to change and adapt by forming new connections between neurones. It does this in response to new information, sensory stimulation, development, damage, or dysfunction. Following damage to the brain, for example following a stroke or brain injury undamaged axons grow new nerve endings to reconnect damaged neurones forming new neural pathways.
(c)	neuroplasticity; changes in neural pathways which enable the brain to respond to changes in the environment and to compensate for injury or disease	• Neuroplasticity occurs throughout life enabling neurones in the brain to compensate for injury, or change and adjust their activity in response to new situations or changes in the environment. • Developmental plasticity occurs when neurones in the young brain rapidly sprout branches and form synapses. Then, as the brain begins to process sensory information, some of these synapses strengthen and others weaken. Eventually, some unused synapses are eliminated completely, a process known as synaptic pruning, which leaves behind efficient networks of neural connections. • Developmental plasticity occurs most profoundly in the first few years of life; at birth, each neurone in the cerebral cortex has about 2,500 synapses. By the time an infant is two or three years old, the number of synapses is approximately 15,000 per neuron. This amount is about twice that of the average adult brain. The connections that are not reinforced by sensory stimulation eventually weaken, and the connections that are reinforced become stronger. Eventually, efficient pathways of neural connections are produced. • Throughout the life of a human or other mammal, these neural connections are fine-tuned through the organism's interaction with its surroundings. During early childhood, which is known as a critical period of development, the nervous system must receive certain sensory inputs in order to develop properly. Once a critical period ends, there is a large drop in the number of connections that are maintained; the ones that do remain are the ones that have been strengthened by the appropriate sensory experiences. This massive "pruning back" of excess synapses often occurs during adolescence. • In humans the development of language requires extensive postnatal experience to produce and decode speech sounds that are the basis of language. For this experience to be effective it must occur in early life. The requirement for hearing and practicing during a critical period is apparent in studies of language acquisition in congenitally deaf children. • Whereas most babies begin producing speech-like sounds at about 7 months (babbling), congenitally deaf infants show obvious deficits in their early vocalizations, and such individuals fail to develop language if not provided with an alternative form of symbolic expression such as sign language. • Examples of pathological situations ("feral children") illustrate the importance of early experience. In one well-documented case, a girl was raised by parents until the age of 13 under conditions of almost total language deprivation. Despite intense subsequent training, she never learned more than a rudimentary level of communication.

Continued on next page

USEFUL INTERACTIVE RESOURCES

EDUQAS > **A LEVEL Biology** > **Specification from 2015**

	SPECIFICATION STATEMENT	COMMENT
(d)	how the expression of genes can affect brain development and the impact this may have on an individual's behaviour	As an organism grows and develops chemical reactions activate and deactivate parts of the genome at strategic times and in specific locations. Epigenetics is the study of these chemical reactions and the factors that influence them. Many brain functions are accompanied at a cellular level by changes in gene expression. Epigenetic changes are also involved in brain diseases such as mental illness and addiction. Abused children are at least 50% more likely than the general population to suffer from serious depression as adults, and find it harder to recover from the illness. Adults that were subjected to childhood abuse and neglect are also at significantly higher risk of a range of other conditions including schizophrenia, eating disorders, personality disorders, bipolar disease and general anxiety. They are also more likely to abuse drugs or alcohol. • One hypothesis is that terrible early childhood experiences change certain physical aspects of the brain during a key developmental period – this may have an epigenetic component which causes an alteration in gene expression in the brain, which predisposes adults to increased risk of mental illness. • The average concentration of the hormone cortisol tends to be higher in adults who had traumatic childhoods, even if the individuals are healthy at the time of measurement. Cortisol is released from the adrenal glands in response to stress; the more stressed we are the more cortisol we produce. Adults who were abused, or neglected, as children have higher concentrations of cortisol and therefore higher background stress levels. This may increase their vulnerability to mental illness. • Cortisol production is controlled by the hippocampus in the brain. In response to stress the hippocampus sends impulses to the hypothalamus, which releases two hormones, corticotrophin-releasing hormone and arginine vasopressin (also known as ADH). • These two hormones stimulate the pituitary gland which responds by releasing the hormone adrenocorticotrophin into the blood. When the cells of the adrenal glands take up this hormone, they release cortisol. • As the cortisol circulates in the bloodstream it binds to glucocorticoid receptors on the hippocampus, which responds by sending nerve impulses to the hypothalamus, inhibiting it. • This is an example of negative feedback – the release of cortisol into the bloodstream results in the decline of cortisol production; this stops us from being constantly overstressed. • Adults who suffered traumatic childhoods are constantly over-stressed. They produce too much cortisol all of the time. Something is going wrong with the feedback loop. • Some studies have shown that the concentration of corticotrophin-releasing hormone was higher in individuals who had suffered childhood abuse that those who hadn't.
(e)	how altered gene expression in childhood could predispose adults to an increased risk of mental illness	

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

List of Related External Resources

SPECIFICATION STATEMENT		COMMENT
(a)	innate behaviour and the advantage to organisms of escape reflexes, kineses and taxes as exemplified by woodlice	Innate Behaviours include reflexes, kineses and taxes. • A reflex is a rapid automatic response to a given stimulus and improves the organism's chances of survival. • Kineses are a form of orientation behaviour and are more complex than reflex responses. They involve the whole organism moving but the response is non-directional i.e. it does not move towards or away from the stimulus but instead it moves faster and changes direction. • Taxes involve the whole organism moving in response to a stimulus, where the direction of the movement is related to the direction of the stimulus.
(b)	learned behaviours; including being able to describe habituation, imprinting, classical and operant conditioning	Learning can be defined as a relatively permanent change in behaviour due to past experience. Learning allows behavioural responses to be modified. • Habituation involves learning to ignore stimuli because they are followed neither by reward or punishment. • Imprinting is a type of learning that occurs during a very early or receptive stage (critical periods of brain development) in the life of birds and some mammals. The young of birds, and some mammals, respond to the first larger, moving object they see/smell/touch/hear. They attach to this object and the attachment is reinforced by 'rewards' such as warmth and food. As exemplified by the experiments of Konrad Lorenz. • Associative behaviours include classical and operant conditioning. In these types of behaviour animals associate one type of stimulus with a particular response/ action. • Classical conditioning involves the association between a natural and an artificial stimulus to bring about the same response. As exemplified by the experiments of Pavlov. • Operant conditioning involves the association between a particular behaviour and a reward or punishment (use of reinforcers). As exemplified by the experiments of BF Skinner. • Latent (exploratory) learning is not directed to satisfying a need or obtaining a reward. Many animals explore new surroundings and learn information at a later stage and mean the difference between life and death. • Insight learning does not result from immediate trial and error learning but may be based on information previously learned by other behavioural activities. As exemplified by the experiments of Kohler. • Imitation is a form of social learning allows learned behaviour patterns to rapidly spread between individuals and to be passed down from generation to generation. It involves copying the behaviour of another animal, usually a member of the same species. Differences can arise between populations as a result of imitation of different behaviour patterns in different areas; these are cultural differences e.g. Chimpanzees cracking nuts - some populations use stones, others use sticks/branches.
(c)	primates, including humans, living in very complex societies and exhibiting behaviours such as imitation and insight	

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USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(d)	the advantages and disadvantages of living in social groups	Social behaviours involve interactions between individuals of the same species and many species form highly structured social groups (societies). Within these groups the behaviour of one individual can influence the behaviour of others within the group. • Social behaviour relies on the ability of animals to be able to communicate with one another. There are various ways that animals can communicate but they always involve one individual producing a signal (sign stimulus) that can be detected by another; this may trigger an innate response in the second individual. For example the begging response of a gull chick is triggered by the red spot on its parent's beak. • These are often referred to as stereotyped behaviours or fixed action patters (FAPs) and the sign stimulus activates nerve pathways which bring about co-ordinated movements without any decision making in the brain. • The response of the individual is dependent upon its motivational state. For example: if a cheetah is hungry (motivational state) it will initiate stalking behaviours upon the sight of prey (sign stimulus); if the cheetah is not hungry the sight of prey does not induce stalking behaviours. • Stereotyped behaviours involving FAPs are more complex than simple reflex actions and can be modified by experience. As exemplified by the experiments of Tinbergen.

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USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(e)	the social structure of some insects; based on a caste system and that communication between individuals is brought about by innate behaviours	Ants, bees and termites are social insects living in colonies which are organised based on a caste system. • Typical insect colonies contain thousands of individuals, which are all closely related, but they are divided into different groups (castes) with specific roles. In a honeybee colony there is a single fertile female (the queen), several thousand sterile female workers and a few hundred fertile male drones. One caste may find food, while others care for young or defend the colony. The success of insect societies is largely due to the division of labour which increases the overall efficiency of the group. • Communication between individuals within the colony is by touch, pheromones and by visual orientation displays known as dances. • Worker bees forage for sources of nectar and communicate the distance and direction of the source to other workers by the nature of a dance performed on a vertical surface on the inside of the hive or on the floor at the hive entrance. The worker performs a round dance which indicates that the source of food is less than about 70m from the hive but gives no indication of direction. The waggle dance is performed if the source is greater than about 70m and also includes information as to its distance from the hive and its direction relative to the hive and the position of the sun.

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USEFUL INTERACTIVE RESOURCES

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	SPECIFICATION STATEMENT	COMMENT
(f)	social structure in vertebrates achieved through dominance hierarchies in which animals are able to recognise each other as individuals and possess some abilities to learn	In vertebrates, social groups tend to be based upon dominance hierarchies; higher ranking individual are dominant over lower-ranking ones. • Most dominance hierarchies are linear i.e. they have no members of equal rank; for example a group of hens sharing a hen-house. • Dominance hierarchies only exist where animals are able to recognise each other as individuals and possess some ability to learn. • One advantage of a dominance hierarchy is that it decreases the amount of individual aggression associated with feeding, mate selection and breeding-site selection and ensures that resources are shared out so that the fittest survive. • Once established, a dominance hierarchy is comparatively stable and is maintained normally by aggression as fighting is a last resort; prior to fighting there is a series of ritualised actions – each reflex is stimulated by the last action (sign stimulus) of the other. As exemplified by red deer.

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USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

	SPECIFICATION STATEMENT	COMMENT
(g)	the advantage of territorial and courtship behaviours in increasing reproductive success	Courtship is used to attract a mate. Courtship allows recognition of species, sex and sexually mature/receptive individuals; stimulation and synchronisation of sexual behaviour. • Courtship routines are innate thus they ensure intraspecific mating and therefore likely to produce fertile offspring. As exemplified by sticklebacks. • Many species show sexual dimorphism – the males and females look different e.g. peahens and peacocks. Sexual selection and natural selection will be working against each other – sexual selection will make a characteristic more conspicuous, whilst natural selection will try to make it less conspicuous. There are two main theories behind the driving force of sexual selection: • Intra-sexual selection/male-male combat: In species such as African lions, and southern elephant seals the males are much bigger than the females. In these species the males fight for sexual access to many females. Sexual selection has therefore favoured the evolution of larger more aggressive males. • Inter-sexual selection/female choice: this can be further divided into the physical attractiveness model and the male handicap model.
(h)	the role of sexual selection in the evolution of territorial and courtship behaviours	

USEFUL INTERACTIVE RESOURCES

EDUQAS > A LEVEL Biology > Specification from 2015

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

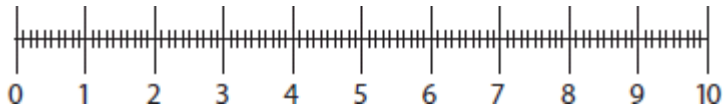
Analysis of results

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.

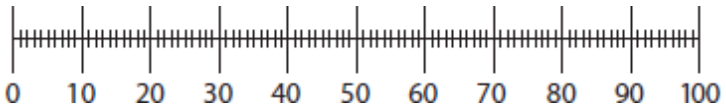
A stage micrometer is used to measure the length of each division at different magnifications. There are two types of stage micrometer available, check which you are using.

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.

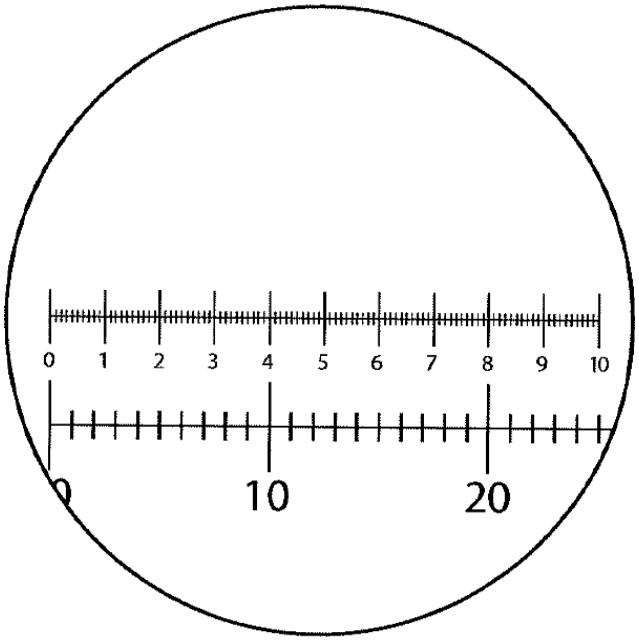
Or

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.



eyepiece

stage micrometer

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 1000 µm
= 2.5 µm

If 1 stage micrometer unit = 0.1 mm

1 eye piece unit $= \frac{2}{80}$ = 0.25 stage micrometer units

1 stage micrometer unit = 0.1 mm

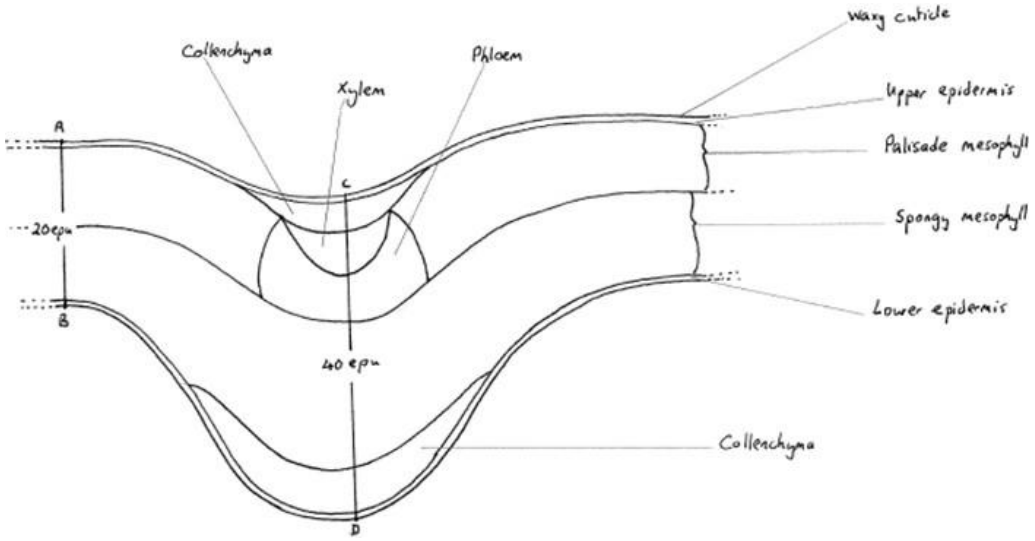
1 eye piece unit = 0.25 x 0.1 mm
= 0.025 mm or 0.025 x 1000 µm
= 2.5 µm

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

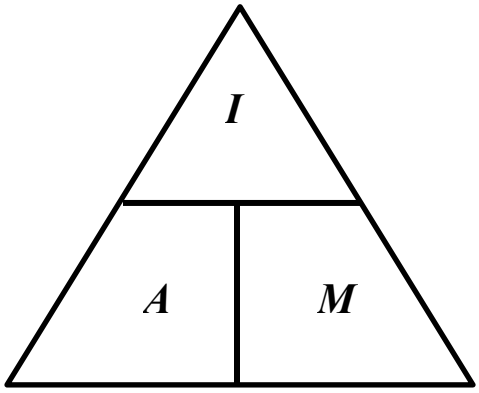
High power drawing of individual cells

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

Practical resources to support the teaching of A level Biology in England

Core Content	Additional Options for Practical Work
Biodiversity	
• the variety of life, both past and present, is extensive, but the biochemical basis of life is similar for all living things • biodiversity refers to the variety and complexity of life and may be considered at different levels • biodiversity can be measured, for example within a habitat or at the genetic level • classification is a means of organising the variety of life based on relationships between organisms and is built around the concept of species • originally classification systems were based on observable features but more recent approaches draw on a wider range of evidence to clarify relationships between organisms • adaptations of organisms to their environments can be behavioural, physiological and anatomical • adaptation and selection are major factors in evolution and make a significant contribution to the diversity of living organisms	http://www.nuffieldfoundation.org/practical-biology/observing-patterns-distribution-simple-plant patterns in plant distribution http://www.nuffieldfoundation.org/practical-biology/investigating-response-worms-soil-improvers investigating the behaviour of animals to different soil conditions http://www.nuffieldfoundation.org/practical-biology/biodiversity-your-backyard using quadrats to measure biodiversity http://www.nuffieldfoundation.org/practical-biology/model-natural-selection-%E2%80%93-spaghetti-worms modelling natural selection http://www.biology-fieldwork.org/woodland/woodland-plants/investigation-comparing-two-areas-of-woodland.aspx Investigation into ground vegetation in two contrasting areas of woodland, including a spreadsheet for calculating Simpson's Diversity Index http://bigpictureeducation.com/video-whats-buttercup Wellcome Trust video and accompanying data for field work http://www.saps.org.uk/secondary/teaching-resources/258 http://www.saps.org.uk/secondary/teaching-resources/127 http://www.saps.org.uk/secondary/teaching-resources/768 online activities to practice sampling techniques before you get into the field, looking at measuring abundance, random sampling and distribution of species across a footpath
Exchange and Transport	
• organisms need to exchange substances selectively with their environment and this takes place at exchange surfaces • factors such as size or metabolic rate affect the requirements of organisms and this gives rise to adaptations such as specialised exchange surfaces and	http://www.nuffieldfoundation.org/practical-biology/effect-size-uptake-diffusion - experiment on rate of diffusion using agar cubes. http://www.nuffieldfoundation.org/practical-biology/estimating-rate-transpiration-

mass transport systems • substances are exchanged by passive or active transport across exchange surfaces • the structure of the plasma membrane enables control of the passage of substances into and out of cells	plant-cutting rate of transpiration and an animation that supports the ideas of water transport http://www.saps.org.uk/secondary/themes/1274 http://www.nuffieldfoundation.org/practical-biology/tracking-active-uptake-minerals-plant-roots active uptake of minerals in plant roots http://www.nuffieldfoundation.org/practical-biology/investigating-effect-temperature-plant-cell-membranes effect of temperature on plant cell membranes (can use colorimeter) http://www.nuffieldfoundation.org/practical-biology/looking-heart heart dissection, looking at structure of organs – link to mass transport system. http://www.nuffieldfoundation.org/practical-biology/modelling-human-ventilation-system modelling human ventilation system http://www.nuffieldfoundation.org/practical-biology/using-spirometer-investigate-human-lung-function using a spirometer to measure lung function http://www.nuffieldfoundation.org/practical-biology/measuring-rate-metabolism measuring metabolic rate
Cells	
• the cell theory is a unifying concept in biology • prokaryotic and eukaryotic cells can be distinguished on the basis of their structure and ultrastructure • in complex multicellular organisms cells are organised into tissues, tissues into organs and organs into systems • during the cell cycle genetic information is copied and passed to daughter cells • daughter cells formed during mitosis have identical copies of genes while cells formed during meiosis are not genetically identical	http://www.nuffieldfoundation.org/practical-biology/looking-heart heart dissection, looking at structure of organs http://www.nuffieldfoundation.org/practical-biology/dissecting-lungs lung dissection tissue and organ structure http://www.nuffieldfoundation.org/practical-biology/comparing-flower-structure-different-angiosperms dissection and comparison of different flower structures http://www.nuffieldfoundation.org/practical-biology/aseptic-techniques aseptic techniques for the culturing of bacteria on agar plates http://www.nuffieldfoundation.org/practical-biology/investigating-mitosis-allium-

	root-tip-squash mitosis in a root tip squash, there is an animation to support this practical http://saps.org.uk/secondary/themes/1290 http://www.nuffieldfoundation.org/practical-biology/preparing-anther-squash meiosis in an anther squash http://www.nuffieldfoundation.org/practical-biology/making-reebops-model-meiosis model of meiosis http://www.saps.org.uk/secondary/teaching-resources/770-microscopy-looking-at-xylem-and-specialised-cells xylem cells
Biological Molecules	
• biological molecules are often polymers and are based on a small number of chemical elements • in living organisms nucleic acids (DNA and RNA), carbohydrates, proteins, lipids, inorganic ions and water all have important roles and functions related to their properties • the sequence of bases in the DNA molecule determines the structure of proteins, including enzymes • enzymes catalyse the reactions that determine structures and functions from cellular to whole-organism level • enzymes are proteins with a mechanism of action and other properties determined by their tertiary structure • enzymes catalyse a wide range of intracellular reactions as well as extracellular ones • ATP provides the immediate source of energy for biological processes	http://www.nuffieldfoundation.org/practical-biology/extracting-dna-living-things extraction of DNA practical work http://www.nuffieldfoundation.org/practical-biology/quantitative-food-test-protein-content-powdered-milk establishing the quantity of protein in powdered milk http://www.britishecologicalsociety.org/wp-content/uploads/Education-Water-lesson.pdf range of practical activities relating to the properties of water http://www.nuffieldfoundation.org/practical-biology/microscale-investigations-catalase-activity-plant-extracts catalase activity in plants http://www.nuffieldfoundation.org/practical-biology/investigating-effect-temperature-activity-lipase temperature impact on lipase http://www.nuffieldfoundation.org/practical-biology/investigating-effect-ph-amylase-activity amylase and the impact of pH http://www.nuffieldfoundation.org/practical-biology/modelling-sliding-filament-hypothesis proteins and muscle movement

Ecosystems	
• ecosystems range in size from the very large to the very small • biomass transfers through ecosystems and the efficiency of transfer through different trophic levels can be measured • microorganisms play a key role in recycling chemical elements • ecosystems are dynamic systems, usually moving from colonisation to climax communities in a process known as succession • the dynamic equilibrium of populations is affected by a range of factors • humans are part of the ecological balance and their activities affect it both directly and indirectly • effective management of the conflict between human needs and conservation help to maintain sustainability of resources •	http://www.nuffieldfoundation.org/practical-biology/nitrogen-fixing-bacteria-free-living-soil - links to nitrogen cycle, recycling chemical elements and impact that humans can have on ecosystems. http://www.biology-fieldwork.org/freshwater/freshwater-animals/investigation-freshwater-energy-flow.aspx An investigation into energy flow using freshwater invertebrates, to construct pyramids of numbers, biomass and energy and calculate efficiency http://www.biology-fieldwork.org/seashore/sand-dunes/investigation-primary-succession-in-sand-dunes.aspx An investigation into primary succession in sand dunes http://www.biology-fieldwork.org/grassland/grassland-plants/fieldwork.aspx Fieldwork techniques for investigating the effects of mowing and trampling in grasslands (human impact on ecosystems) http://www.saps.org.uk/secondary/teaching-resources/127 online activity to explore the how to look at distribution of species across a footpath before you go out into the field
Control Systems	
• homeostasis is the maintenance of a constant internal environment • negative feedback helps maintain an optimal internal state in the context of a dynamic equilibrium. Positive feedback also occurs • stimuli, both internal and external, are detected leading to responses • the genome is regulated by a number of factors • coordination may be chemical or electrical in nature	http://www.nuffieldfoundation.org/practical-biology/investigating-factors-affecting-breathing-rate-locust investigating the factors that affect breathing rate http://www.nuffieldfoundation.org/practical-biology/investigating-factors-affecting-heart-rate-daphnia investigating factors that affect heart rate http://www.nuffieldfoundation.org/practical-biology/observing-effects-exercise-human-body effects of exercise on humans http://www.nuffieldfoundation.org/practical-biology/using-choice-chamber-investigate-animal-responses-stimuli animals response to stimuli

	http://www.nuffieldfoundation.org/practical-biology/investigating-response-calliphora-larvae-light larvae response to light
Genetics and Evolution	
• transfer of genetic information from one generation to the next can ensure continuity of species or lead to variation within a species and possible formation of new species • reproductive isolation can lead to accumulation of different genetic information in populations potentially leading to formation of new species • sequencing projects have read the genomes of organisms ranging from microbes and plants to humans. This allows the sequences of the proteins that derive from the genetic code to be predicted • gene technologies allow study and alteration of gene function in order to better understand organism function and to design new industrial and medical processes	http://www.nuffieldfoundation.org/practical-biology/preparing-anther-squash meiosis in an anther squash http://www.nuffieldfoundation.org/practical-biology/making-reebops-model-meiosis model of meiosis http://www.saps.org.uk/secondary/teaching-resources/706 new effective technique for cloning cauliflowers http://www.nuffieldfoundation.org/practical-biology/cloning-living-organism taking cuttings from plants http://www.nuffieldfoundation.org/practical-biology/gene-induction-%C3%9F-galactosidase-e-coli induction of genes (genetic control) http://www.nuffieldfoundation.org/practical-biology/following-gene-transfer-conjugation-bacteria horizontal gene transfer in bacteria http://www.yourgenome.org/ range of activities and animations from Sanger Institute
Energy for biological processes	
• in cellular respiration, glycolysis takes place in the cytoplasm and the remaining steps in the mitochondria • ATP synthesis is associated with the electron transfer chain in the membranes of mitochondria and chloroplasts • in photosynthesis energy is transferred to ATP in the light- dependent stage and the ATP is utilised during synthesis in the light-independent stage	http://www.nuffieldfoundation.org/practical-biology/how-do-plants-and-animals-change-environment-around-them#node-2978 investigating levels carbon dioxide produced by animals and plants in light and dark conditions http://www.nuffieldfoundation.org/practical-biology/investigating-light-dependent-reaction-photosynthesis using DCPIP as an electron acceptor – investigating light dependent reaction

	http://www.saps.org.uk/secondary/teaching-resources/235 investigating photosynthesis using algal balls and an animation that outlines respiration and photosynthesis http://www.saps.org.uk/secondary/themes/1281 http://www.nuffieldfoundation.org/practical-biology/measuring-rate-metabolism measuring metabolic rate http://www.nuffieldfoundation.org/practical-biology/measuring-respiratory-quotient measuring the respiratory quotent http://www.saps.org.uk/secondary/teaching-resources/181 thin layer chromatography for photosynthetic pigments

Practical resources to support the teaching of practical skills in Biology A levels in England

Practical techniques	Additional options for practical work
use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH)	http://www.saps.org.uk/secondary/teaching-resources/235 investigating photosynthesis using algal balls (pH and colour)
use appropriate instrumentation to record quantitative measurements, such as a colorimeter or potometer	http://www.nuffieldfoundation.org/practical-biology/measuring-rate-water-uptake-plant-shoot-using-potometer using a potometer http://www.saps.org.uk/secondary/teaching-resources/1263 a simpler set of potometer apparatus http://www.nuffieldfoundation.org/practical-biology/quantitative-food-test-protein-content-powdered-milk establishing the quantity of protein in powdered milk – can use a colorimeter in this practical http://www.nuffieldfoundation.org/practical-biology/investigating-effect-temperature-plant-cell-membranes effect of temperature on plant cell membranes (can use colorimeter) http://www.saps.org.uk/secondary/teaching-resources/235 and http://www.saps.org.uk/secondary/teaching-resources/1224 investigating photosynthesis using algal balls (can use a colorimeter)
use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions	
use of light microscope at high power and low power, including use of a graticule	http://www.nuffieldfoundation.org/practical-biology/investigating-mitosis-allium-root-tip-squash mitosis in a root tip squash http://www.nuffieldfoundation.org/practical-biology/preparing-anther-squash meiosis in an anther squash http://www.saps.org.uk/secondary/teaching-resources/770-microscopy-looking-at-xylem-and-specialised-cells xylem cells, trichomes

	http://www.saps.org.uk/secondary/teaching-resources/1325 preparing a temporary slide to show and measure phloem and xylem
produce scientific drawing from observation with annotations	http://www.nuffieldfoundation.org/practical-biology/investigating-mitosis-allium-root-tip-squash mitosis in a root tip squash http://www.nuffieldfoundation.org/practical-biology/preparing-anther-squash meiosis in an anther squash http://www.nuffieldfoundation.org/practical-biology/comparing-flower-structure-different-angiosperms dissection and comparison of different flower structures http://www.saps.org.uk/secondary/teaching-resources/770-microscopy-looking-at-xylem-and-specialised-cells xylem cells, trichomes http://www.saps.org.uk/secondary/teaching-resources/1325 phloem and xylem
use qualitative reagents to identify biological molecules	http://www.nuffieldfoundation.org/practical-biology/quantitative-food-test-protein-content-powdered-milk establishing the quantity of protein in powdered milk – can use a colorimeter in this practical
separate biological compounds using thin layer/paper chromatography or electrophoresis	http://www.saps.org.uk/secondary/teaching-resources/181thin layer chromatography for photosynthetic pigments
safely and ethically use organisms to measure: - plant or animal responses	http://www.nuffieldfoundation.org/practical-biology/microscale-investigations-catalase-activity-plant-extracts catalase activity in plants http://www.nuffieldfoundation.org/practical-biology/how-do-plants-and-animals-change-environment-around-them#node-2978 investigating levels carbon dioxide produced by animals and plants in light and dark conditions http://www.nuffieldfoundation.org/practical-biology/investigating-response-worms-soil-improvers investigating the behaviour of animals to different soil conditions

- physiological functions	http://www.nuffieldfoundation.org/practical-biology/using-choice-chamber-investigate-animal-responses-stimuli animals response to stimuli http://www.nuffieldfoundation.org/practical-biology/investigating-response-calliphora-larvae-light larvae response to light http://www.nuffieldfoundation.org/practical-biology/using-spirometer-investigate-human-lung-function using a spirometer to measure lung function http://www.nuffieldfoundation.org/practical-biology/investigating-factors-affecting-breathing-rate-locust investigating the factors that affect breathing rate http://www.nuffieldfoundation.org/practical-biology/investigating-factors-affecting-heart-rate-daphnia investigating factors that affect heart rate http://www.nuffieldfoundation.org/practical-biology/observing-effects-exercise-human-body effects of exercise on humans http://www.getinthezone.org.uk/ Practical activities (kits were delivered to free to all schools in 2012) with link to online database for analysis
use microbiological aseptic techniques, including the use of agar plates and broth	http://www.nuffieldfoundation.org/practical-biology/aseptic-techniques standard practice for aseptic techniques http://www.nuffieldfoundation.org/practical-biology/incubating-and-viewing-plates standard practice for viewing and incubating agar plates http://www.nuffieldfoundation.org/practical-biology/making-nutrient-agars making up nutrient agars http://www.nuffieldfoundation.org/practical-biology/pouring-agar-plate how to pour agar plates

safely use instruments for dissection of an animal organ, or plant organ	http://www.nuffieldfoundation.org/practical-biology/looking-heart heart dissection, looking at structure of organs http://www.nuffieldfoundation.org/practical-biology/dissecting-lungs lung dissection tissue and organ structure http://www.nuffieldfoundation.org/practical-biology/comparing-flower-structure-different-angiosperms dissection and comparison of different flower structures http://www.saps.org.uk/secondary/teaching-resources/1325-a-level-set-practicals-dissection-and-microscopy-of-a-plant-stem dissection of plants
use sampling techniques in fieldwork	http://www.nuffieldfoundation.org/practical-biology/observing-patterns-distribution-simple-plant patterns in plant distribution http://www.nuffieldfoundation.org/practical-biology/biodiversity-your-backyard using quadrats to measure biodiversity http://www.biology-fieldwork.org/woodland/woodland-plants/fieldwork-collecting-vegetation-data.aspx Sampling strategies and use of quadrats for sampling ground vegetation in woodlands http://www.biology-fieldwork.org/woodland/woodland-invertebrates/fieldwork-sampling-woodland-invertebrates.aspx Sampling strategies and capture techniques for sampling woodland invertebrates. http://www.biology-fieldwork.org/woodland/woodland-invertebrates/investigation-sampling-snail-populations.aspx Use of mark-release-recapture and Lincoln Index for estimating the size of populations http://bigpictureeducation.com/video-whats-buttercup Wellcome Trust video and accompanying data for field work http://bigpictureeducation.com/animation-surveying-populations Animation shows sampling methods in different environments

	http://www.saps.org.uk/secondary/teaching-resources/258 http://www.saps.org.uk/secondary/teaching-resources/127 http://www.saps.org.uk/secondary/teaching-resources/768 online activities to practice sampling techniques before you get into the field, looking at measuring abundance, random sampling and distribution of a species across a footpath
use ICT such as computer modelling, or data logger to collect data, or use software to process data	http://www.dnadarwin.org/ explore the molecular evidence for evolution through practical bioinformatics activities that use data analysis tools and molecular data.

Additional links to teaching resources

Society of Biology www.societyofbiology.org

Society of Biology and Nuffield Resource http://www.nuffieldfoundation.org/practical-biology

Field Studies Council http://www.biology-fieldwork.org/

British Ecological Society http://www.britishecologicalsociety.org/education/

Biochemical Society http://www.biochemistry.org/Education/Teachers.aspx and www.sciberbrain.org and http://www.biochemistry.org/Portals/0/Education/Docs/Biochem_Booklet_web.pdf

Science and Plants for Schools http://www.saps.org.uk and

http://www.saps.org.uk/secondary/teaching-resources/1304-a-level-set-practicals (a collection of new practical resources to support the practical endorsement)

Society for General Microbiology http://www.sgm.ac.uk and http://www.microbiologyonline.org.uk

Wellcome Trust www.wellcome.ac.uk/education

Resources contributed by:

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