GCE

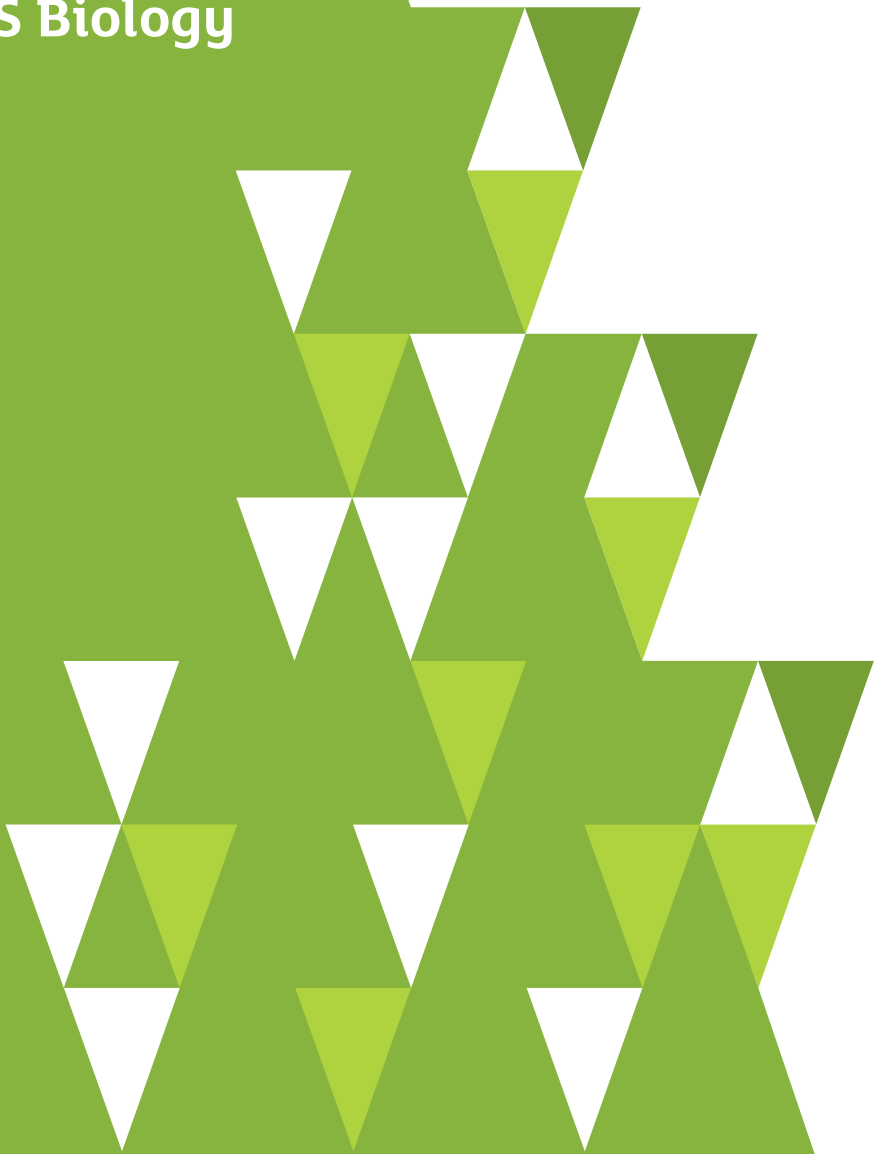
Rewarding Learning

Revised GCE

Biology

Practical Guidance Booklet

Practical Skills in AS Biology
AS 3



Updated September 2020

September 2020

This practical support booklet has been updated to reflect minor changes in evidence and guidance that have been communicated at recent Agreement Trials and in the Principal Moderator's reports. The updated document should help to clarify points made about the evidence required for each practical.

For ease of use, the table below shows which practicals have had changes.

Practical	**Detail of changes**
Enzymes	Clarification on required evidence.
Enzyme immobilization	Minor amend to required evidence.
Using a colorimeter	Minor amend to guidance; new section to include production of a (starch) calibration curve as an acceptable colorimeter practical.
Measuring cell size	Minor amend to guidance to allow teachers to supply cells to be measured; clarification on required evidence.
Osmosis investigation	Clarification on required evidence.
Measuring the average solute potential of cells at incipient plasmolysis	Clarification on required evidence.
Root tip squash	Amends to guidance; minor amend to required evidence.
Block diagram	Amend to guidance/evidence to allow the use of section through a plant root.
Heart dissection	Minor amend to required evidence.
Simpson's Index	Clarification on required evidence.

Introduction

The information provided here is intended to provide support for teachers delivering the revised CCEA GCE Biology course (first teaching from September 2016).

For most practical tasks this guidance provides detailed background information rather than providing a series of 'recipe' practical investigations (although these are included for some tasks). The internet (for example, www.nuffieldfoundation.org) and many other sources provide additional information, in carrying out 'A' level Biology tasks.

The evidence required for each task is listed at the end of each task.

Note: This document does not provide health and safety information for the safe carrying out of practical tasks identified in the specification. It is the responsibility of teachers to ensure that they and their students are aware of any health and safety issues that are relevant in any particular task.

Using qualitative reagents to identify biological molecules

Substance	Name of test	Procedure	Colour change if positive result
Reducing sugars	Benedict's test	Add an equal volume of Benedict's reagent to the test solution and heat	Blue to brick red precipitate
Non-reducing sugars	Benedict's test	• Hydrolyse sample by heating with dilute HCl in a water bath • Once cooled, neutralise by adding sodium hydrogen carbonate • Test with Benedict's solution	Blue to brick red precipitate
Starch	Iodine test	Add iodine solution	Yellow-brown to blue-black
Protein	Biuret test	Add potassium hydroxide to the test sample then add a few drops of copper sulfate solution	Blue to lilac/purple/ mauve

Note: the Benedict's test is partially quantitative. The Benedict's reagent will change through the sequence blue-green-yellow-orange-brick red, depending on how much reducing sugar is present in the sample.

It is good practice to develop holistic biological skills when the opportunity arises. For example, starch grains can be observed in a thin section of potato tissue. A thin section of potato is mounted in water on a slide and a coverslip placed on top. Add a drop of iodine to the slide at the edge of the coverslip. A piece of folded filter paper, or similar, at the other side of the coverslide is used to draw the iodine across the slide staining the starch grains in the potato section as it does so.

Evidence: Table with details of tests outlined and results obtained for particular substances.

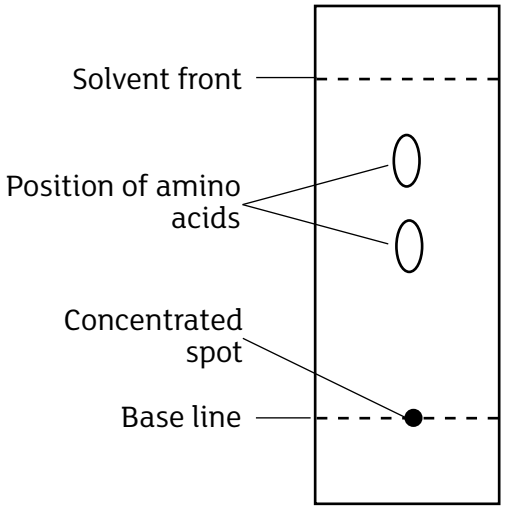
Paper chromatography of amino acids

Paper chromatography is a technique by which soluble compounds can be separated and identified. Chromatography makes it possible to separate complex organic compounds such as mixtures of free amino acids or plant pigments. It is also possible to separate and identify small quantities of the solutes in an unknown mixture.

A drop or 'spot' of the solution containing the mixture of the solutes to be separated is 'spotted' repeatedly about 3-4 cm away from one end of the chromatography paper. The concentrated 'spot' is then allowed to dry. The end of the chromatography paper is placed in a suitable solvent, making sure the 'spot' (usually placed on a pencil line 'origin') remains above the level of solvent. To ensure the chromatography paper remains in place it is normally suspended from the lid of the chromatography tank (or similar piece of apparatus).

The solvent flows up the paper dissolving and carrying the amino acids with it. The solvent flow is allowed to continue until it approaches the top of the chromatogram. The paper is removed from the tank, the solvent front is marked on the paper and then it is dried.

Once dried the chromatography paper (chromatogram) can be stained to observe the different amino acids. The amino acids that are more soluble in the solvent travel further than those that are less soluble, therefore achieving separation of the different amino acids.



Amino acids can be identified by their Rf value. The Rf value is the relative distance an amino acid has moved relative to the solvent front and is calculated by dividing the distance a particular amino acid has moved relative to the solvent front.

$$Rf = \frac{\text{distance moved by amino acid}}{\text{distance moved by solvent front}}$$

An Rf value is always less than 1 and will be approximately the same for a particular amino acid in a chromatogram when using a particular solvent.

Evidence: Table of results, copy (photograph) of chromatogram, and calculations of Rf values to identify particular amino acids.

Enzyme investigations (any two investigations can be used)

(a) Investigating the effect of temperature, pH, substrate concentration and enzyme concentration on enzyme activity.

There are many different enzyme practical investigations in use across CCEA centres and there is no requirement to do any particular one or group of investigations.

A typical example is outlined below.

The effect of pH on the activity of trypsin

Background knowledge – The protein-digesting enzyme, trypsin, will hydrolyse the protein gelatine. Gelatine is a major constituent of jelly. When a coloured jelly such as a strawberry-flavoured variety is exposed to trypsin the red colour is released as the gelatine is broken down. The intensity of the colouring released in different experimental tubes can be compared using a colorimeter.

Procedure:

- Label six boiling tubes 1-6.
- Cut six 1 cm^3 cubes of jelly.
- Add 10 cm^3 of 2% trypsin to each boiling tube.
- Add 10 cm^3 of the appropriate buffer to five of the boiling tubes and distilled water to the remaining one.

Boiling tube	1	2	3	4	5	6
pH of buffer	4	5	6	7	8	water

- Place the boiling tubes in a water bath at 25°C.
- After 5 minutes add a cube of jelly to each of the boiling tubes 1 to 5.
- After 24 hours remove a sample from tube 6 to act as a blank for the colorimeter. Using the appropriate filter, set the percentage transmission on the colorimeter to 100%.
- Shake the contents of boiling tube 1 and remove a sample, place in the colorimeter and record the percentage transmission.
- Repeat for the other boiling tubes 1 to 5.
- Tabulate the results and use an appropriate graphical technique to present the results.

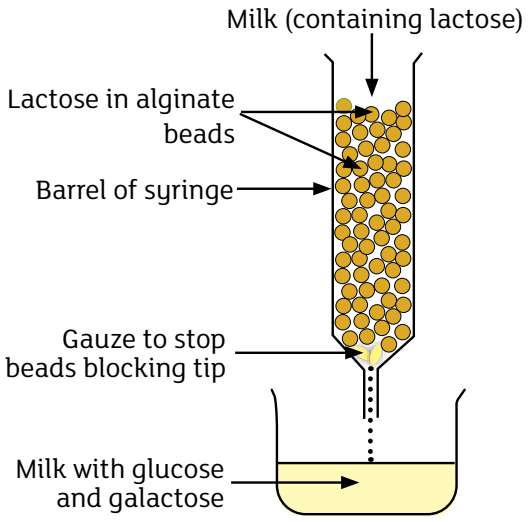
Evidence: For this and many other enzyme investigations a table and graph of results plus a brief conclusion describing and explaining the results using appropriate AS terminology.

(b) Demonstrate enzyme immobilisation

Beta-galactosidase (lactase) is an enzyme that breaks down lactose into glucose and galactose. Pouring milk (containing lactose) through a column of immobilised beta-galactosidase will result in the enzyme breaking down the lactose into glucose and galactosidase.

Procedure:

- Add 8 cm^3 of sodium alginate solution (2%) to a small beaker.
- Add 2 cm^3 of beta-galactosidase (lactase) to the beaker.
- Add one drop of food colouring – this allows the reaction to be seen more clearly.
- Mix thoroughly but keep bubbles to a minimum.
- Draw this mixture into the barrel of a 10 cm^3 plastic syringe.
- Add 1.5g of calcium chloride to 100 cm^3 of distilled water in 250 cm^3 beaker.
- Add the enzyme mixture dropwise from the syringe to the calcium chloride solution. Allow the immobilised enzyme beads that start to form to harden for about 10 minutes. Remove the beads and rinse thoroughly with water.
- Rinse the syringe and remove the plunger and fix the barrel to a retort stand.
- Place a small piece of gauze near the tip of the syringe to prevent the beads from blocking the syringe nozzle.
- Add the beads to the syringe.
- Test the milk after it has been filtered through the beads with Clinistix or a similar specific test for glucose.



Evidence: A description of results - or before and after photographs of diagnostic strip - and a conclusion explaining the results.

Using a colorimeter

A colorimeter can be used in a number of experiments (one of the three following practicals can be submitted as a colorimetry practical

Using a colorimeter to follow the course of a starch-amylase catalysed reaction

A colorimeter measures the change of light intensity as it passes through a solution. Colorimeters can record the amount of light that is absorbed (**absorbance**) by the solution or the amount of light that passes through (**transmission**). The light that is not absorbed by the sample passes on to the photo-sensitive cell and this is converted into a digital readout.

It is important to **calibrate the colorimeter**. For example, if the colorimeter is going to follow the course of amylase breaking down starch to maltose, a weak solution of iodine could be calibrated as the end-point or 'blank', (100% transmission).

When following the course of a starch-amylase catalysed reaction, **a red filter** is usually used as this maximises the percentage transmission/absorbance change over the course of the investigation.

[Note: this experiment cannot be used as an enzyme practical as it does not investigate any factors affecting enzyme activity]

Evidence: A table and graph of results plus a brief conclusion describing and explaining the results.

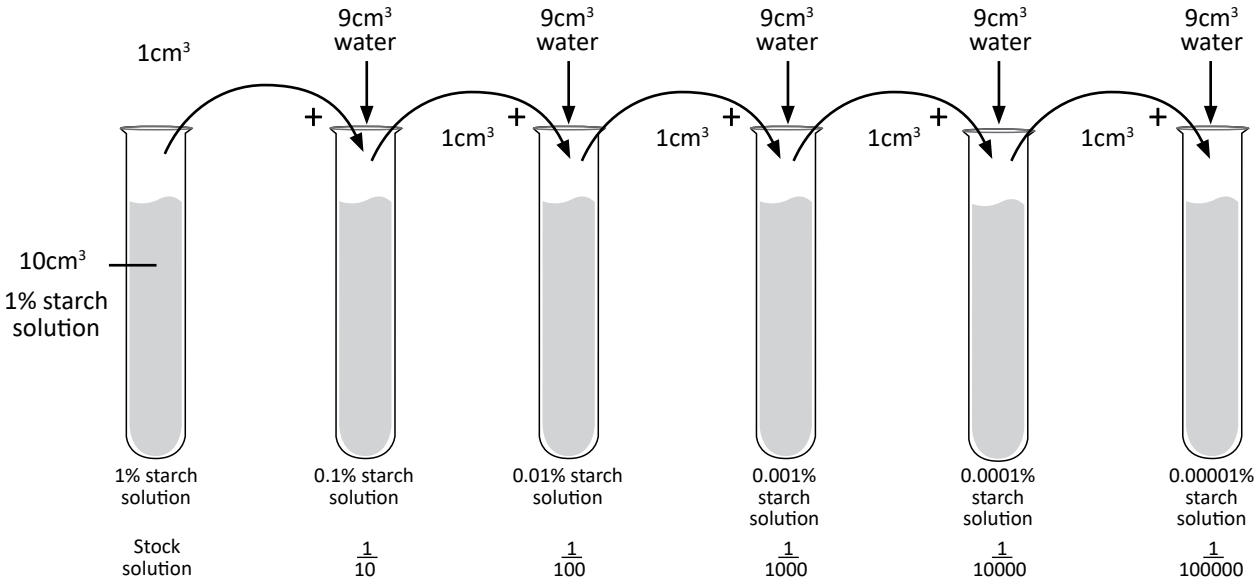
Using a colorimeter to produce a starch calibration curve

Calibration graph - In investigations using a colorimeter it is often necessary to produce calibration graphs (curves) if the investigation will involve calculating specific quantities of a substance. A calibration graph can then be drawn that will allow colorimeter readings to be expressed in terms of substance concentrations.

The stages in producing a calibration graph involve:

- starting with a known 'standard' or 'stock' concentration of substance, for example starch;
- making a range of starch concentrations using serial dilutions or using a range of known starch concentrations;
- measuring the % transmission (or % absorbance) for each of these values; and
- plotting a graph with % transmission on the Y-axis and starch concentration on the X-axis.

Serial dilutions – In serial dilutions each solution is less concentrated than the previous one by a set factor. For example, when using a dilution factor of 10 (each solution is ten times less concentrated than the previous one) 1 cm^3 of the solution is added to 9 cm^3 water and so on as shown in the following diagram.



Serial starch dilutions

Evidence: A table of colorimeter readings at a range of starch concentrations, and the graph of the starch calibration curve.

Using a colorimeter to investigate the effect of a factor, for example, temperature, on the permeability of cell-surface membranes in beetroot

Beetroot contains the pigment betalain that gives it the characteristic dark red-purple colour. Intact beetroot placed in water will not lead to a colour change in the water. However, if some of the cell-surface membranes in the beetroot tissue are damaged, red pigment will seep out of the cells and the water then will turn red. The more damage to the tissue the more the colour change in the water.
Procedure:

- Cut several small sections of beetroot of equal size using a cork borer.
- Rinse the beetroot in water until the water remains clear.
- Set up a number of water baths at, e.g. 20°C, 40°C, 60°C, 80°C.
- Add 10 cm^3 of water to each of 5 test tubes and place one in each water bath for 5 minutes to allow the temperature of the water to equilibrate.
- Place a section of surface-dried beetroot in each of the test tubes.
- Leave in the water bath for 10 minutes.
- Set up a colorimeter using a blue/blue-green filter. Calibrate distilled water as 0 absorbance (or 100% transmission).

- After the 10 minutes, sample the water surrounding the beetroot sections and check its absorbance (or transmission) for each temperature.

- Add the results to an appropriate results table and draw a graph of % absorbance (or transmission) against temperature.

- Explain your results.

Evidence: A table and graph of results plus a brief conclusion describing and explaining the results.

Using a graticule and stage micrometer in measuring cell size at both low and high powers

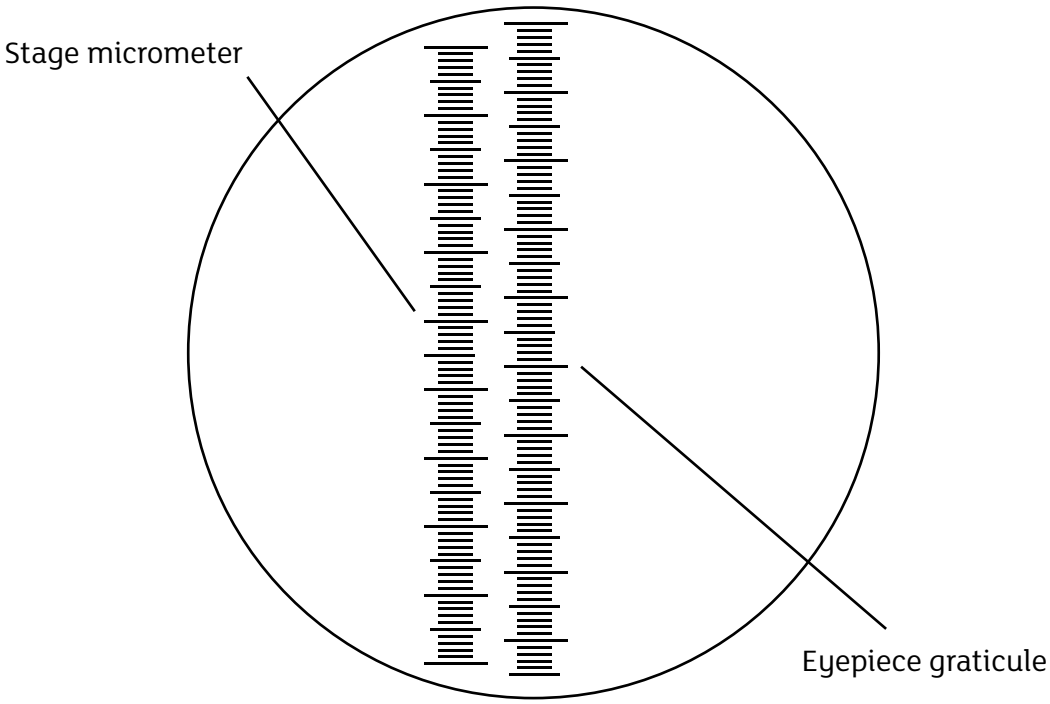
This involves using a special eyepiece lens that contains a scale to measure cell length. The eyepiece scale (**graticule**) needs to be calibrated before measurements are made. To do this a stage **micrometer** (a special slide that has a calibrated scale of known length on its upper surface) is used.

Many stage micrometers are 1 mm in length and have their scale divided into 100. Therefore each unit on the stage micrometer = **10 micrometres (µm)**, as each division is 1 mm (1000 micrometres) divided by 100.

Some stage micrometers have a scale 1 cm in length (not 1 mm) but the principle of calibration is the same. In a 1 cm stage micrometer scale, each small division is 100 µm.

Calibrating at low and high power

Calibrating at low / mid power (for example, x 100) - with the eyepiece graticule and stage micrometer in place, align the two scales so that the left edges of each scale (the 0 values) are superimposed. Then check for a position where the divisions of each scale are overlapping further along the scales.



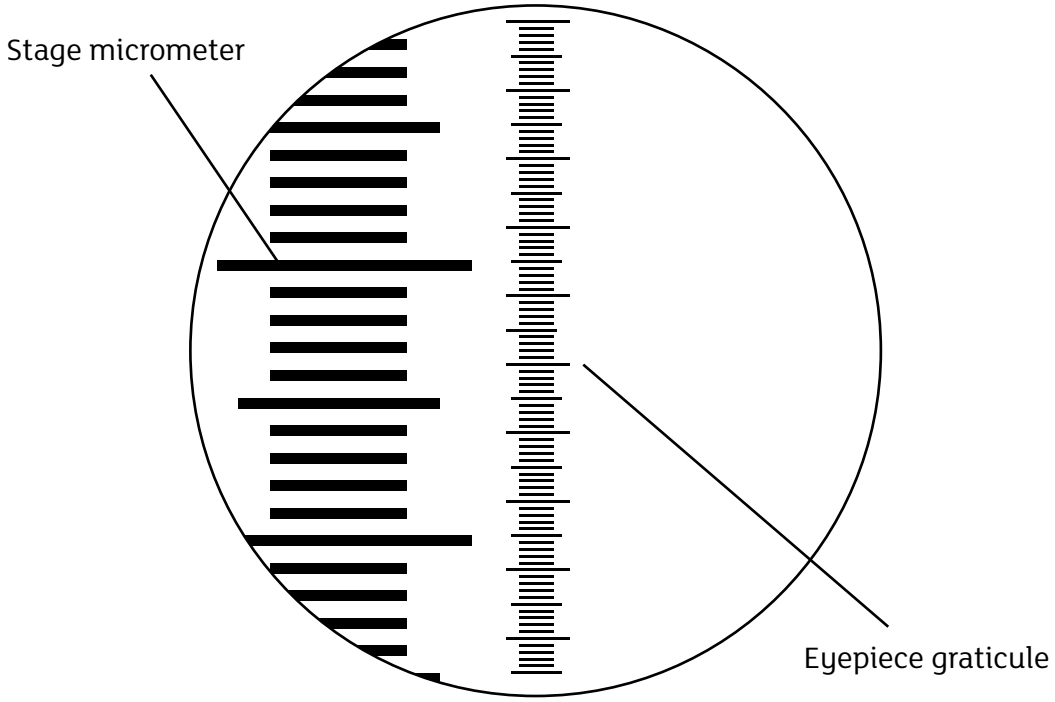
Calibrating the eyepiece graticule at low power

Diagram info – the top of both scales are aligned, each scale is divided into 100 units with major subdivisions every ten and a minor one every five. In this diagram the end of the stage micrometer is exactly level with the 94th division in the eyepiece graticule

The smallest divisions in the eyepiece graticule are often referred to as **Small Eyepiece Units (SEUs)**.
In the above diagram, 100 SEUs are equal to 94 divisions on the stage micrometer.
If the stage micrometer is 1 mm in length, then each division is 10 micrometers.

Therefore 1 SEU = 94 x 10 / 100 = 9.4 µm.

Calibrating at high power (for example, x 400) When calibrating at high power the stage micrometer will be magnified much more and the full length of the scale will no longer be visible within the field of view.



Calibrating the eyepiece graticule at high power

Diagram instructions – adjust the left hand scale slightly so that the 26th small division lines up with the end of the smaller scale. As before the top end of the two scales align.

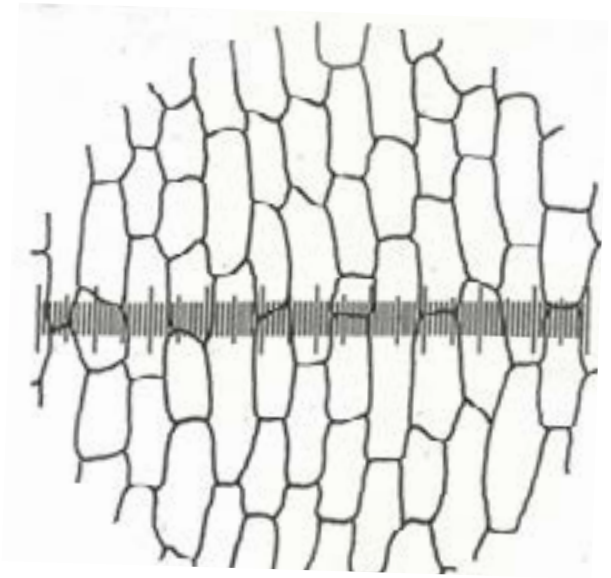
In the diagram showing calibration at high power, 100 SEUs = 26 divisions on the stage micrometer (with each division on the stage micrometer = 10 µm).

Therefore 1 **SEU** = 26 x 10 / 100 = **2.6 µm**

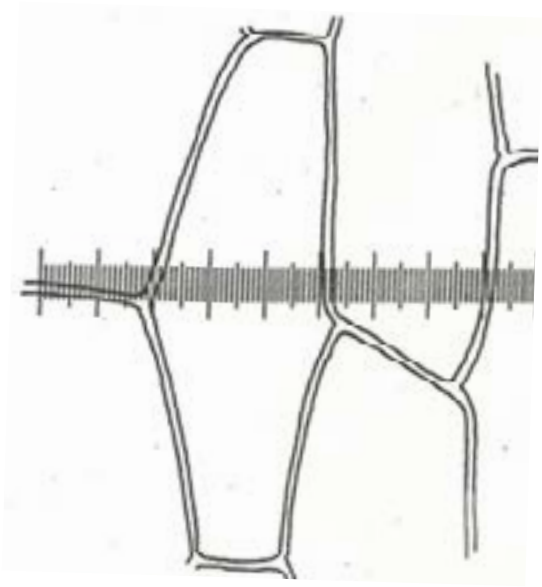
Once the calibration is complete, remove the stage micrometer and replace with a slide containing the specimen that contains the cells to be measured. These cells can be provided by the teacher.

The following diagram shows how to measure cell width after the eyepiece graticule has been calibrated.

View at x100 of a plant tissue through an eyepiece containing a graticule scale



View at x400 of a plant tissue through an eyepiece containing a graticule scale



Using the eyepiece graticule to measure cell length

Where possible cell measurements should be calculated at high power (rather than low/medium power) as it is more precise/accurate.

In the HP diagram the width of the cell is (approx.) 31 SEUs.
Therefore the cell width is 31 x 2.6 µm = 80.6 µm.

Evidence: Calculations of calibrations using the stage micrometer and eyepiece graticule and the subsequent measurement of cells using the calibrated values. Each step in the calculations should be shown.

Osmosis investigations

Measuring the average water potential of cells in plant tissue

There are many plant tissues, for example, potato and carrot, that can be used in this investigation. A typical procedure is described below:

- Add a range of sucrose solutions (and water) to separate beakers.
- Cut cylinders of potato and weigh.
- Add a cylinder to each beaker.
- After 24 hours remove the potato and reweigh.
- The percentage change in mass should be calculated for the cylinder in each solution.
- Plot the percentage change in mass against sucrose solution.
- Where the line of best fit crosses the X-axis, the water potential in the potato is equal to the solute potential of the sucrose solution.
- The solute potential of the sucrose solution at the point of intercept can be calculated from a conversion table.

Evidence: A table and graph of results plus a brief conclusion describing and explaining the results. The conclusion should include the water potential of the tissue(s) and how it was determined.

Measuring the average solute potential of cells at incipient plasmolysis

When a cell is at incipient plasmolysis the cell-surface membrane is just making contact, and no more, with the cell wall. Theoretically there is no pressure being exerted on the cell wall. Therefore, the solute potential of the cell is the same as its water potential.

In reality, it is impossible to judge when a cell is exactly at incipient plasmolysis so a point where 50% of cells are turgid and 50% plasmolysed is taken as a compromise.

Procedure:

- Add sections of onion epidermal tissue to pure water to make sure all the onion cells are turgid.
- Place sections of the onion epidermal cells in beakers, with each beaker containing either water or one of a range of sucrose solutions.
- Leave the epidermal tissue in the beakers for 30 minutes.
- After 30 minutes remove the onion epidermal tissue and place on a microscope slide.

- Observe the onion tissue under the microscope and calculate the percentage of cells that are plasmolysed for each solution.

- Draw a graph of percentage plasmolysis against sucrose solution. Use the graph to identify the point at which 50% of the cells are plasmolysed. At this point the average solute potential of the onion cells is the same as the solute potential of the sucrose. The solute potential of the sucrose at that concentration can be calculated from a conversion table.

Evidence: A table and graph of results plus a brief conclusion describing and explaining the results. The conclusion should include the solute potential of the tissue(s) and how it was determined.

Preparing and staining root tip squashes to observe mitosis

Many species of plants can be used for this investigation, but the root tips of broad beans are a good, and relatively easy to manipulate, source to use.

Harvest broad beans 7 – 10 days after planting in a seed tray. By this stage the beans will have germinated and the young shoots will have extended through the top of the soil. Short lateral roots about 1 cm in length growing out from the main tap root are the best to use for observing mitosis.

Procedure:

- Add a small section of root containing lateral roots to a boiling tube containing acetic orcein;

- place the boiling tube in a water bath at 60 °C for 30 minutes;

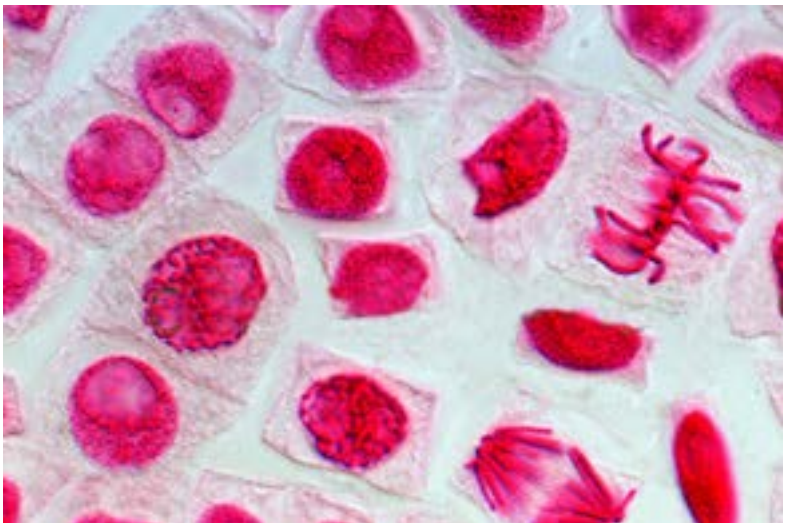
- after 30 minutes remove a section of root from the boiling tube and use a scalpel to remove the last few mm or so from one of the lateral roots. Add this short section to a microscope slide and add more acetic orcein if necessary to stop the root tip from drying out;

- add a cover slip and gently tap with a blunt end of a pointed needle. This will 'squash' the root tip into a single layer of cells; and

- observe under a microscope.

Other staining techniques - there are many different techniques used to stain chromosomes and prepare root tip squashes for observing mitosis. Using toluidine blue with garlic tips works well. Common features between each process are that the chromosomes are stained and that part of the procedure used softens / breaks up the root tissue allowing it to be easily 'squashed' into a single layer.

Finding cells undergoing mitosis - using low / medium power (for example, x100), scan the root tip section and look for the zone of division. Cells in the zone of division are characteristically small and cuboidal in shape with the nucleus appearing relatively large. Once the zone of division is located switch to high power (x400) to observe cells at different stages of mitosis.



The photograph shown shows cells undergoing mitosis. The cell top-right of centre is in metaphase and the cell lower-right of centre is in late anaphase / telophase. Note the large nucleus / cell size ratio as is typical of cells undergoing mitosis.

Drawing cells undergoing mitosis - select a group of two or three cells together, including at least one that is undergoing mitosis and draw.

Candidates can be given a pre-prepared slide or photo from which they can draw and label cells in various stages of division, if the root tip squash does not produce clear results. Candidates can take pictures of successful or unsuccessful squashes and include it in their evidence. If drawing from a photograph supplied by the teacher, a copy of the photograph being used to draw from should be included to help the moderation process (one copy per centre included in the sample is sufficient).

Evidence: Drawings of cells undergoing mitosis as seen through the microscope. The stage(s) of mitosis should be identified on the drawings.

Completing accurate block diagrams of sections of the ileum, leaf or plant root observed under the microscope.

Block diagrams need to accurately represent the microscope view or the photograph used. They do not need detail of individual cells. If possible, a copy of the photograph being used to draw the block diagram from, should be included to help the moderation process (one copy per centre included in sample is sufficient).

Good block diagrams:

- have all the obvious (tissue) layers included;
- have layers added in the correct proportions;
- have continuous (not sketchy) lines; and
- have labels as appropriate.

Evidence: A block diagram of a section through the ileum, leaf or plant root, meeting the above requirements

Dissecting a mammalian heart

External anatomy – the major blood vessels are at the top of the heart and the coronary arteries run diagonally down the heart from the base of the aorta.

The pulmonary artery and aorta are very close to each other at the very top of the heart:

- The **aorta** is the larger of the two vessels. The pulmonary artery is adjacent to the aorta but is smaller with thinner walls.

- The two **vena cava** often appear more like flaps rather than discrete blood vessels, when they are cut close to the heart. One returns blood from the upper part of the body and the other returns blood from the lower part of the body. These are on the top right hand side of the heart (top left as you examine it assuming the front of the heart is facing you) with the vena cava coming from the lower part of the body being slightly lower and further back in the heart.

- The **pulmonary vein** is on the top left of the heart (top right as you examine it).

The internal anatomy - to examine the heart's anterior it is necessary to make incisions (cuts) with a scalpel or dissecting scissors through the ventral (front) wall from the top of each atrium to the base of the ventricle:

- From the position of the right atrium cut through the heart wall down through the right atrium and right ventricle. Keep the cut as close as possible to the septum. After making the cut pull the two sides apart to expose the two heart chambers on the right side of the heart.

- It should be possible to identify the **papillary muscles**, the **chordae tendinae** ('heartstrings') and the **tricuspid valve** (an atrio-ventricular valve) – seen as three tissue flaps.

- Identify the origin of the pulmonary artery, leading out of the right ventricle, and follow it up, cutting as necessary, until you find the semilunar valves.

- Repeat for the left side of the heart. The **bicuspid valve**, the atrio-ventricular valve on the left side of the heart has only two flaps.

- Identify the origin of the aorta, leading out of the right ventricle, and follow it up until you find the semilunar valves.

Evidence: A labelled drawing of the external view of the heart and a labelled diagram or photograph of the dissected heart.

Sampling techniques (in the field), including measuring abiotic or biotic factors (up to two investigations may be counted).

Candidates are expected to be familiar with a range of qualitative and quantitative techniques used to investigate the distribution and relative abundance of plants and animals in a habitat.

Sampling

There are several ways to estimate the amount, or **abundance**, of organisms present in a particular habitat. The abundance of organisms can be estimated in terms of:

- **Density** – the **number** of individuals present – usually sampled using quadrats and the most common way of sampling **animals**.

- **Percentage cover –** usually for **plants** as it is often difficult to distinguish between individual plants.

- **Frequency** – species are recorded as being **present** or **absent** at a particular sampling point. Percentage frequency indicates the percentage of all quadrats or sampling points that a species occurs in; **pin frames** are often used when recording frequency.

Types of sampling

Random sampling - if the area to be sampled is uniform or if there is an absence of any clear pattern in species distribution, **then random sampling** is appropriate.

Systematic sampling - systematic sampling should be used when there appears to be zonation / clear transition from one habitat type to another, for example, up a rocky shore. The sampling takes place along a line or **transect**.

There are a number of different types of transect used in systematic sampling:

- **Line transect** – sampling continually or at intervals (for example, every 5 m) along a transect line, for example a tape. Only individuals actually touching the transect line are counted.

- **Belt transect** – sampling along a transect line using **quadrats** rather than the tape itself.

- **Interrupted belt transect** – sampling is at intervals along the overall 'transect' due to the large distances involved, for example when sampling a large sand dune system running over a km or more. Interrupted belt transects are several belt transects at intervals within an overall transect.

Biotic and abiotic factors

Abiotic factors are non-living or physical factors, for example, soil moisture, soil organic content, soil temperature, soil pH, and light intensity. Factors that relate to the soil are called **edaphic factors**. **Biotic factors** are factors linked to living organisms. These include competition from other organisms and grazing.

Measuring abiotic factors

1. Soil moisture (water) content

Procedure:
- collecting a sample of soil using a soil borer or other appropriate apparatus;
- weigh a sample of the soil and place in an oven and dry to a constant weight at 105 °C; and
- percentage soil moisture (water) content can be calculated using the formula:

[(initial soil mass – soil mass after drying) / initial soil mass] x 100%

2. Soil organic content

Procedure:
- place the oven-dried soil in a crucible and reweigh;
- burn off the organic content (humus) ;
- allow to cool and reweigh;
- percentage organic content can be calculated using the formula:

[(dry soil mass – soil mass after burning) / initial soil mass] x 100%

3. Soil pH

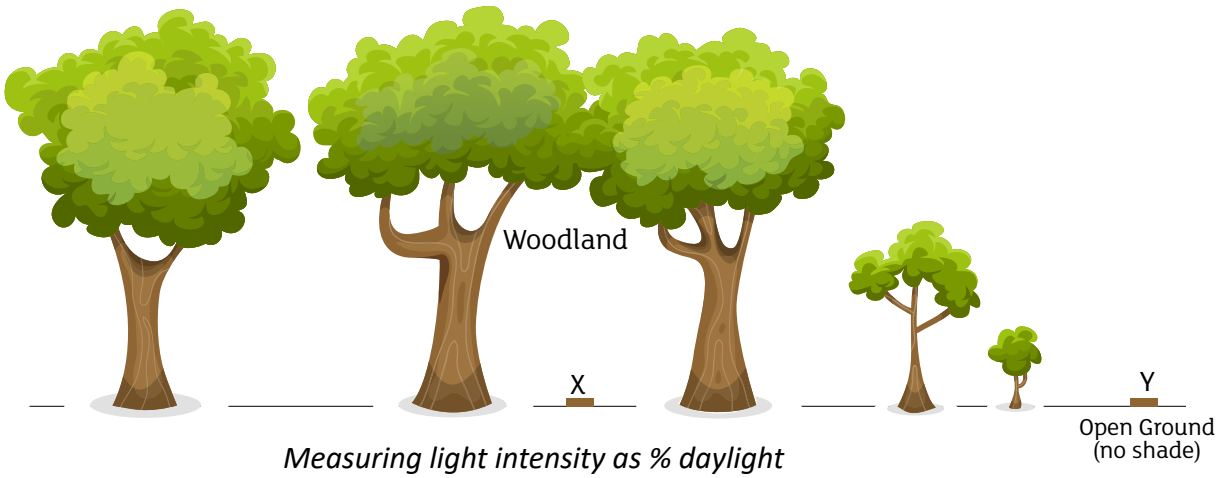
Soil pH can be measured using a **soil testing kit** (using indicator dyes) or using a **pH electrode** attached to a digital meter.

4. Soil temperature

Soil temperature can be measured using special **soil thermometers**.

5. Light intensity

Light can be measured using a **light meter.** Often relative light intensity (for example, the light reaching ground level in a particular habitat as a percentage of light reaching 'open' ground) is more meaningful than absolute light intensity. This is measured as light intensity at ground level for a sample point divided by the light intensity in the open (no shade) x 100. In the diagram below,



Measuring light intensity as % daylight

percentage light intensity for the woodland floor can be calculated as (light intensity at X / light intensity at Y) x 100%.

Sampling animals

Candidates should be familiar with the appropriate use of **pitfall traps**, **sweep nets** and **pooters**.

Evidence: Tables and graphs of an ecological investigation involving the use of sampling technoques and the measurement of abiotic/biotic factors where appropriate. (If calculating a Simpson's Index value, the degree of biodiversity should be commented on by reference to the Simpson's Index value.)

COUNCIL FOR THE **CURRICULUM, EXAMINATIONS** AND **ASSESSMENT**
29 Clarendon Road, Clarendon Dock, Belfast BT1 3BG
Tel: +44 (0)28 9026 1200 Fax: +44 (0)28 9026 1234
Email: info@ccea.org.uk Web: www.ccea.org.uk

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