

GCSE Biology: Required practical handbook

Version 3.8

The methods provided in this *Required practical handbook* are suggested examples, designed to help your students fulfil the apparatus and techniques requirements outlined in the specifications. Written papers will include questions requiring knowledge gained from carrying out the specified practicals.

Please note: it is the Apparatus and techniques requirements which are compulsory and must be fulfilled. Teachers are encouraged to adapt or develop activities, resources and contexts to suit their equipment and to provide the appropriate level of engagement and challenge for their own students.

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Introduction

Students need to undertake the required practical activities listed in the GCSE Biology specification (8461) so that they have the opportunity to experience all of the apparatus and techniques required by Ofqual.

In this guide, we suggest methods and activities for carrying out the required practical activities to help you plan the best experience for your students.

All of the activities we describe have been written and trialled by practising teachers and use apparatus and materials that are commonly available in most schools.

Why do practical work?

Practical work is at the heart of science – that’s why we have placed it at the heart of each of our GCSE science specifications.

There are three separate, but interconnected, reasons for doing practical work in schools.

1. To support and consolidate scientific concepts. Doing practical work enables students to make sense of new information and observations, and provides them with insights into the development of scientific thinking.
2. To develop investigative skills. These transferable skills include:
 - devising and investigating testable questions
 - identifying and controlling variables
 - analysing, interpreting and evaluating data.
3. To build and master practical skills such as:
 - using specialist equipment to take measurements
 - handling and manipulating equipment with confidence and fluency
 - recognising hazards and planning how to minimise risk.

This guide signposts opportunities for developing these working scientifically skills (WS). Working scientifically is explained in more detail in the GCSE Biology specification on page 9.

Helping you to plan

This guide includes:

- teachers' notes providing information and tips on setting up and running practicals
- technical information providing guidance for technicians preparing for the practicals
- student sheets providing a possible method for students to carry out the practical.

The student sheets contain a blank space for students to add the learning outcomes. By focusing on the reasons for carrying out a particular practical, you will help your students to:

- understand the subject better
- develop the skills of a scientist
- master the manipulative skills required for further study or jobs in STEM subjects.

At least 15% of the marks in the written exams will draw on the knowledge and understanding students have gained by carrying out the required practical activities. It is therefore essential that you plan your practical activities with reference to the specification and make students aware of the key content that they need to learn.

You can find examples of the type of practical questions students can expect in our guide, *Practicals in exams*.

We have designed the methods in this guide specifically to help your students fulfil the Apparatus and techniques requirements outlined in the specification. We encourage you to adapt or develop these activities, resources and contexts to suit your circumstances and to tailor the level of engagement and challenge to your students. To help you do this, we've provided the guide in Word.

The practical science statement

Unlike the A-levels, there will be no practical endorsement. Instead, we will provide the head of each school or college with a practical science statement to sign, confirming that reasonable steps have been taken to ensure that each student has:

- completed the required practical activities detailed in the specification
- made a contemporaneous record of such work done during the activities and the knowledge, skills and understanding derived from those activities.

The head of centre will need to return the signed statement to us by the date we will publish on our website, on our [practicals page](#). We will also contact schools and colleges directly with the deadline date and send timely reminders if we don't receive the form. Failure to send this form counts as malpractice/maladministration, and may result in formal action or a warning for the school or college.

Not having done some of the practicals, despite the school's best efforts, will not stop a student from entering for the GCSE. However, it may affect their grade, because there may be questions in the exams that they won't be able to answer.

Apparatus and techniques

The following table lists the biology Apparatus and techniques (AT). Students must be given the opportunity to experience all of these during their GCSE Biology course, regardless of the awarding body specification they study. The list includes opportunities for choice and use of appropriate laboratory apparatus for a variety of experimental problem-solving and/or enquiry-based activities.

Use and production of appropriate scientific diagrams to set up and record apparatus and procedures used in practical work is common to all science subjects and should be included wherever appropriate.

AT 1–7 are common with both of our GCSE Combined Science specifications. AT 8 is for GCSE Biology only.

Where possible, we have added links to the Apparatus and techniques in our A-level Biology course, to show how the skills progress from GCSE to A-level.

| | Apparatus and techniques |
|------|---|
| AT 1 | Use of appropriate apparatus to make and record a range of measurements accurately, including length, area, mass, time, temperature, volume of liquids and gases, and pH (links to A-level AT a). |
| AT 2 | Safe use of appropriate heating devices and techniques including use of a Bunsen burner and a water bath or electric heater (links to A-level AT a). |
| AT 3 | Use of appropriate apparatus and techniques for the observation and measurement of biological changes and/or processes. |
| AT 4 | Safe and ethical use of living organisms (plants or animals) to measure physiological functions and responses to the environment (links to A-level AT h). |
| AT 5 | Measurement of rates of reaction by a variety of methods including production of gas, uptake of water and colour change of indicator. |

| | |
|------|---|
| AT 6 | Application of appropriate sampling techniques to investigate the distribution and abundance of organisms in an ecosystem via direct use in the field (links to A-level AT k). |
| AT 7 | Use of appropriate apparatus, techniques and magnification, including microscopes, to make observations of biological specimens and produce labelled scientific drawings (links to A-level AT d and e). |
| AT 8 | Use of appropriate techniques and qualitative reagents to identify biological molecules and processes in more complex and problem-solving contexts including continuous sampling in an investigation (links to A-level AT f). |

Suggested practical apparatus list

Through their study of the new GCSE sciences students must be given the opportunity to experience a wide range of apparatus. Hands-on experience will help them acquire the practical skills defined by the DfE in their Apparatus and techniques criteria.

We have designed all the activities to use standard equipment and materials that can be found in most school laboratories.

The lists are not exhaustive, and we encourage teachers to modify the activities to suit their students' needs and learning objectives, and the resources available in their school/college.

Lab equipment

- 0.5 m² quadrats
- 10 cm³ plastic syringes
- 10 cm³ measuring cylinders
- 100 cm³ conical flasks
- 250 cm³ beakers
- 30 cm rulers
- 30 m tape measures
- 5 cm³ measuring cylinders/syringes
- boiling tubes
- Bunsen burners
- cork borer
- digital top pan balances (accurate to 0.01g)
- disposable plastic pipettes
- dropping bottles
- filter funnels and filter paper
- filter paper discs
- forceps
- gauze mats
- glass stirring rods
- glass spreaders
- heatproof mats
- incubator

-
- light sources (LED or standard. Not energy saving)
 - metre rulers
 - microscope slide coverslips
 - microscope slides
 - microscopes
 - pestles and mortars
 - petri dishes
 - Perspex rulers
 - spotting tiles
 - stopwatches
 - test tube racks
 - test tubes
 - thermometers (stirring)
 - tripods
 - water baths (electrical or Bunsen burners and beakers)
 - wax pencils
 - white tiles

Specialist supplies

- 1% VirKon disinfectant
- amylase solution
- Benedict's solution
- biuret solution
- buffered solution
- culture of *E. coli* bacteria (K12 or B strain)
- distilled water
- ethanol
- iodine solution
- lipase solution (5%)
- nutrient agar plates
- Cresol red
- pond weed (*Elodea* or *Cabomba* are recommended)
- sodium carbonate solution (0.05 M)
- sodium hydrogen carbonate solution (0.2%)
- starch solution
- Sudan III stain solution
- white mustard seeds

Risk assessment

Safety is an overriding requirement for all practical work. Although all of the suggested practical activities have been suggested by teachers who have successfully carried them out in the lab, schools and colleges are responsible for ensuring that appropriate safety procedures are followed whenever their students undertake practical work, and should undertake full risk assessments.

Required practicals summary

The practicals that have been selected will be familiar, using apparatus and materials that are readily available in most schools. This table summarises the ten practicals required for Biology GCSE.

A student who has completed all of the practicals will have had the opportunity to experience all of the apparatus and techniques required for the specification. Opportunities for developing mathematical skills and working scientifically skills have also been signposted.

| Microscopy | Spec ref. | Skills |
|--|--|--|
| Use a light microscope to observe, draw and label a selection of plant and animal cells. A magnification scale must be included. | Biology 4.1.1.5 Trilogy 4.1.1.5 Synergy 4.1.3.2 | AT 1 – use appropriate apparatus to record length and area. AT 7 – use a microscope to make observations of biological specimens and produce labelled scientific drawings. MS 1d, 3a |
| Microbiology (Biology only) | Spec ref. | Skills |
| Investigate the effect of antiseptics or antibiotics on bacterial growth using agar plates and measuring zones of inhibition. | Biology 4.1.1.6 | AT 1 – use appropriate apparatus to record length and area. AT 3 – use appropriate apparatus and techniques to observe and measure the process of bacterial growth. AT 4 – safe and ethical use of bacteria to measure physiological function and response to antibiotics and antiseptics in the environment. AT 8 – the use of appropriate techniques and qualitative reagents in problem-solving contexts to find the best antibiotic to use or the best concentration of antiseptic to use. MS 5c WS 2.1, WS 2.2, WS 2.4 |
| Osmosis | Spec ref. | Skills |
| Investigate the effect of a range of concentrations of salt or | Biology 4.1.3.2 Trilogy | AT 1 - use appropriate apparatus to record mass and time. AT 3 - use appropriate apparatus and techniques to |

| | | |
|---|--|---|
| sugar solutions on the mass of plant tissue. | 4.1.3.2 Synergy 4.1.3.3 | observe and measure the process of osmosis. AT 5 - measure the rate of osmosis by water uptake. MS 1a, MS 1c, MS 2b, MS 4a, MS 4b, MS 4c, MS 4d WS 2.1, WS 2.2, WS 2.4, WS 2.6, WS 2.7 WS 3.1, WS 3.2 |
| Enzymes | Spec ref. | Skills |
| Investigate the effect of pH on the rate of reaction of amylase enzyme. Students should use a continuous sampling technique to determine the time taken to completely digest a starch solution at a range of pH values. Iodine reagent is to be used to test for starch every 30 seconds. Temperature must be controlled by use of a water bath or electric heater. | Biology 4.2.2.1 Trilogy 4.2.2.1 Synergy 4.2.1.5 | AT 1 – use appropriate apparatus to record the volumes of liquids, time and pH. AT 2 – safe use of a water bath or electric heater. AT 5 – measure the rate of reaction by the colour change of iodine indicator. AT 8 – use of qualitative iodine reagent to identify starch by continuous sampling. (Biology only). MS 1a, MS 1c WS 2.1, WS 2.4, WS 2.5, WS 2.6. WS 3.1, WS 3.2 |
| Food Tests | Spec ref. | Skills |
| Use qualitative reagents to test for a range of carbohydrates, lipids and proteins. To include: Benedict's test for sugars; iodine test for starch; and Biuret reagent for protein. | Biology 4.2.2.1 Trilogy 4.2.2.1 Synergy 4.2.1.5 | AT 2 – safe use of a Bunsen burner and a boiling water bath. AT 8 – use of qualitative reagents to identify biological molecules. (Biology only) WS 2.4 |
| Photosynthesis | Spec ref. | Skills |
| Investigate the effect of light intensity on the rate of photosynthesis using an aquatic | Biology 4.4.1.2 Trilogy 4.4.1.2 | AT 1 - use appropriate apparatus to record the rate of production of oxygen gas produced; and to measure and control the temperature of the water in the 'heat shield' beaker. |

| | | |
|---|--|---|
| organism such as pondweed. | Synergy 4.2.2.6 | AT 2 – safe use of a thermometer to measure and control temperature of water bath. AT 3 - use appropriate apparatus and techniques to observe and measure the process of oxygen gas production. AT 4 – safe and ethical use and disposal of living pondweed to measure physiological functions and responses to light. AT5 – measuring rate of reaction by oxygen gas production. MS 1a, MS 1c, MS 4a, MS 4c, MS 3a, MS 3d (HT) WS 2.1, WS 2.2, WS 2.5, WS 2.6 WS 3.1, WS 3.2 |
| Reaction time | Spec ref. | Skills |
| Plan and carry out an investigation into the effect of a factor on human reaction time. | Biology 4.5.2.1 Trilogy 4.5.2 Synergy 4.2.1.6 | AT 1 - use appropriate apparatus to record. AT 3 – selecting appropriate apparatus and techniques to measure the process of reaction time. AT 4 – safe and ethical use of humans to measure physiological function of reaction time and responses to a chosen factor. MS 4a |
| Plant responses (Biology only) | Spec ref. | Skills |
| Investigate the effect of light or gravity on the growth of germinated seedlings. Record results as both length measurements and as careful, labelled biological drawings to show the effects. | Biology 4.5.4.1 | AT 1 - use appropriate apparatus to record length and time. AT 3 – selecting appropriate apparatus and techniques to measure the growth of shoots or roots. AT 4 – safe and ethical use of plants to measure physiological function of growth in response to light or gravity. AT 7 – observations of biological specimens to produce labelled scientific drawings. WS 2.2, WS 2.3, WS 2.6, WS 2.7 WS 3.1 |
| Field investigations | Spec ref. | Skills |

| | | |
|---|--|--|
| <p>Measure the population size of a common species in a habitat. Use sampling techniques to investigate the effect of a factor on the distribution of this species.</p> | <p>Biology 4.7.2.1</p> <p>Trilogy 4.7.2.1</p> <p>Synergy 4.4.2.4</p> | <p>AT 1 - use appropriate apparatus to record length and area.</p> <p>AT 3 - use transect lines and quadrats to measure distribution of a species.</p> <p>AT 4 - safe and ethical use of organisms and response to a factor in the environment.</p> <p>AT 6 – application of appropriate sampling techniques to investigate the distribution and abundance of organisms in an ecosystem via direct use in the field.</p> <p>AT 8 – use of appropriate techniques in more complex contexts including continuous sampling in an investigation (Biology only).</p> <p>MS 1d, MS 2b, MS 2d, MS 2f, MS 3a, MS 4c</p> <p>WS 2.1, WS 2.2, WS 2.3.</p> |
| <p>Decay (Biology only)</p> | <p>Spec ref.</p> | <p>Skills</p> |
| <p>Investigate the effect of temperature on the rate of decay of fresh milk by measuring pH change.</p> | <p>Biology 4.7.2.3</p> | <p>AT 1 – use appropriate apparatus to record temperature and pH.</p> <p>AT 3 – the use of appropriate apparatus to measure anaerobic decay.</p> <p>AT 4 – safe use of microorganisms.</p> <p>AT 5 – measurement of rate of decay by pH change.</p> <p>MS 1c, MS 4a, MS 4c</p> <p>WS 2.1, WS 2.4, WS 2.6, WS 2.7</p> |

GCSE Biology required practical activity: Microscopy

Teachers' notes

| Required practical activity | Apparatus and techniques |
|--|--------------------------|
| Use a light microscope to observe, draw and label a selection of plant and animal cells. A magnification scale must be included. | AT 1, AT 7 |

Using a light microscope to observe, draw and label cells in an onion skin.

Materials

In addition to access to general laboratory equipment, each group of student needs:

- a small piece of onion
- a knife
- a white tile
- forceps
- a microscope slide
- a coverslip
- a microscope
- iodine solution in a dropping bottle
- prepared animal and plant cells.

Technical information

0.01M Iodine solution may be purchased ready-made or can be made up following the instructions on CLEAPSS recipe sheet 50.

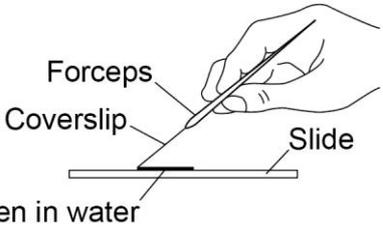
Additional information

The techniques involved should be demonstrated to the students. The students should be allowed time to practice the technique of preparing a wet slide.

It is particularly important that they practise the technique of lowering the cover slip on to the slide so that no air bubbles are trapped.

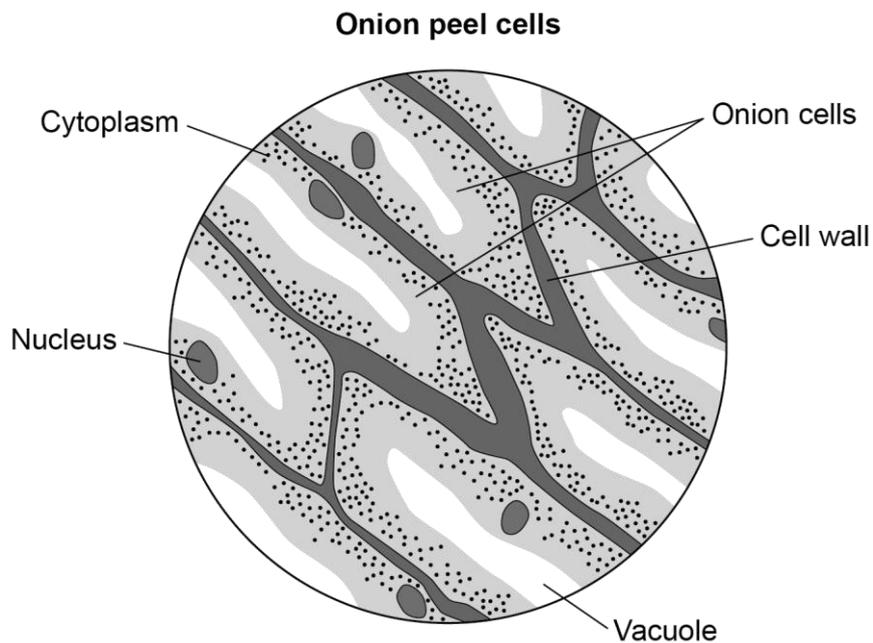
For the exams, students will need to be able to carry out calculations involving magnification, real size and image size using the formula:

$$\text{magnification} = \frac{\text{size of image}}{\text{size of real object}}$$

| Techniques requiring practice | Additional information |
|--|---|
| Lowering the coverslip on to the slide |  <p>The diagram illustrates the technique of lowering a coverslip onto a slide. A hand is shown using forceps to hold the coverslip at an angle, allowing it to slide smoothly onto the specimen on the slide. Labels include: Forceps, Coverslip, Slide, and Specimen in water.</p> |
| Using the microscope | Students should be given guidance in how to use an optical microscope, with particular reference to the coarse and fine focus controls. |

Students should be able to see the following using $\times 400$ magnification.

This diagram may help to identify the different cell parts.



Risk assessment

-
- Risk assessment and risk management are the responsibility of the school or college.
 - Safety goggles should be used when handling iodine solution.
 - Wash off any spillages on the skin immediately.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Microscopy

Student sheet

| Required practical activity | Apparatus and techniques |
|--|--------------------------|
| Use a light microscope to observe, draw and label a selection of plant and animal cells. A magnification scale must be included. | AT 1, AT 7 |

Using a light microscope to observe, draw and label cells in an onion skin

Prepare a microscope slide to show the contents of cells from onion skin.

Use an optical microscope to observe and draw the onion cells. You will also need to identify structures within the cells.

Your teacher will provide a selection of other plant and animal cells to view.

| Learning outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Method

You are provided with the following:

- a small piece of onion
- a knife
- a white tile
- forceps
- a microscope slide
- a coverslip
- a microscope
- iodine solution in a dropping bottle

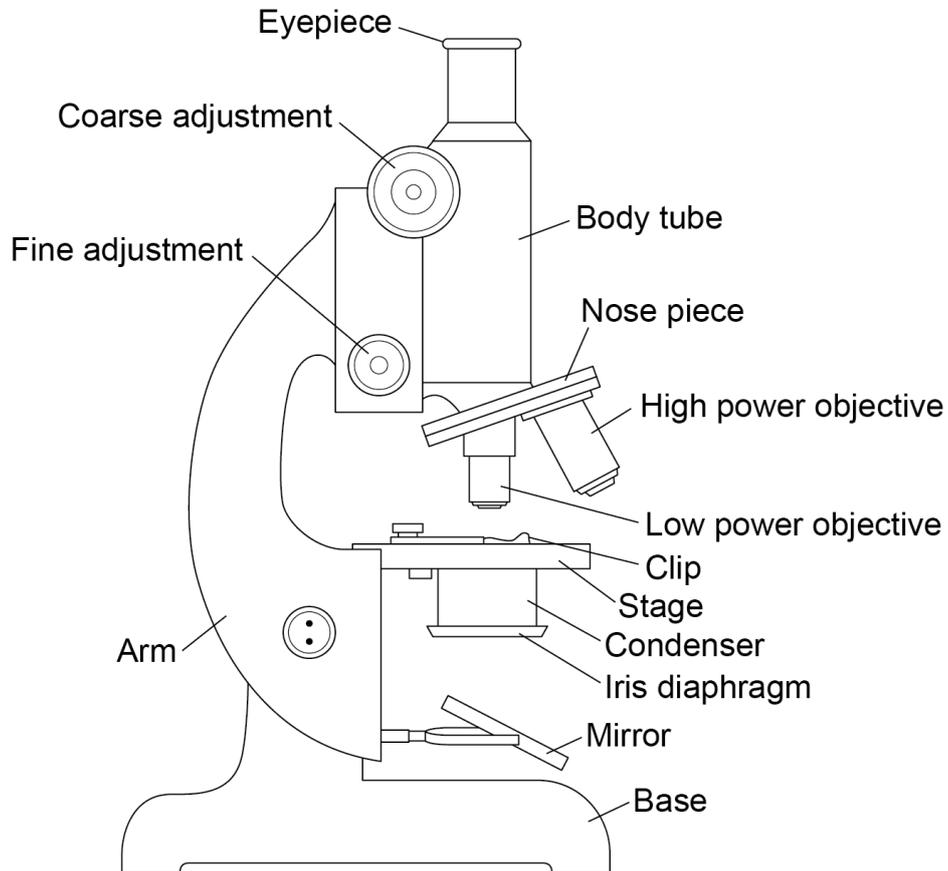
-
- prepared animal and plant cells

Read these instructions carefully before you start work.

1. Use a dropping pipette to put one drop of water onto a microscope slide.
2. Separate one of the thin layers of the onion.
3. Peel off a thin layer of epidermal tissue from the inner surface.
4. Use forceps to put this thin layer on to the drop of water that you have placed on the microscope slide.
5. Make sure that the layer of onion cells is flat on the slide.
6. Put two drops of iodine solution onto the onion tissue.
7. Carefully lower a coverslip onto the slide. Do this by:
 - placing one edge of the coverslip on the slide
 - use the forceps to lower the other edge onto the slide.
8. There may be some liquid around the edge of the coverslip. Use a piece of paper to soak this liquid up.
9. Put the slide on the microscope stage.

Using the microscope to look at animal and plant cells

The diagram shows a typical microscope.



This microscope has a mirror to reflect light up through the slide. Some microscopes have a built-in light instead of a mirror.

10. Use the lowest power objective lens. Turn the nosepiece to do this.
11. The end of the objective lens needs to almost touch the slide. Do this by turning the coarse adjustment knob. Look from the side (**not** through the eyepiece) when doing this.
12. Now looking through the eyepiece, turn the coarse adjustment knob in the direction to increase the distance between the objective lens and the slide. Do this until the cells come into focus.
13. Now rotate the nosepiece to use a higher power objective lens.
14. Slightly rotate the fine adjustment knob to bring the cells into a clear focus and use the high-power objective to look at the cells.

-
15. Make a clear, labelled drawing of some of these cells. Make sure that you draw and label any component parts of the cell.
 16. Write the magnification underneath your drawing.
 17. Use this technique to draw a range of animal and plant cells on prepared slides.

GCSE Biology required practical activity: Microbiology

Teachers' notes

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Investigate the effect of antiseptics or antibiotics on bacterial growth using agar plates and measuring zones of inhibition. | AT 1, AT 3, AT 4, AT 8 |

Investigating the effect of antiseptics on the growth of bacteria

Materials

Teacher

In addition to access to general laboratory equipment, teachers will need the following for demonstration purposes:

- a nutrient agar plate
- a Bunsen burner
- a heatproof mat
- a disposable plastic pipette (sterile)
- a culture of bacteria (*E. coli*– K12 or B strain)
- a sterile glass spreader
- filter paper discs.

Student

- three antiseptics (such as mouthwash, TCP, and antiseptic cream)
- 1% VirKon disinfectant
- forceps
- clear tape
- hand wash
- a wax pencil
- access to an incubator (set to maximum of 25°C).

Technical information

Cultures of *E. coli* bacteria, nutrient agar, and suitable disinfectants for the bench spray and the 'discard beaker' can be bought from educational suppliers. The instructions, and any risk assessment information, which accompany them should be followed carefully. Please note: when using *E. coli*, **penicillin will not produce clearing**. You could use *Micrococcus luteus* instead of *E. coli* which is bright yellow and grows much better at 25 °C.

Plastic petri dishes should be used as these can be destroyed by melting in an autoclave or sterilising pressure cooker, in a specialist autoclave bag (or roasting bag), immediately after obtaining the results. Discs can be cut from filter paper using a hole-punch. Glass spreaders are made by bending a 3–4 mm diameter glass rod into an L-shape.

Plates should be secured with extra sticky tape before student viewing. It is important that condensation in the plates can still escape.

For sterilising glass pipettes or glass spreaders, wrap in greaseproof paper or foil and heat treat at 160°C for 2 hours. Sterile plastic pipettes and spreaders can be purchased.

1% VirKon disinfectant should be used as they have validation of sterilisation.

If ethanol sterilisation is to be used, the ethanol should be kept well away from any naked flames.

The incubator should be kept secure, either in a locked prep room, or locked if it is in the lab.

DISINFECTION: all equipment and materials and work surfaces must be disinfected using excess 1% VirKon for at least 10 minutes. Pipettes and spreaders should be placed into a discard beaker of 1% VirKon immediately after use. An A4 piece of paper that has been laminated to make it waterproof (or a similar sized piece of plastic) is a suitable work surface. The work surface should be placed in a tray of 1% VirKon, so that it is fully covered, for 10 minutes. The surface should be blotted dry with a paper towel before use. Always wear eye protection when using VirKon solution.

Additional information

The aseptic techniques shown in the table below could be demonstrated but students do not need to prepare the plates themselves or spread the lawn of the bacteria.

| Techniques requiring practice | Additional information |
|---|--|
| Flaming the neck of the culture bottle. | This must be done whilst still holding the pipette and the lid of the culture bottle in your other hand (neither should be placed down on the bench at any point). The bottle must not be held still in the flame as the glass will crack – it should be rotated as it is very briefly passed through the flame. |

| | |
|---|--|
| Lifting the lid of the agar plate at an angle. | The lid should only be opened at the side facing the Bunsen burner to avoid contamination |
| Placing drops of culture from the pipette onto the agar. | This needs to be done while carefully holding the lid over the plate. |
| Spreading the bacteria thoroughly around the agar plate right to the edges. | This is best done by holding the glass spreader still up to the edge of the plate and rotating the plate. The lid of the plate must be held over it at the same time to avoid contamination. |
| Placing the filter paper discs onto the agar plate in the right positions. | Students should hold the first disc with the forceps. They should lift the lid of the agar plate at an angle (as before) and place the disc flat onto the central dot in the first third of the plate. The lid of the agar plate should be replaced whilst the next disc is collected. This is repeated so that all three discs are in position. |

It is important to work carefully but quickly to minimise contamination.

Time can be saved by using commercially produced antibiotic discs rather than having the students prepare the discs themselves.

Clear zones are not always perfectly circular so students should measure the diameter twice (at 90° to each other) and calculate a mean diameter for each clear zone.

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- Care should be taken to ensure that appropriate aseptic techniques are used when handling microorganisms.
- There should be facilities available in the laboratory for students to wash their hands thoroughly before and after handling microbes.
- Care should be taken if using ethanol in this experiment. Refer to Hazcard 40A.
- Students should ensure that their work spaces and hands are thoroughly disinfected with 1% VirKon before and after the experiment. Refer to technical notes regarding disinfection.
- Care must be taken to ensure that the lids on the agar plates are secured in place (but not completely sealed). Students must not remove the lids when making their clear zone measurements. Tape plates with two/three small pieces of sticky tape so that lids remain attached to the base.
- All equipment that has come into contact with the microorganisms should be suitably destroyed or sterilised immediately after the experiment.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Microbiology

Student sheet

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Investigate the effect of antiseptics or antibiotics on bacterial growth using agar plates and measuring zones of inhibition. | AT 1, AT 3, AT 4, AT 8 |

Investigating the effect of antiseptics on the growth of bacteria

Measure the diameter of the 'clear zone' around the disc. This is where there is no bacteria growing. The larger the clear zone, the more effective the antiseptic.

| Learning outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk assessment

- Ensure that your work spaces and hands are thoroughly cleaned before and after the experiment.
- Care must be taken when handling microorganisms such as bacteria. You will use techniques called aseptic techniques during this experiment to avoid contamination.
- Contamination can occur when microorganisms from:
 - the surroundings get into your experiment and spoil your results
 - your experiment get into the surroundings and cause a potential health hazard.

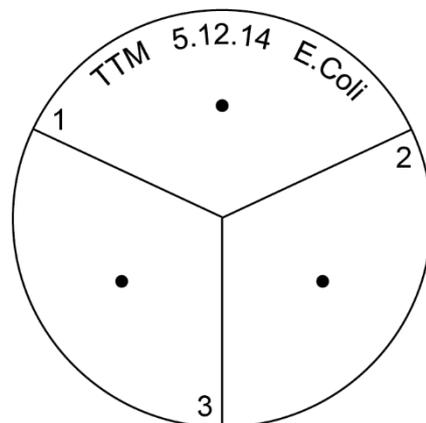
Method

You are provided with the following:

- a nutrient agar plate
- a heatproof mat
- filter paper discs
- three antiseptics (such as mouthwash, TCP, and antiseptic cream)
- disinfectant bench spray
- 1% VirKon disinfectant
- forceps
- clear tape
- hand wash
- a wax pencil
- access to an incubator (set to 25°C).

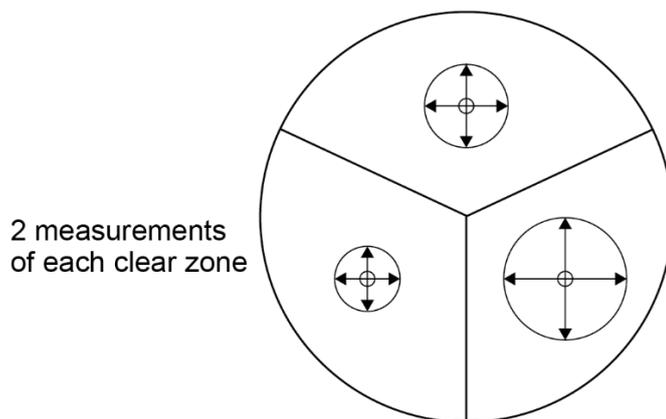
Read these instructions carefully before you start work.

1. Spraying the bench where you are working with disinfectant spray. Then wipe with paper towels.
2. Mark the underneath of a nutrient agar plate (not the lid) with the wax pencil as follows (make sure that the lid stays in place to avoid contamination):
 - divide the plate into three equal sections and number them 1, 2 and 3 around the edge
 - place a dot into the middle of each section
 - around the edge write your initials, the date and the name of the bacteria (*E. coli*)
3. Wash your hands with the antibacterial hand wash.



4. Put different antiseptics onto the three filter paper discs. This can be done by either soaking them in the liquid **or** spreading the cream or paste onto them.

5. Carefully lift the lid of the agar plate at an angle. Do not open it fully.
6. Use forceps to carefully put each disc onto one of the dots drawn on with the wax pencil.
7. Make a note of which antiseptic is in each of the three numbered sections of the plate.
8. Secure the lid of the agar plate in place using two small pieces of clear tape.
Do **not** seal the lid all the way around as this creates anaerobic conditions. Anaerobic conditions will prevent the *E. coli* bacteria from growing and can encourage some other very nasty bacteria to grow.
9. Incubate the plate at 25 °C for 48 hours.
10. Measure the diameter of the clear zone around each disc by placing the ruler across the centre of the disc. Measure again at 90° to the first measurement so that the mean diameter can be calculated.



11. Record your results in a table such as the one here.

| Type of antiseptic | Diameter of clear zone in mm | | |
|----------------------|------------------------------|---|------|
| | 1 | 2 | Mean |
| Mouthwash (1) | | | |
| TCP (2) | | | |
| Antiseptic cream (3) | | | |

GCSE Biology required practical activity: Osmosis

Teachers' notes

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Investigate the effect of a range of concentrations of salt or sugar solutions on the mass of plant tissue. | AT 1, AT 3, AT 5 |

Investigating osmosis in potato tissue

Materials

In addition to access to general laboratory equipment, each group of students' needs:

- a potato
- a cork borer or potato chipper/ vegetable stick cutter
- a ruler
- a 10 cm³ measuring cylinder
- labels
- three boiling tubes
- a test tube rack
- paper towels
- a sharp knife
- a white tile
- a range of sugar solutions (1.0 M - 0.25 M)
- distilled water
- a top-pan balance (accurate to at least 0.01 g).

Technical information

Make up a solution of 1.0 M sucrose solution by adding distilled water to 342.4 g of sugar (dissolve by heating) and making up to 1 litre in a volumetric flask. Dilute this appropriately to produce a range of solutions from 1.0 M - 0.25 M. This should provide enough for a class as each student or group will need 10 cm³ of each solution, in addition to 10 cm³ of distilled water.

To avoid students having to use sharp implements the potato cylinders can be prepared for them. They must be freshly prepared.

Ensure that potato cylinders do not have any skin on them as this affects the movement of water molecules.

Additional information

This investigation can be time-consuming especially if students are waiting for access to a balance. Although a range of five solutions is required to produce a useful graph, each group could measure just three potato cylinders. The class data could then be collated before plotting the graph. Where the line of best fit crosses the x-axis is an approximation of the concentration inside the potato tissue.

The length of time that the potato cylinders are left in the sugar solutions can be adjusted to suit lesson timings. Better results are achieved if they are left for more than 30 minutes.

Note that fungi may grow in the test tubes containing potato in weaker solutions of salt or sugar. Test tube contents should be disposed of after viewing, the day after the potatoes have been placed in the solutions. Any test tubes showing visible growth of fungi should be sterilised by autoclaving.

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- Care should be taken with the use of cork borers and scalpels when students are cutting their own potato cylinders. Small kitchen knives could be used if available.
- Care should be taken with the use of an electrical balance in the presence of water.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Osmosis

Student sheet

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Investigate the effect of a range of concentrations of salt or sugar solutions on the mass of plant tissue. | AT 1, AT 3, AT 5 |

Investigating osmosis in potato tissue

Osmosis is the movement of water through a selectively permeable membrane. The water moves from an area of high concentration of water to an area of lower concentration of water.

Plant tissues can be used to investigate osmosis. This experiment uses potato, but other tissue such as sweet potato, carrot or beetroot can be used.

Potato tissue is cut into equal sized cylinders. The potato tissue is left overnight in sugar solution and distilled water. The changes in length and mass can then be accurately compared.

| Learning outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk assessment

Care should be taken:

- cutting potato cylinders
- with the use of an electrical balance in the presence of water.

Method

You are provided with the following:

- a potato
- a cork borer or potato chipper/vegetable stick cutter
- a ruler
- a 10 cm³ measuring cylinder
- labels
- three boiling tubes
- a test tube rack
- paper towels
- a sharp knife
- a white tile
- a range of sugar solutions
- distilled water
- a top-pan balance.

Read these instructions carefully before you start work.

1. Use a cork borer to cut five potato cylinders of the same diameter.
2. Trim the cylinders so that they are all the same length (about 3 cm).
3. Accurately measure and record the length and mass of each potato cylinder.
4. Measure 10 cm³ of the 1.0 M sugar solution and put into the first boiling tube. Label boiling tube as: 1.0. M sugar.
5. Repeat step 4 to produce the additional labelled boiling tubes containing solutions of 0.75 M, 0.5 M. and 0.25 M.
6. Measure 10 cm³ of the distilled water and put into the fifth boiling tube. Label boiling tube as water.
7. Add one potato cylinder to each boiling tube. Make sure you know the length and mass of each potato cylinder in each boiling tube.

8. Record the lengths and masses of each potato cylinder in a table such as the one below.

| | 1.0 M sugar solution | 0.75 sugar solution | 0.5 M sugar solution | 0.25 M sugar solution | Distilled water |
|-----------------------|----------------------|---------------------|----------------------|-----------------------|-----------------|
| Initial length (mm) | | | | | |
| Final length (mm) | | | | | |
| Change in length (mm) | | | | | |
| Initial mass (g) | | | | | |
| Final mass in (g) | | | | | |
| Change in mass in (g) | | | | | |

9. Leave the potato cylinders in the boiling tubes overnight in the test tube rack.

10. Remove the cylinders from the boiling tubes and carefully blot them dry with the paper towels.

11. Re-measure the length and mass of each cylinder (make sure you know which is which).

Record your measurements in the table. Then calculate the changes in length and mass of each potato cylinder.

12. Plot a graph with:

- 'Change in mass in g' on the y-axis
- 'Concentration of sugar solution' on the x-axis.

13. Plot another graph with:

- 'Change in length in mm' on the y-axis
- 'Concentration of sugar solution' on the x-axis.

Compare the two graphs that you have drawn.

GCSE Biology required practical activity: Enzymes

Teachers' notes

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| <p>Investigate the effect of pH on the rate of reaction of amylase enzyme.</p> <p>Students should use a continuous sampling technique to determine the time taken to completely digest a starch solution at a range of pH values. Iodine reagent is to be used to test for starch every 30 seconds.</p> <p>Temperature must be controlled by use of a water bath or immersible electric heater.</p> | AT 1, AT 2, AT 5, AT 8 |

Investigating the effect of pH on the enzyme amylase

Materials

In addition to access to general laboratory equipment, each group of students needs:

- test tubes
- a test tube rack
- water baths (electrical or Bunsen burners and beakers)
- spotting tiles
- a 5 cm³ measuring cylinder
- syringes or 10 cm³ measuring cylinders
- a glass rod
- a stop watch
- starch solution
- amylase solution
- buffered solutions
- iodine solution
- thermometers.

Technical information

A 1% solution of amylase and a 1% suspension of starch are appropriate for this experiment.

Amylase will slowly lose activity so it is best to make up a fresh batch, using the powdered enzyme, for each lesson. Otherwise any results collected on different days will not be comparable. Alternatively, use AMG (amylolucosidase) which breaks down starch more effectively.

Starch suspension should also be made fresh. This can be done by making a cream of 5 g of soluble starch in cold water and pouring into 500 cm³ of boiling water. Stir well and boil until you have a clear solution.

A 0.01 M solution of iodine is suitable for starch testing.

Buffer solutions should be made using CLEAPSS recipe 18 (The Universal Buffer: Recipe 1). The optimum pH for amylase is pH 6. A range of buffer solutions between pH 5 - 8 would be appropriate.

Additional information

It is best to check that the amylase breaks down the starch at an appropriate rate before students do this experiment. At around the optimum pH of 6, the end point should be reached within 1–2 minutes.

It might be appropriate for each student to test only one pH, working in a pair or a group, so that results can be pooled. This would ensure that the tests were performed in the same lesson, and therefore are more comparable.

A wider range of pH could be investigated and class results could be collated. This would require more water baths, but students could make their own using beakers and Bunsen burners etc.

Some amylases used in detergents are not denatured even at temperatures close to boiling water. Some amylases are also inhibited by buffers.

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- All solutions, once made up, are low hazard. Refer to Hazcard 33 for amylase.
- Iodine solution may irritate the eyes so safety goggles should be worn. Refer to Hazcards 54A and 54B.
- Universal buffer solution is an irritant. Refer to Hazcards 9, 14, 72, and 91.
- Safety goggles should be worn in the presence of hot water in water baths.

-
- Care should be taken with the use of naked flames in this experiment if students are using Bunsen burners to make water baths.
 - Care should be taken with the presence of water and electrical equipment, if electrical water baths are being used.
 - Note that some people are allergic to enzymes.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Enzymes

Student sheet

| Required practical activity | Apparatus and techniques |
|--|--------------------------|
| <p>Investigate the effect of pH on the rate of reaction of amylase enzyme.</p> <p>Students should use a continuous sampling technique to determine the time taken to completely digest a starch solution at a range of pH values. Iodine reagent is to be used to test for starch every 30 seconds.</p> <p>Temperature must be controlled by use of a water bath or electric heater.</p> | AT 1, AT 2, AT 5, AT 8 |

Investigating the effect of pH on the enzyme amylase

The enzyme amylase controls the breakdown of starch in our digestive system. We are able to simulate digestion using solutions of starch and amylase in test tubes. We can also determine the optimum conditions required.

The presence or absence of starch can be determined using iodine solution. In this experiment, we can measure how long the amylase takes to break down the starch at different pHs.

| Learning outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk assessment

- Safety goggles should be worn throughout.
- Take care with boiling water.

Method

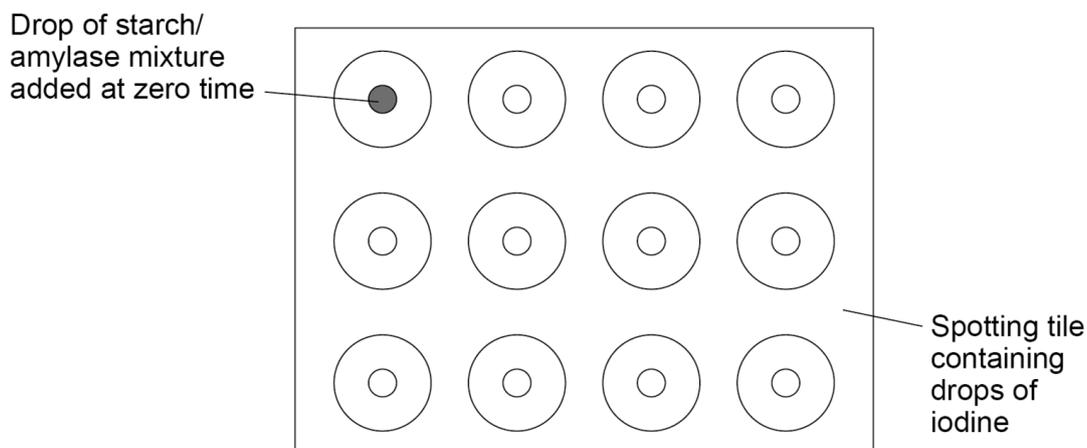
You are provided with the following:

- test tubes
- a test tube rack
- water bath (electrical or Bunsen burner and beakers)
- spotting tiles
- 5cm³ measuring cylinder
- syringes
- a stop clock
- starch solution
- amylase solution
- buffered solutions covering a range of pH, each with a labelled syringe/ plastic pipette
- iodine solution
- syringes.

Read these instructions carefully before you start work.

1. Place one drop of iodine solution into each depression on the spotting tile.
2. Place labelled test tubes containing the buffered pH solutions, amylase solution and starch solutions in to the water bath
3. Allow the solutions to reach 25 °C
4. Add 2cm³ of one of the buffered solutions to a test tube.
5. Use the syringe to place 2 cm³ of amylase into the buffered pH solution.
6. Use another syringe to add 2 cm³ of starch to the amylase/buffer solution.
7. Immediately start the stop clock and leave it on throughout the test.
8. Mix using a glass rod.
9. After 30 seconds, remove one drop of the mixture with a glass rod.
Place this drop on the first depression of the spotting tile with the iodine solution.

The iodine solution should turn blue-black.



10. Rinse the rod.

11. Use the glass rod to remove one drop of the mixture every 30 seconds. Put each drop onto the iodine solution in the **next** depression on the spotting tile. Rinse the glass rod with water after each drop. Continue until the iodine solution and the amylase/buffer/starch mixture remain orange.

12. Repeat the procedure with solutions of other pHs

13. Record your results in a table such as the one here.

| pH of solution | Time taken for amylase to completely break down the starch in seconds (s) |
|----------------|---|
| | |

14. Plot a graph with:

- 'Time taken to break down starch (s)' on the y-axis
- 'pH of solution' on the x-axis

or

15. Calculate the rate of reaction and plot a graph with:

- 'Rate of reaction' on the y-axis
- 'pH of the solution' on the x-axis.

GCSE Biology required practical activity: Food tests

Teachers' notes

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Use qualitative reagents to test for a range of carbohydrates, lipids and proteins. To include: Benedict's test for sugars; iodine test for starch; Biuret reagent for protein. | AT 2, AT 8 |

Using qualitative reagents to test for a range of carbohydrates, lipids and proteins

Materials

In addition to access to general laboratory equipment, each group of students' needs:

- food to be tested
- a pestle and mortar
- a stirring rod
- a filter funnel and filter paper
- 5 × beaker, 250 ml
- a conical flask
- 4 × test tube
- Benedict's solution
- iodine solution (0.01 M)
- Sudan III stain solution
- Biuret solution
- kettle for boiling water
- a thermometer
- safety goggles.

Technical information

Benedict's qualitative reagent (CLEAPSS)

Benedict's solution or DNSA (see Recipe sheet 34) should be used to test for reducing sugars.

Glucose, lactose and maltose are reducing sugars and give a positive test. Sucrose is a non-reducing sugar and does not give a positive result.

No hazard warning symbol is required on the bottle as the concentrations of each of the constituents are low.

Qualitative Biuret Reagent (CLEAPSS)

This does **not** keep so only prepare what is required.

General hazards:

- Sodium hydroxide (solid) and 2 M solution. See Hazcard 91.
- Copper sulphate, see Hazcard 27C.

Preparing 1 litre of Qualitative Biuret reagent:

- wear safety goggles
- weigh out 0.75 g of copper(II) sulfate(VI)-5 -water
- prepare 1 litre of 2 M potassium or sodium hydroxide solution
- dissolve the copper(II) sulfate(VI) in the alkali and label the solution CORROSIVE

A purple or pink colouration indicates the presence of protein.

Iodine solution (CLEAPSS)

A 0.01 M solution is suitable as a test reagent for starch and may be purchased ready-made or can be made up following the instructions on CLEAPSS recipe sheet 50.

The concentration of solutions decreases with storage. Check that the solutions work before use in the laboratory.

Sudan III stain solution

Dissolve 0.5 g of dye in 70 ml of ethanol and 30 ml of water, using a warm water bath, and filter. Ethanol is HIGHLY FLAMMABLE (see Hazcards 32 and 40). Label the solution HIGHLY FLAMMABLE.

Wear safety goggles.

Additional information

The techniques involved should be demonstrated to the students. Students should be allowed time to practice the techniques by testing pure substances first in order to see the expected colour change. The following are suggested for this purpose:

- Biuret test – albumen solution (eg 1% concentration)
- Benedict’s solution – glucose solution (eg 1% concentration)
- iodine solution – starch solution (eg 1% concentration)
- Sudan III – any suitable oil.

In particular, students will need to practice the following:

| Techniques requiring practice | Additional information |
|-------------------------------|---|
| Use of a pestle and mortar | When crushing the food it may help to add a small amount of sharp sand |
| Filtration | Students may need to be taught how to fold the filter paper correctly |
| Use of water bath | Students may need to learn how to use a beaker of hot water as a water bath |

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- Biuret solution contains copper sulfate, which is poisonous, and sodium hydroxide, which is caustic.
- Safety goggles should be worn when carrying out the tests.
- Wash off spills on skin immediately.
- Ensure that there is no eating or drinking during testing.

Suggested foods for testing

Proteins: milk, yogurt, cheese, meat, tofu, apple, potato, yeast, cooked beans, eggs.

Lipids: olive oil, sesame seed oil, grape seed oil, margarine, butter, lard, milks (full fat, semi-skimmed, skimmed), egg white solution, egg yolk solution.

Carbohydrates: potato, bread, cooked noodles, biscuits, sugar, apples, flour, corn starch.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Food tests

Student sheet

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Use qualitative reagents to test for a range of carbohydrates, lipids and proteins. To include: Benedict's test for sugars; iodine test for starch; Biuret reagent for protein. | AT 2, AT 8 |

1. Testing for sugars

In this experiment you will test one or more foodstuffs for the presence of carbohydrates.

| Learning outcomes |
|---|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk assessment

- Safety goggles should be worn when carrying out the tests.
- Wash off spills on skin immediately.
- Take care with boiling water.

Method

You are provided with the following:

- food to be tested
- a pestle and mortar
- a stirring rod
- filter funnel and filter paper
- 2 × beaker, 250 ml
- a conical flask

-
- 2 × test tube
 - Benedict's solution
 - iodine solution
 - kettle for boiling water
 - a thermometer
 - safety goggles.

Read these instructions carefully before you start work.

1. Use a pestle and mortar to grind up a small sample of food.
2. Transfer the ground up food into a small beaker. Then add distilled water.
3. Stir the mixture so that some of the food dissolves in the water.
4. Filter using a funnel with filter paper to obtain as clear a solution as possible.
The solution should be collected in a conical flask.
5. Half fill a test tube with some of this solution.
6. Add 10 drops of Benedict's solution to the solution in the test tube.
7. Put hot water from a kettle in a beaker. The water should **not** be boiling.
Put the test tube in the beaker for about five minutes.
8. Note any colour change.
If a reducing sugar (such as glucose) is present, the solution will turn green, yellow, or brick-red. The colour depends on the sugar concentration.
9. Take 5 ml of the solution from the conical flask and put it into a clean test tube.
10. Add a few drops of iodine solution and note any colour change.
If starch is present, you should see a black or blue-black colour appear.
11. Record your results in a table such as the one below.

| Name of food tested | Colour produced with Benedict's solution | Colour produced with iodine solution |
|---------------------|--|--------------------------------------|
| | | |

2. Testing for lipids

In this experiment you will test one or more foodstuffs for the presence of lipids (fats).

| Learning outcomes |
|---|
| <p>1</p> <p>2</p> <p>3</p> <p>Teachers to add these with particular reference to working scientifically</p> |

Risk assessment:

- Safety goggles should be worn when carrying out the tests.
- Sudan III contains ethanol, which is highly flammable. Keep the solution away from naked flames.
- Wash off spills on skin immediately.

Method

You are provided with the following:

- food to be tested
- a pestle and mortar
- a stirring rod
- 2 × beaker, 250 ml
- a test tube
- Sudan III stain solution.
- safety goggles.

Read these instructions carefully before you start work.

1. Use a pestle and mortar to grind up a small sample of food.
2. Transfer the ground up food into a small beaker. Then add distilled water.
3. Stir the mixture so that some of the food dissolves in the water. Do not filter.
4. Half fill a test tube with some of this solution.
5. Add 3 drops of Sudan III stain to the solution in the test tube. Shake gently to mix.
6. If fat is present: a red-stained oil layer will separate out and float on the water surface.

3. Testing for proteins

In this experiment you will test one or more foodstuffs for the presence of protein.

| Learning outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk assessment:

- Safety goggles should be worn when carrying out the tests.
- Biuret solution contains copper sulphate, which is poisonous, and sodium hydroxide, which is caustic.
- Wash off spills on skin immediately.

Method

You are provided with the following:

- food to be tested
- a pestle and mortar

-
- a stirring rod
 - a filter funnel and filter paper
 - 2 × beaker, 250 ml
 - a test tube
 - Biuret solution
 - safety goggles.

Read these instructions carefully before you start work.

1. Use a pestle and mortar to grind up a small sample of food.
2. Transfer the ground up food into a small beaker. Then add distilled water.
3. Stir the mixture so that some of the food dissolves in the water.
4. Filter using a funnel with filter paper to obtain as clear a solution as possible.
The solution should be collected in a conical flask.
5. Put 2 cm³ of this solution into a test tube.
6. Add 2 cm³ of Biuret solution to the solution in the test tube. Shake gently to mix.
7. Note any colour change. Proteins will turn the solution pink or purple.

GCSE Biology required practical activity: Photosynthesis

Teachers' notes

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Investigate the effect of light intensity on the rate of photosynthesis using an aquatic organism such as pondweed. | AT 1, AT 3, AT 4, AT 5 |

Investigating the effect of light intensity on photosynthesis in pondweed

Materials

In addition to access to general laboratory equipment, each group of students' needs:

- a boiling tube
- freshly cut 10 cm piece of pondweed
- a light source
- a ruler
- a test tube rack
- a stop watch
- 0.2% solution of sodium hydrogen carbonate solution
- a glass rod.

Technical information

Native species of *Cabomba* or *Elodea* could be used as the pondweed in this investigation. Both can be bought from tropical fish shops and some large garden centres.

Cabomba is recommended as it is the most reliable as it produces the most bubbles. *Cabomba* should be kept in a well aerated tank prior to its use. If *Elodea* is used, it is suggested that the plant is placed in a beaker of water in front of a lamp for 2–3 hours before starting the investigation. Alternatively students could immobilise *Scenedesmus* algae in alginate. This gives a measured amount of photosynthetic material which can be placed directly in to bicarbonate indicator.

High intensity light sources (at least 1000 lumens) need to be used for the practical.

It is best to use an LED light source as they give off less heat, although care should be taken to ensure that they emit the correct wavelengths required for photosynthesis. If these are not available, use a normal light bulb but place a beaker of water in between the boiling tube and the

light source to reduce the chance of temperature affecting the results. Low energy light bulbs should not be used as the light intensity may be too low to promote measurable photosynthesis.

Additional information

Graphs can be drawn of number of bubbles per minute against distance from light source.

Light intensity is proportional to $1/\text{distance}^2$. Higher attaining students may want to draw their graphs of number of bubbles against light intensity instead.

If no bubbles appear from the cut end of the pondweed when placed closest to the light source, cut a few millimetres off the end or, if necessary, use a new freshly-cut piece of pondweed.

Students could work within a group in order to investigate a wider range of distances and with increments of 5 cm instead of 10 cm. Group results could be collated.

Note

Students need to be aware of a method to measure the volume of oxygen produced by photosynthesis. The method described here can be modified by placing the elodea under a filter funnel in a beaker of water. A 10 cm³ measuring cylinder containing water is inverted over the spout of the filter funnel. Any oxygen produced by the elodea passes through the funnel and is collected in the measuring cylinder. The volume of oxygen produced in different light intensities can be measured over a time.

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- 0.2% sodium hydrogen carbonate solution is low hazard. Refer to Hazcard 95C.
- Care should be taken when handling glassware.
- Care should be taken with the use of lamps that may get hot.
- Use a large beaker of water in front of hot light sources.
- Care should be taken when using mercury-containing light bulbs (eg compact fluorescent tubes).
- Use light sources that absorb any UV light given off by the bulb/tube.
- Care should be taken with the presence of water and the electrical power supply for the lamp.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Photosynthesis

Student sheet

| Required practical activity | Apparatus and techniques |
|--|--------------------------|
| Investigate the effect of a factor on the rate of photosynthesis using an aquatic organism such as pondweed. | AT 1, AT 3, AT 4, AT 5 |

Investigating the effect of light intensity on photosynthesis in pondweed

Plants use carbon dioxide and water to produce glucose and oxygen. This process is called photosynthesis. The rate of photosynthesis is affected by many factors, such as:

- light intensity
- light wavelength.

Aquatic plants produce visible bubbles of oxygen gas into the surrounding water when they photosynthesise. These bubbles can be counted as a measure of the rate of photosynthesis. Pondweed is an example of an aquatic plant.

The effect of light intensity can be investigated by varying the distance between pondweed and a light source. The closer the light source, the greater the light intensity.

| Learning outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk assessment:

Care should be taken:

- when handling glassware
- with the use of lamps that may get hot
- with the presence of water and the electrical power supply for the lamp.

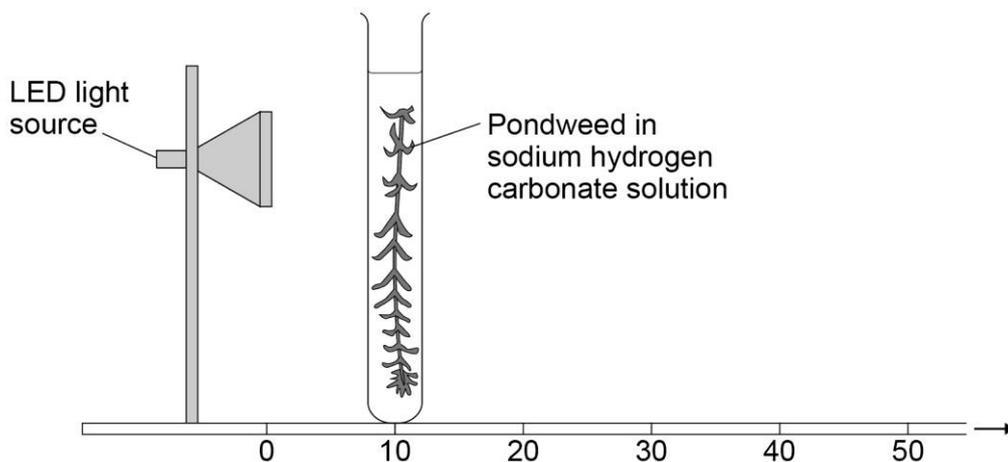
Method

You are provided with the following:

- a boiling tube
- freshly cut 10 cm piece of pondweed
- a light source
- a ruler
- a test tube rack
- a stop watch
- 0.2% solution of sodium hydrogen carbonate
- a glass rod.

Read these instructions carefully before you start work.

1. Set up a test tube rack containing a boiling tube at a distance of 10 cm away from the light source
2. Fill the boiling tube with the sodium hydrogen carbonate solution.
3. Put the piece of pondweed into the boiling tube with the cut end at the top. Gently push the pondweed down with the glass rod.
4. Leave the boiling tube for 5 minutes.
5. Start the stop watch and count the number of bubbles produced in one minute.



6. Record the results in a table such as the one here.

| Distance between pondweed and light source in cm | Number of bubbles per minute | | | |
|--|------------------------------|---|---|------|
| | 1 | 2 | 3 | Mean |
| 10 | | | | |
| 20 | | | | |
| 30 | | | | |
| 40 | | | | |

7. Repeat the count twice more. Then use the data to calculate the mean number of bubbles per minute.
8. Repeat steps **1–7** with the test tube rack and boiling tube at distances of 20 cm, 30 cm and 40 cm from the light source.

GCSE Biology required practical activity: Reaction Time

Teachers' notes

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Plan and carry out an investigation into the effect of a factor on human reaction time. | AT 1, AT 3, AT 4 |

Investigating whether practice reduces human reaction times

Materials

In addition to access to general laboratory equipment, each group of students' needs a:

- metre ruler
- chair
- table
- partner.

Technical information

Students should use their weaker hand for the ruler drop test. They should ensure that they have not done any practicing before the start of the experiment but start taking measurements immediately so that the effects of any practicing can be seen.

Ruler measurements can be converted to reaction times using the conversion table below.

Additional information

Other factors that could be investigated include the effect of caffeine or exercise on reaction times.

The following conversion table can be given to students in order to determine reaction times.

Graphs of reaction time against attempt number can be drawn.

| Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) | Reading from ruler (cm) | Reaction time (s) |
|-------------------------|-------------------|-------------------------|-------------------|-------------------------|-------------------|-------------------------|-------------------|-------------------------|-------------------|-------------------------|-------------------|-------------------------|-------------------|-------------------------|-------------------|-------------------------|-------------------|-------------------------|-------------------|
| 1 | 0.05 | 21 | 0.21 | 41 | 0.29 | 61 | 0.35 | 81 | 0.41 | | | | | | | | | | |
| 2 | 0.06 | 22 | 0.22 | 42 | 0.29 | 62 | 0.36 | 82 | 0.41 | | | | | | | | | | |
| 3 | 0.08 | 23 | 0.22 | 43 | 0.30 | 63 | 0.36 | 83 | 0.41 | | | | | | | | | | |
| 4 | 0.09 | 24 | 0.22 | 44 | 0.30 | 64 | 0.36 | 84 | 0.41 | | | | | | | | | | |
| 5 | 0.10 | 25 | 0.23 | 45 | 0.30 | 65 | 0.36 | 85 | 0.42 | | | | | | | | | | |
| 6 | 0.11 | 26 | 0.23 | 46 | 0.31 | 66 | 0.37 | 86 | 0.42 | | | | | | | | | | |
| 7 | 0.12 | 27 | 0.23 | 47 | 0.31 | 67 | 0.37 | 87 | 0.42 | | | | | | | | | | |
| 8 | 0.13 | 28 | 0.24 | 48 | 0.31 | 68 | 0.37 | 88 | 0.42 | | | | | | | | | | |
| 9 | 0.14 | 29 | 0.24 | 49 | 0.32 | 69 | 0.38 | 89 | 0.43 | | | | | | | | | | |
| 10 | 0.14 | 30 | 0.25 | 50 | 0.32 | 70 | 0.38 | 90 | 0.43 | | | | | | | | | | |
| 11 | 0.15 | 31 | 0.25 | 51 | 0.32 | 71 | 0.38 | 91 | 0.43 | | | | | | | | | | |
| 12 | 0.16 | 32 | 0.26 | 52 | 0.33 | 72 | 0.38 | 92 | 0.43 | | | | | | | | | | |
| 13 | 0.16 | 33 | 0.26 | 53 | 0.33 | 73 | 0.39 | 93 | 0.44 | | | | | | | | | | |
| 14 | 0.17 | 34 | 0.26 | 54 | 0.33 | 74 | 0.39 | 94 | 0.44 | | | | | | | | | | |
| 15 | 0.18 | 35 | 0.27 | 55 | 0.34 | 75 | 0.39 | 95 | 0.44 | | | | | | | | | | |
| 16 | 0.18 | 36 | 0.27 | 56 | 0.34 | 76 | 0.39 | 96 | 0.44 | | | | | | | | | | |
| 17 | 0.19 | 37 | 0.28 | 57 | 0.34 | 77 | 0.40 | 97 | 0.45 | | | | | | | | | | |
| 18 | 0.19 | 38 | 0.28 | 58 | 0.34 | 78 | 0.40 | 98 | 0.45 | | | | | | | | | | |
| 19 | 0.20 | 39 | 0.28 | 59 | 0.35 | 79 | 0.40 | 99 | 0.45 | | | | | | | | | | |
| 20 | 0.21 | 40 | 0.29 | 60 | 0.35 | 80 | 0.40 | 100 | 0.45 | | | | | | | | | | |

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- Care should be taken to avoid injury from the falling ruler.
- Care should be taken to ensure that students do not experience any discomfort when being used as the subjects of investigation.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Reaction Time

Student sheet

| Required practical activity | Apparatus and techniques |
|---|--------------------------|
| Plan and carry out an investigation into the effect of a factor on human reaction time. | AT 1, AT 3, AT 4 |

Investigating whether practice reduces human reaction times

Messages travel very quickly around your body through the nervous system. This is so that you are able to respond to changes in the environment. The time it takes for you to respond to such a change is called your reaction time.

Athletes spend hours practising to try to reduce their reaction time. This is to help them improve their performance in their particular sport. Responding quicker to the starter's pistol in a race can gain you the advantage over other runners.

You will conduct a simple, measurable experiment called the ruler drop test. From this you can determine whether your reaction time can be reduced with practice.

| Learning outcomes |
|---|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk Assessment

- Care should be taken to avoid injury from the falling ruler.

Method

You are provided with the following:

- a metre ruler
- a chair
- a table
- a partner.

Read these instructions carefully before you start work:

1. Use your weaker hand for this experiment. If you are right handed then your left hand is your weaker hand.
2. Sit down on the chair with good upright posture and eyes looking across the room.
3. Place the forearm of your weaker arm across the table with your hand overhanging the edge of the table.
4. Your partner will hold a ruler vertically with the bottom end (the end with the 0 cm) in between your thumb and first finger.
Practice holding the ruler with those two fingers.
5. Your partner will take hold of the ruler and ask you to remove your fingers.
6. Your partner will hold the ruler so the zero mark is level with the top of your thumb. They will tell you to prepare to catch the ruler.
7. Your partner will then drop the ruler **without** telling you.
8. You must catch the ruler as quickly as you can when you sense that the ruler is dropping.
9. After catching the ruler, look at the number level with the top of your thumb.
Record this in a table such as the one here.

| Drop test attempts | Ruler measurements in cm | | Reaction times in seconds | |
|--------------------|--------------------------|----------|---------------------------|----------|
| | Person 1 | Person 2 | Person 1 | Person 2 |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |

10. Have a short rest and then repeat the test. Record the number on the ruler as attempt 2.
11. Continue to repeat the test several times.
12. Swap places with your partner. Repeat the experiment to get their results.
13. Use a conversion table to convert your ruler measurements into reaction times.

GCSE Biology required practical activity: Plant responses

Teachers' notes

| Required practical activity | Apparatus and techniques |
|--|--------------------------|
| Investigate the effect of light or gravity on the growth of newly germinated seedlings. Record results as both length measurements and as carefully, labelled biological drawings to show the effects. | AT 1, AT 3, AT 4, AT 7 |

Investigating the effect of light intensity on the growth of mustard seedlings

Materials

In addition to access to general laboratory equipment, each group of students' needs:

- white mustard seeds
- petri-dishes
- cotton wool
- a ruler
- water
- access to a light windowsill and a dark cupboard.

Technical information

Cotton wool should be damp but not in excess water. The amount of cotton wool and water needed should be determined before students do the experiment.

Seeds will require a day or so to germinate (depending on how warm it is). Alternative seeds, such as cress or *Brassica rapa*, could be used instead of white mustard seeds. However, white mustard seeds are bigger and easier to handle so these are recommended.

Partial light can be achieved by alternating a day on the windowsill with a day in the dark cupboard.

Additional information

To measure the height of the seedlings students should rest the ruler on the cotton wool and then gently hold the seedling to its full height against the ruler. Care should be taken to ensure the seedlings are not damaged during measuring.

Note

There are a number of variations that can make basic investigation more challenging. These include:

1. The effect of different light conditions can be measured by placing germinated seedlings in a covered box illuminated from one side. It is possible to establish the effect of different light intensities by measuring the angle of growth.
2. The effect of gravity on plant growth is best observed by placing newly germinated bean seeds in a beaker or gas jar stuffed with kitchen towel. The beans should be pressed up against the glass to make growth visible. The beans will continue to grow if the kitchen towel is kept moist. The shoots will begin to grow upwards and the roots downwards. If the beans are rotated through 90° after 48 hours further growth will show the effect of gravity on the shoot and root. This investigation provides good opportunities for making biological drawings.

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- The equipment and techniques used in this experiment are not hazardous.
- Wash hands after handling seeds.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Plant responses

Student sheet

| Required practical activity | Apparatus and techniques |
|--|--------------------------|
| Investigate the effect of light or gravity on the growth of newly germinated seedlings. Record results as both length measurements and as carefully, labelled biological drawings to show the effects. | AT 1, AT 3, AT 4, AT 7 |

Investigating the effect of light intensity on the growth of mustard seedlings

Light affects the distribution of auxins within the stems of newly germinated seeds. The effect of light on this growth can be determined by measuring the height of shoots with a ruler.

| Learning outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk Assessment

- Wash hands after handling seeds.

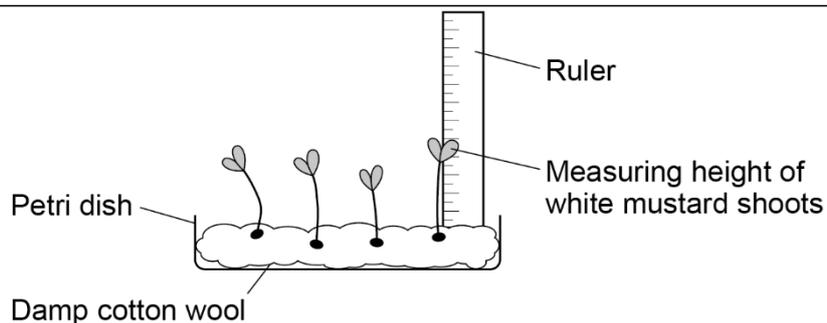
Method

You are provided with the following:

- white mustard seeds
- petri-dishes
- cotton wool
- a ruler
- water
- access to a light windowsill and a dark cupboard.

Read these instructions carefully before you start work.

1. Set up three petri dishes containing cotton wool soaked in equal amounts of water.
2. Put ten mustard seeds in each petri dish.
3. Put the petri dishes in a warm place. They must **not** be disturbed or moved.
4. Allow the mustard seeds to germinate.
Add more water if the cotton wool gets dry (equal amounts of water to each petri dish).
5. Each petri dish should have the same number of seedlings after the seeds have germinated.
Remove excess seedlings from any dish that has too many.
For example, one dish has eight seedlings which are the fewest compared to the other petri dishes. Therefore, remove seedlings from the other petri dishes so that each dish has eight.
6. Move the petri dishes into position.
 - One should be placed on a windowsill in full sunlight.
 - One should be placed in partial light.
 - The third should be placed in darkness.
7. Measure the height of each seedling. Do this every day, for **at least** a week.



Record the heights in a table such as the one here.

You will need a table each for:

- full sunlight
- partial light
- darkness.

| Day | Height of seedling in full sunlight in mm | | | | | | | | Mean |
|-----|---|---|---|---|---|---|---|---|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| 1 | | | | | | | | | |
| 2 | | | | | | | | | |
| 3 | | | | | | | | | |
| 4 | | | | | | | | | |
| 5 | | | | | | | | | |
| 6 | | | | | | | | | |
| 7 | | | | | | | | | |

8. Calculate the mean height of the seedlings each day.

9. Plot a graph with:

- 'Mean height in mm' on the y-axis
- 'Day' on the x-axis.

The graph should include data for full sunlight, partial light and darkness.

Compare the data.

GCSE Biology required practical activity: Field investigations

Teachers' notes

| Required practical activity | Apparatus and techniques |
|--|------------------------------|
| Measure the population size of a common species in a habitat. Use sampling techniques to investigate the effect of a factor on the distribution of this species. | AT 1, AT 3, AT 4, AT 6, AT 8 |

There are two parts to this Investigation:

- 1. Investigating the population size of a plant species using random sampling**
- 2. Investigating the effect of a factor on plant distribution using a transect line.**

Materials

In addition to access to general laboratory equipment, each student needs:

- a 25 cm x 25cm quadrat
- a 30 m tape measure
- a clipboard
- a pen
- paper.

Technical information

1. Investigating the population size of a plant species using random sampling

Choose an area of grass with sufficient space to carry out this survey. You will need at least 400 m² to accommodate a class.

Lay out two tape measures (or marked strings) 20m in length so that they form right angles. These two tape measures represent the two sides of a 20m x 20m square. Place two bags containing numbers at the point where the two tape measures meet. Help the students to identify the species being investigated – plantain is very common although dandelion could also be used.

Organise the students into groups of three. One student will select a number from one of the bags and move that distance in metres along the tape. A second student will select a number from the other bag and move that distance along the other tape. The third student with the quadrat uses the other two students as markers in order to place the quadrat on the ground. The group then return

their numbers to the bags and return to their quadrat in order to count and record the number of plantain in the quadrat.

Get students to repeat this process in order to count the number of plantain in 10 quadrats. Students can then use this data to estimate the population of the survey area.

For example, in a case where a 50 plantain were counted in 10 samples the total population can be estimated using this equation:

$$\text{estimated population size} = \frac{\text{area sampled}}{\text{total area}} \times \text{number of plantain counted}$$

The area sampled from 10 quadrats is $0.25\text{m} \times 0.25\text{m} = 10 \times 0.0625 \text{ m}^2 = 0.625 \text{ m}^2$

The total area of the survey = $20\text{m} \times 20\text{m} = 400 \text{ m}^2$

$$\text{Estimated population} = \frac{0.625}{400} \times 50 = 32,000$$

2. Investigating the effect of a factor on plant distribution using a transect line

A transect line from a tree to an open area can be used to record the change in the number of a particular species as light intensity changes. Students can record either % grass cover or the number of plantain in each quadrat. Students need to lay out a tape measure in a straight line so that a quadrat can be placed at regular intervals. A light meter should be used to record the light intensity at each quadrat. This will allow students to plot a graph of distribution against light intensity.

A shorter transect line could be used if space is limited and quadrats could be placed closer together.

Several students can work independently along one transect line if the number of tape measures is limited. Alternatively, string or rope can be used as the transect line by marking intervals along it with a marker pen.

Additional information

Exactly what counts as a plantain should be demonstrated to students to ensure they are counting whole plants (rosettes) and not just counting flowers. Students will need to kneel or crouch down and may need to use their hands to determine how many plants are within the quadrat (especially in the longer grass of the un-trampled area).

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- CLEAPSS members can use safety guidance in “SRA 08, School Grounds”.

-
- It is advisable not to undertake this experiment if the conditions are very wet as students may slip on wet grass.
 - The areas to be used should be checked beforehand to ensure that no hazardous materials, such as broken glass, are present. This is especially necessary where items could be hidden in the longer grass.
 - Care should be taken when using tape measures that may recoil back if not carefully locked in place.
 - Care should be taken to ensure that students place the quadrats carefully along the transect line and do not throw them around, as this could cause injury to other students.
 - Students should wash their hands thoroughly after the activity, before there is any hand to mouth transfer. Protects against plant and fungal allergens etc.

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Field investigations

Student sheet

| Required practical activity | Apparatus and techniques |
|--|------------------------------|
| Measure the population size of a common species in a habitat. Use sampling techniques to investigate the effect of a factor on the distribution of this species. | AT 1, AT 3, AT 4, AT 6, AT 8 |

This investigation has two parts:

1. Investigating the population size of a plant species using random sampling
2. Investigating the effect of a factor on plant distribution using a transect line.

| Learning outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Risk Assessment

- Wash hands after handling seeds.

Method

You are provided with the following:

- a 25cm x 25cm quadrat
- 2 x 30 m tape measure
- a clipboard
- a pen
- paper.

Read these instructions carefully before you start work:

1. Investigating the population size of a plant species using random sampling.

Your teacher will have prepared a survey area for you and will show you how to identify plantain plants. You will need to work in threes.

1. Collect two numbers, one from each bag.
2. Use the numbers and the tape measures to locate the first position for your quadrat.
3. Lay the 25cm x 25 cm quadrat on the ground.
4. Replace the numbers in the bags.
5. Count and record the number of plantain inside the quadrat.
6. Collect two more numbers from the bags and use them to locate the next site.
7. Replace the numbers in the bags for other students to use.
8. Count and record the number of plantain inside the quadrat. Repeat steps 1 – 5 until you have recorded the numbers of plantain in 10 quadrats.
10. Your teacher will show you how to estimate the population of plantain using the equation:

$$\text{estimated population size} = \frac{\text{area sampled}}{\text{total area}} \times \text{number of plantain counted}$$

2. Investigating the effect of a factor on plant distribution using a transect line

Your teacher will help you identify a species of plant to identify.

1. Lay the 30m tape measure in a line from the base of a tree to an open area of ground.
2. Put the 25cm x 25cm quadrat against the transect line. One corner of the quadrat should touch the 0 m mark on the tape measure.
3. Count the number of plants within the quadrat and record them in a table.

| Distance along the transect line in m | Number of plants | Light intensity |
|---------------------------------------|------------------|-----------------|
| 0 | | |
| 5 | | |
| 10 | | |
| 15 | | |
| 20 | | |
| 25 | | |
| 30 | | |

4. Move the quadrat 5 m up the transect line and count the number of plants again. Record in the table.
5. Continue to place the quadrat at 5 m intervals and count the number of plants in each quadrat.
6. Gather data from your class to produce a graph of plant numbers against light intensity.

GCSE Biology required practical activity: Decay

Teachers' notes

| Required practical activity | Apparatus and techniques |
|--|--------------------------|
| Investigate the effect of temperature on the rate of decay of fresh milk by measuring pH change. | AT 1, AT 3, AT 4, AT 5 |

Investigating the effect of temperature on the rate of decay of fresh milk by measuring pH change.

Please note: Because the natural process of decay in milk is slow, it is difficult for students to monitor in normal class time. Instead we have suggested an approach which speeds up the process through the addition of lipase. Therefore the procedure suggested should serve as a 'model' for the investigation of decay in milk. The fall in pH in natural decay would be due to the production of lactic acid. In this model, the fall in pH is due mainly to the production of fatty acids.

Materials

In addition to access to general laboratory equipment, each group of students' needs:

- a small beaker containing full fat milk or single cream (not UHT)
- a small beaker containing sodium carbonate solution (0.05 mol dm^{-3})
- a small beaker containing 5% lipase solution
- 250 cm^3 beakers, to be used as water baths
- test tubes
- a test tube rack
- a marker pen
- 10 cm^3 plastic syringes
- a stirring thermometer
- stop clock
- Cresol red, in a dropper bottle
- an electric kettle, for heating water
- ice, for investigating temperatures below room temperature.

Technical information

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.

Lipase solution should be freshly made, but it can be kept for a few days in a refrigerator.

Cresol red is an indicator that is purple in alkaline solutions of about pH 8.8. When the pH drops below pH 7.2 Cresol red becomes yellow.

Additional information

If thermostatically controlled water baths are available, this would be preferable to using hot water in beakers.

Ideally, at least five different temperatures should be investigated, ranging around 60 °C.

If timer is short, class results may be pooled rather than individual students carrying out a number of different temperatures and repeats.

An alternative investigation can be carried out using Resazurin solution with whole milk. If live yoghurt is added to the mixture the microbial population is boosted to such an extent that you can see results within an hour.

Risk assessment

- Risk assessment and risk management are the responsibility of the school or college.
- 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 95a; it is an IRRITANT at concentrations over 1.8 M. Please see Hazcard 95a.
- There is an allergen risk with all enzymes. See CLEAPSS website for details.
- For Cresol red, see CLEAPSS Hazcard 32

Trialling

The practical should be trialled before use with students.

GCSE Biology required practical activity: Decay

Student sheet

| Required practical activity | Apparatus and techniques |
|--|--------------------------|
| Investigate the effect of temperature on the rate of decay of fresh milk by measuring pH change. | AT 1, AT 3, AT 4, AT 5 |

Investigating the effect of temperature on the rate of decay of fresh milk by measuring pH change

You will use an alkaline solution of milk. When lipase is added to the milk the fat in the milk is broken down into fatty acids. This makes the pH lower.

Cresol red is an indicator that is purple in alkaline solutions of about pH 8.8. When the pH drops below pH 7.2 Cresol red becomes yellow.

| Learning Outcomes |
|--|
| 1 |
| 2 |
| 3 |
| Teachers to add these with particular reference to working scientifically |

Method

You are provided with the following:

- a small beaker containing full fat milk or single cream
- a small beaker containing sodium carbonate solution
- a small beaker containing lipase solution
- 250 cm³ beakers, to be used as water baths
- test tubes
- a test tube rack
- a marker pen
- 10 cm³ plastic syringes
- a stirring thermometer
- a stop clock
- Cresol red, in a dropper bottle
- an electric kettle, for heating water
- ice, for investigating temperatures below room temperature.

Read these instructions carefully before you start work.

1. Half fill one of the 250 cm³ beakers with hot water from the kettle. This will be the water bath.
2. Label two test tubes:
 - one '**lipase**'
 - one '**milk**'
3. In the 'lipase' test tube put 5 cm³ of lipase solution.
4. In the 'milk' test tube put five drops of Cresol red solution.
5. Use a calibrated dropping pipette to add 5 cm³ of milk to the 'milk' test tube.
6. Use another pipette to add 7 cm³ of sodium carbonate solution to the 'milk' test tube.
The solution should be purple.
7. Put a thermometer into the 'milk' test tube.

8. Put both test tubes into the water bath. Wait until the contents reach the same temperature as the water bath.
9. Use another dropping pipette to transfer 1 cm³ of lipase into the 'milk' test tube. Immediately start timing.
10. Stir the contents of the 'milk' test tube until the solution turns yellow.
11. Record the time taken for the colour to change to yellow, in seconds.
12. Repeat steps **1–11** for different temperatures of water bath.
You can obtain temperatures below room temperature by using ice in the beaker instead of hot water.
13. Record your results in a table such as the one here. Plot a graph of your results.

| Temperature of milk in °C | Time taken for solution to turn yellow, in seconds | | | |
|---------------------------|--|---------|---------|------|
| | Trial 1 | Trial 2 | Trial 3 | Mean |
| | | | | |