

OXFORD

INTERNATIONAL  
AQA EXAMINATIONS

# INTERNATIONAL AS AND A-LEVEL BIOLOGY

(9610)

Practical handbook

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This is the **Biology** version of this practical handbook.

The sections on tabulating data, significant figures, uncertainties, graphing, biological drawings, statistical tests in Biology, and subject specific vocabulary are particularly useful for students and could be printed as a student booklet by schools.

The information in this document is correct, to the best of our knowledge as of September 2018.

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# INTRODUCTION

Practical work brings science to life, helping students make sense of the universe around them. That's why we've put practical work at the heart of our Biology, Chemistry and Physics International AS and A-levels. Practical science allows scientific theory to transform into deep knowledge and understanding – scientific thinking. Through investigation, students uncover the important links between their personal observations and scientific ideas.

“In the best schools visited, teachers ensured that pupils understood the ‘big ideas’ of science. They made sure that pupils mastered the investigative and practical skills that underpin the development of scientific knowledge and could discover for themselves the relevance and usefulness of those ideas.”

Ofsted report

Maintaining curiosity in science

November 2013, No. 130135

## THE PURPOSE OF THIS PRACTICAL HANDBOOK

This handbook has been developed to support you in advancing your students to fluency in science.

Over the years, there have been many rules developed for practical work in Biology, Chemistry and Physics. Some have been prescriptive, some have been intended as guidance. Although we have always attempted to be consistent within subjects, differences have emerged over time. For example, students taking Biology may also be taking Physics and find themselves confronted with contradictory rules and guidance.

This practical handbook is an attempt to harmonise the rules and guidance for International Biology, Chemistry and Physics. There are occasions where these will necessarily be different, but we will try to explain why on the occasions where that happens.

We have worked with teachers, technicians and examiners to produce this handbook. This has been an evolving document, but one that we hope you will be able to use with your students, whether they're doing International AS or A-level Biology, Chemistry or Physics, or a combination of subjects, to improve their practical skills: in the classroom, in the laboratory, in exams and on to university or the workplace.

Unless specified, all guidance is common to Biology, Chemistry and Physics at both International AS and A-level and subject-specific examples are for illustration only. However, the extent to which a particular aspect is assessed will differ. Teachers should refer to the specifications and specimen materials on our website for more information.

## THE PURPOSE OF PRACTICAL WORK

There are three interconnected, but separate reasons for doing practical work in schools and colleges. They are:

1. To support and consolidate **scientific concepts** (knowledge and understanding).  
This is done by applying and developing what is known and understood of abstract ideas and models. Through practical work we are able to make sense of new information and observations, and provide 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.

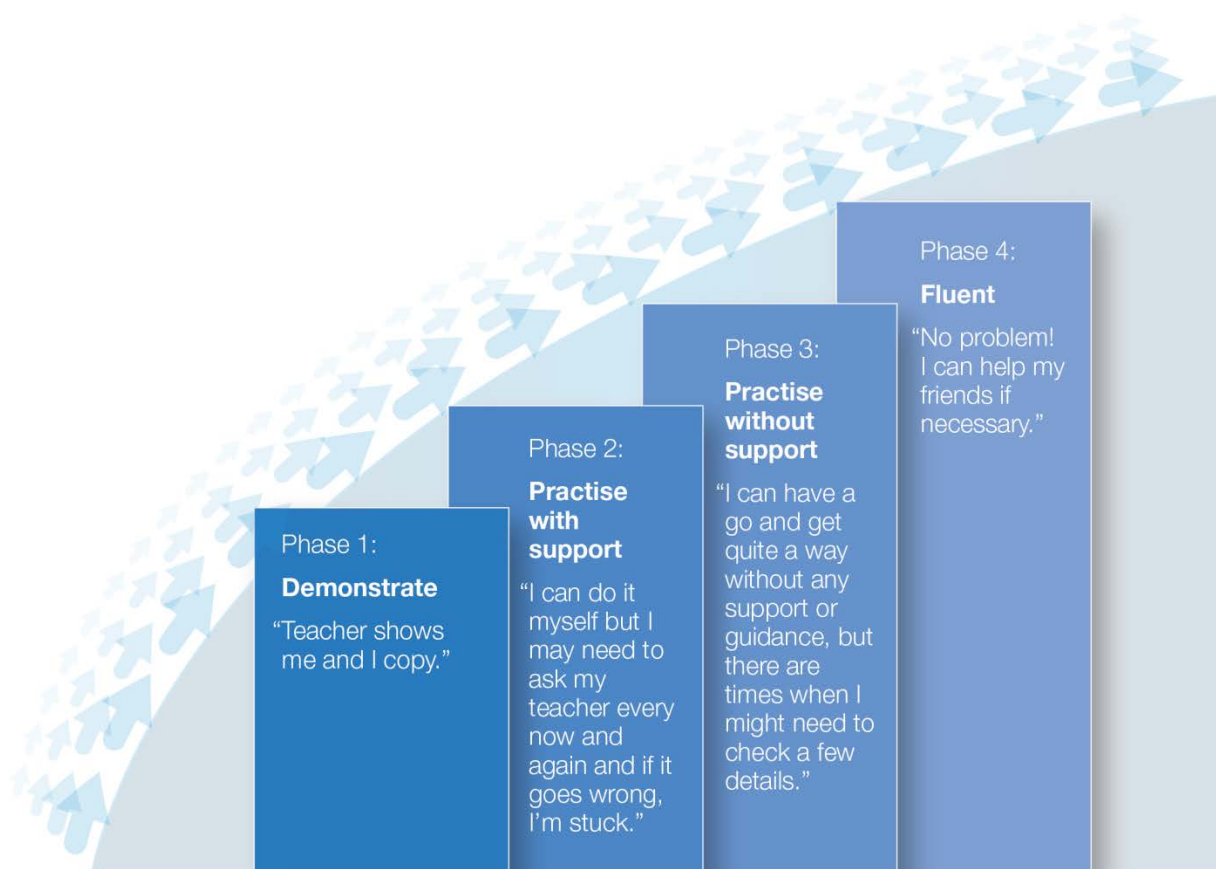
By focusing on the reasons for carrying out a particular practical, teachers will help their students understand the subject better, to develop the skills of a scientist and to master the manipulative skills required for further study or jobs in STEM subjects.

The International AS and A-levels in Biology, Chemistry and Physics separate the ways in which practical work is assessed. This is discussed in the next section.

## FLUENCY IN SCIENCE PRACTICAL WORK

At the beginning of a year 12 course, students will need support and guidance to build their confidence. This could involve, for example, breaking down practicals into discrete sections or being more explicit in instructions. Alternatively, a demonstration of a key technique followed by students copying may support students' development. This could be a better starting point than 'setting students loose' to do it for themselves.

**Progression in the mastery of practical skills and techniques shows increasing independence and confidence.**



**Safety is always the responsibility of the teacher. No student should be expected to assess risks and then carry out their science practical without the support and guidance of their teacher.**

# PRACTICAL SKILLS ASSESSMENT IN QUESTION PAPERS

The International AS and A-level papers will contain the following types of questions which relate to practical work:

1. Questions set in a practical context, where the question centres on the science, not the practical work.

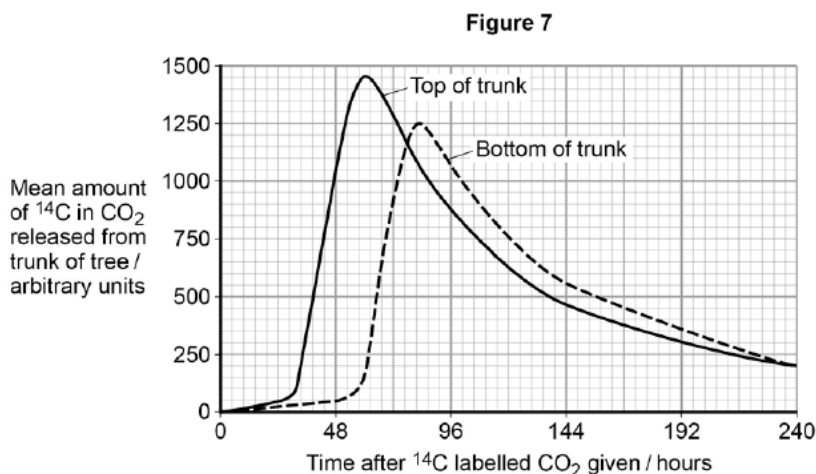
## EXAMPLE (A-LEVEL BIOLOGY SPECIMEN PAPER 1)

Scientists measured translocation in the phloem of trees. They used carbon dioxide labelled with radioactive  $^{14}\text{C}$ .

They put a large, clear plastic bag over the leaves and branches of each tree and added  $^{14}\text{CO}_2$ . The main trunk of the tree was not in the plastic bag.

At regular intervals after adding the  $^{14}\text{CO}_2$  to the bag, the scientists measured the amount of  $^{14}\text{CO}_2$  released from the top and bottom of the main trunk of the tree. On the surface of the trunk of these trees, there are pores for gas exchange.

Figure 7 shows the scientists' results.



These questions are set in the context of practical work that has been carried out.

However, the questions relate more to the basic Biology behind the situation, or mathematical skills.

09 . 2 Name the process that produced the  $^{14}\text{CO}_2$  released from the trunk. [1 mark]

\_\_\_\_\_

09 . 3 How long did it take the  $^{14}\text{C}$  label to get from the top of the trunk to the bottom of the trunk? Explain how you reached your answer. [2 marks]

\_\_\_\_\_

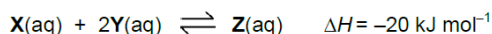
\_\_\_\_\_

09 . 4 What other information is required in order to calculate the mean rate of movement of the  $^{14}\text{C}$  down the trunk? [1 mark]

\_\_\_\_\_

**EXAMPLE (AS CHEMISTRY SPECIMEN PAPER 1)**

**4** Colourless solutions of **X(aq)** and **Y(aq)** react to form an orange solution of **Z(aq)** according to the following equation.



A student added a solution containing 0.50 mol of **X(aq)** to a solution containing 0.50 mol of **Y(aq)** and shook the mixture.

After 30 seconds, there was no further change in colour.

The amount of **Z(aq)** at equilibrium was 0.20 mol.

**0 4 . 1** Deduce the amounts of **X(aq)** and **Y(aq)** at equilibrium.

**[2 marks]**

Amount of **X(aq)** = \_\_\_\_\_ mol      Amount of **Y(aq)** = \_\_\_\_\_ mol

This question requires an understanding of the underlying chemistry, not the practical procedure undertaken.

**EXAMPLE (A-LEVEL PHYSICS SPECIMEN PAPER 3)**

**0 2 . 6** The experiment is performed with a capacitor of nominal value 680  $\mu\text{F}$  and a manufacturing tolerance of  $\pm 5\%$ . In this experiment the charging current is maintained at 65  $\mu\text{A}$ . The data from the experiment produces a straight-line graph for the variation of pd with time. This shows that the pd across the capacitor increases at a rate of 98  $\text{mV s}^{-1}$ .

Calculate the capacitance of the capacitor.

**[2 marks]**

capacitance = \_\_\_\_\_  $\mu\text{F}$

This question is set in a practical context, and particular readings need to be used to calculate the answer, but the specific practical set-up is not important.



2. Questions that require specific aspects of a practical procedure to be understood in order to answer a question about the underlying science.

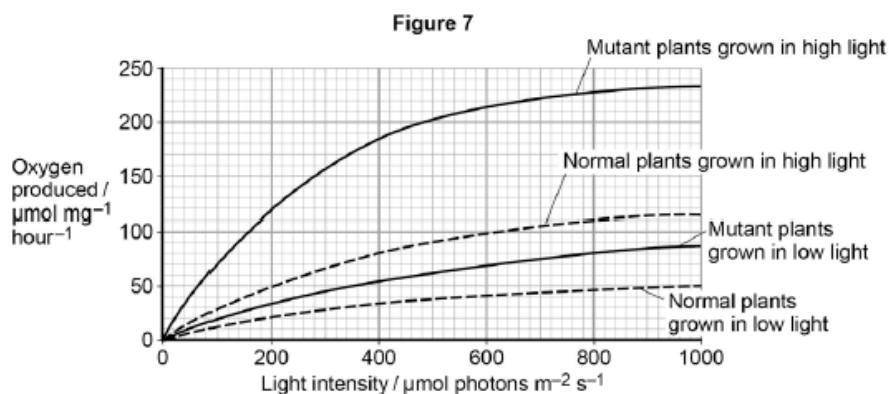
**EXAMPLE (A-LEVEL BIOLOGY SPECIMEN PAPER 2)**

8 Chloroplasts contain chlorophyll a and chlorophyll b. Scientists found tobacco plants with a mutation that caused them to make more chlorophyll b than normal tobacco plants. They investigated the effect of this mutation on the rate of photosynthesis.

The scientists carried out the following investigation.

- They grew normal and mutant tobacco plants. They grew some of each in low light intensity and grew others in high light intensity.
- They isolated samples of chloroplasts from mature plants of both types.
- Finally, they measured oxygen production by the chloroplasts they had isolated from the plants.

Figure 7 shows the scientists' results.

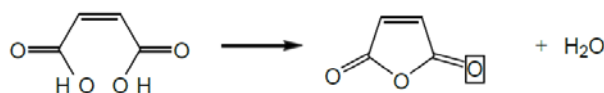


0 8 . 1 Explain why the scientists measured the rate of production of oxygen in this investigation. [2 marks]

This question requires the students to understand how oxygen production can be used as a proxy measure for photosynthesis, but no other details of the practical procedure are important.

**EXAMPLE (AS CHEMISTRY SPECIMEN PAPER 2)**

0 1 . 4 The effect of gentle heat on maleic acid is shown below.



A student predicted that the yield of this reaction would be greater than 80%.  
In an experiment, 10.0 g of maleic acid were heated and 6.53 g of organic product were obtained.  
Is the student correct? Justify your answer with a calculation using these data. [2 marks]

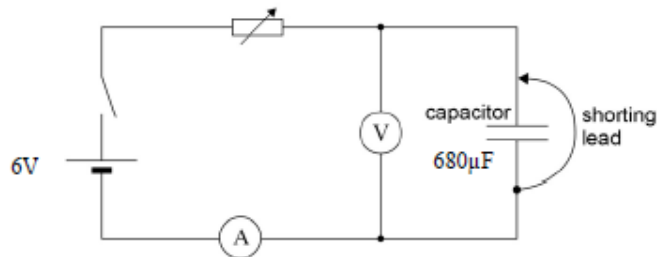
To answer this question, the student must understand the process of yield calculation (which will have been gained through practical work), but again the details of the practical procedure are unimportant.

EXAMPLE (A-LEVEL PHYSICS SPECIMEN PAPER 5)

0 2

This question is about capacitor charging and discharging.  
A student designs an experiment to charge a capacitor using a constant current as shown in Figure 3.

Figure 3



The variable resistor is then adjusted to give a suitable ammeter reading. The shorting lead is removed and the stop clock is started at the same instant.

A student measured the potential difference (pd) across the capacitor at 10 s intervals while adjusting the variable resistor to keep the charging current constant.

The power supply has a negligible internal resistance and the variable resistor has a maximum resistance of 100 kΩ.

0 2 . 1

The student chooses a digital voltmeter for the experiment. A digital voltmeter has a very high resistance.

Explain why it is important to use a voltmeter with very high resistance.

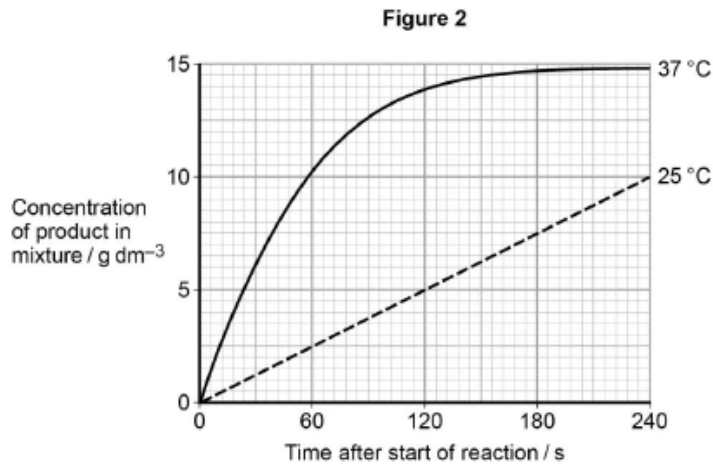
[1 mark]

To answer this question, the student must understand why a voltmeter needs to have a very high resistance.

3. Questions directly on the required practical procedures.

**EXAMPLE (AS BIOLOGY SPECIMEN PAPER 1)**

2 A technician investigated the effect of temperature on the rate of an enzyme-controlled reaction. At each temperature, he started the reaction using the same volume of substrate solution and the same volume of enzyme solution.  
Figure 2 shows his results.



0 2 . 1 Give one other factor the technician would have controlled. [1 mark]

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0 2 . 2 Calculate the rate of reaction at 25 °C. [2 marks]

Similarly, in this example, the students should have done a very similar experiment.

The first question is simple recall of the factors involved in the rate of enzyme controlled reactions.

The second requires the calculation of a gradient, which is a skill students will have learned through their practical and other work.

EXAMPLE (A-LEVEL CHEMISTRY SPECIMEN PAPER 3)

3 A peptide is hydrolysed to form a solution containing a mixture of amino acids. This mixture is then analysed by silica gel thin-layer chromatography (TLC) using a toxic solvent. The individual amino acids are identified from their  $R_f$  values.

Part of the practical procedure is given below.

1. **Wearing plastic gloves to hold a TLC plate**, draw a pencil line 1.5 cm from the bottom of the plate.
2. Use a capillary tube to apply a very small drop of the solution of amino acids to the mid-point of the pencil line.
3. Allow the spot to dry completely.
4. In the developing tank, add the developing solvent to **a depth of not more than 1 cm**.
5. Place your TLC plate in the developing tank.
6. Allow the developing solvent to rise up the plate **to the top**.
7. Remove the plate and quickly mark the position of the solvent front with a pencil.
8. Allow the plate to dry **in a fume cupboard**.

0 3 · 1 Parts of the procedure are in bold text.

For each of these parts, consider whether it is essential and justify your answer. **[4 marks]**

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Students who have completed the related required practical will have a greater understanding of each of the steps in the procedure and will be able to explain each in turn.

This type of question is likely to be fairly rare, to avoid predictable assessments.

EXAMPLE (A-LEVEL PHYSICS SPECIMEN PAPER 5)

0 2 · 6 The student decides to confirm the value of the capacitance by first determining the time constant of the circuit when the capacitor discharges through a fixed resistor.

Describe an experiment to do this. Include in your answer:

- a circuit diagram
- an outline of a procedure
- an explanation of how you would use the data to determine the time constant.

**[4 marks]**

This question focuses on a particular aspect of one of the required practicals, and is related to the discharging of a capacitor through a fixed resistor.

4. Questions applying the skills from the required practical procedures and the apparatus and techniques list.

**EXAMPLE (A-LEVEL BIOLOGY SPECIMEN PAPER 5)**

2 Fibrin is a protein.  
Congo red is a dye that binds to fibrin molecules and colours them red. When a suspension of Congo-red fibrin is digested, the dye goes into solution.

You are provided with

- fibrin powder that has been dyed with Congo red
- trypsin, an enzyme that hydrolyses fibrin
- any other laboratory apparatus that you might need.

Plan an investigation to find the effect of pH on the rate of hydrolysis of fibrin by Trypsin.

0 2 . 1 Describe how you would change the independent variable. Include the steps that you would take to ensure that confounding variables were kept constant and any controls that you would set up. [4 marks]

This question expects students to understand enzyme controlled reactions, which is one of the required practicals. It is not necessary for students to have used Congo Red to understand the requirements.

**EXAMPLE (A-LEVEL CHEMISTRY SPECIMEN PAPER 3)**

0 1 . 3 The boiling points of the organic compounds in a reaction mixture are shown in Table 1.

**Table 1**

<b>Compound</b>	ethanol	ethanal	ethanoic acid
<b>Boiling point / °C</b>	78	21	118

Use these data to describe how you would obtain a sample of ethanal from a mixture of these three compounds. Include in your answer a description of the apparatus you would use and how you would minimise the loss of ethanal. Your description of the apparatus can be either a description in words or a labelled sketch. [5 marks]

This question expects students to understand distillation which is one of the required practicals. It is not necessary for students to have carried out this precise experiment to understand the requirements.

**EXAMPLE (AS PHYSICS SPECIMEN PAPER 2)**

0 2

**Data analysis question**

Capillary action can cause a liquid to rise up a hollow tube. Figure 3 shows water that has risen to a height  $h$  in a narrow glass tube because of capillary action.

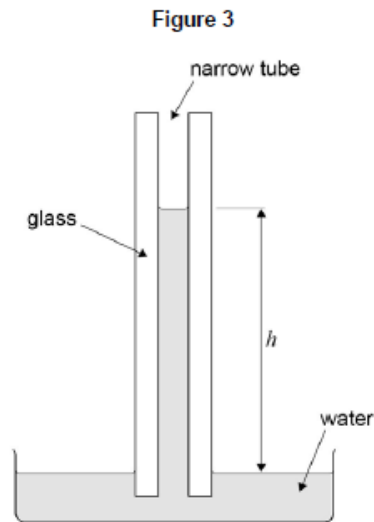


Figure 3

Figure 4 shows the variation of  $h$  with temperature  $\theta$  for this particular tube.

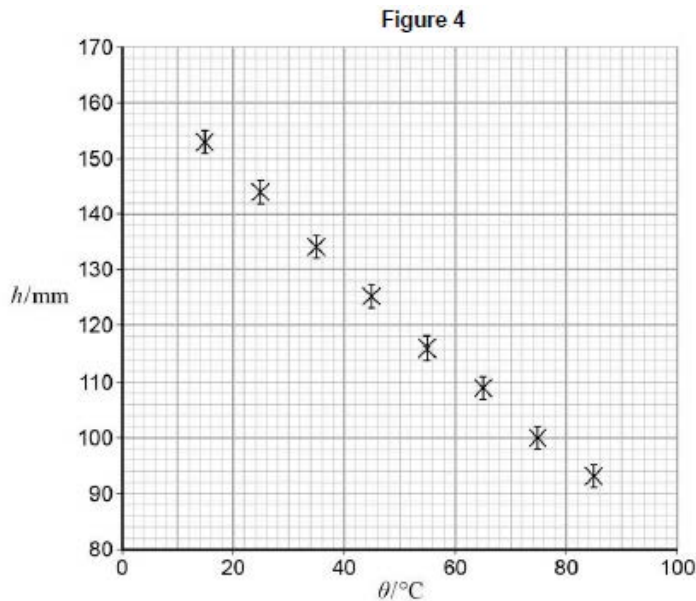


Figure 4

The uncertainty in the measurement of  $h$  is shown by the error bars. Uncertainties in the measurements of temperature are negligible.

0 2

. 1 Draw a best-fit straight line for these data (Figure 4).

[1 mark]

0 2

. 2 It is suggested that the relationship between  $h$  and  $\theta$  is

$$h = h_0 - (h_0 k) \theta$$

where  $h_0$  and  $k$  are constants.

Determine  $h_0$ .

[1 mark]

This question requires students to apply the skills of data analysis that they will have gained through their practical work in the required practicals and apply it to an unusual situation.

# GUIDELINES TO SUPPORTING STUDENTS IN PRACTICAL WORK

Developed in collaboration with National Foundation for Educational Research and CLEAPSS

## **CLARIFY THE IMPORTANCE OF KEEPING A LAB BOOK OR OTHER RECORDS OF PRACTICAL WORK**

Explain that students need a record of their achievements to guide their learning. Lab books also can be an opportunity to develop a skill used both by scientists and in business. They allow students to accurately and clearly record information, ideas and thoughts for future reference which is a very useful life skill.

## **WARN STUDENTS AGAINST PLAGIARISM AND COPYING**

Explain the meaning of the term plagiarism and that the use of acknowledged sources is an encouraged and acceptable practice, but trying to pass off other people's work as their own is not, and will not help them learn. Show students how sources should be cited.

## **EXPLAIN THE LEARNING CRITERIA FOR EACH SKILL**

This will help students learn and allow them to know when they have met the criteria. The student lab book contains the criteria, but they own the process and have the responsibility for collecting appropriate evidence of success.

## **USE CLEARLY DEFINED LEARNING OUTCOMES**

For example, if you are running a practical session to teach students how to use a microscope and staining techniques safely and efficiently, then make sure they know why they are learning this. This will also make it much easier for them to know when they have met the criteria.

## **START WITH SIMPLE TASKS INITIALLY**

Students need to become confident with the apparatus and concepts of practical work before they can proceed to more complicated experiments. It may be more effective to start with simple manipulation skills and progress to the higher order skills.

## **TEACH PRACTICAL WORK IN YOUR PREFERRED ORDER**

Teach the skills as you see fit and that suit your circumstances – the assessment process is aimed to be flexible and help you teach practical work, not to dictate how it should be done.

## **USE FEEDBACK AND PEER ASSESSMENT**

Feedback is essential to help students develop skills effectively. Allowing self and peer review will allow time for quality feedback as well as provide powerful learning tools. However, this is a decision for teachers. The scheme is designed to be flexible while promoting best practice.

Research shows that feedback is the best tool for learning in practical skills. Students who normally only receive numerical marks as feedback for work will need to be trained in both giving and receiving comment-based feedback. Provided it is objective, focused on the task and meets learning outcomes, students will quickly value this feedback.

Feedback does not need to be lengthy, but it does need to be done while the task is fresh in the students' mind. Not everything needs written feedback but could be discussed with students, either individually or as a class. For example, if a teacher finds that many students cannot calculate percentage change, the start of the next lesson could be used for a group discussion about this.

The direct assessment of practical work is designed to allow teachers to integrate student-centred learning (including peer review), into day-to-day teaching and learning. This encourages critical skills. Research indicates these are powerful tools for learning. For example, teachers could ask students to evaluate each other's data objectively. The students could identify why some data may be useful and some not. This can be a very good way of getting students to understand why some conventions are used, and what improves the quality of results. This also frees up marking time to concentrate on teaching.

## **DON'T GIVE MARKS**

We have deliberately moved away from banded criteria and marks to concentrate on the mastery of key practical competencies. The purpose of marking should be changed to emphasise learning. Students should find it easier to understand and track their progress, and focus their work. We would expect most students, with practice and the explicit teaching of skills and techniques to have the confidence to approach practical work related exam questions.

## **USE GROUP WORK**

This is a very useful skill, allowing students to build on each other's ideas. For example, planning an experiment can be done as a class discussion. Alternatively, techniques such as snowballing can be used, in which students produce their own plan then sit down in a small group to discuss which are the best collective ideas. From this, they revise their plan which is then discussed to produce a new 'best' plan.



# USE OF LAB BOOKS

Students do **not** need to write up every practical that they do in detail. However, it is good practice to have a record of all they do. A lab book could contain this record. It is a student's personal book and may contain a range of notes, tables, jottings, reminders of what went wrong, errors identified and other findings. It is a live document that can function as a learning journal.

Lab books are **not** a requirement of the OxfordAQA AS and A-level specifications in Biology, Chemistry or Physics. They are, however, highly valued by colleagues in higher education.

Each institution has its own rules on lab book usage. The following guidelines are based on those from a selection of companies and universities that use lab books. They are designed to help students and teachers in preparing to use lab books for university but do not represent the only way that books could be used for International AS and A-level sciences. Teachers may wish to vary or ignore the following points to suit their purposes.

## THE PURPOSE OF A LAB BOOK

A lab book is a complete record of everything that has been done in the laboratory. As such, it becomes important both to track progress of experiments, and also, in industry and universities, to prove who developed an idea or discovered something first.

A lab book is a:

- source of data that can be used later by the experimenter or others
- complete record of what has been done so that experiments could be understood or repeated by a competent scientist at some point in the future
- tool that supports sound thinking and helps experimenters to question their results to ensure that their interpretation is the same one that others would come to
- record of why experiments were done.

## TYPE OF BOOK

A lab book is often a hard-backed book with bound pages. Spiral bound notebooks are not recommended as it is too easy to rip a page out and start again. It is generally advisable that a lab book has a cover that won't disintegrate the moment it gets slightly wet.

## STYLE

Notes should be recorded as experiments are taking place. They should not be a "neat" record written at a later date from scraps of paper. However, they should be written clearly, in legible writing and in language which can be understood by others.

Many lab books are used in industry as a source of data, and so should be written in indelible ink.

To ensure that an observer can be confident that all data are included when a lab book is examined, there should be no blank spaces. Mistakes should be crossed out and re-written. Numbers should not be overwritten, erased, nor should Tippex be used. Pencil should not be used for anything other than graphs and diagrams.

### **EACH PAGE SHOULD BE DATED**

Worksheets, graphs, printed information, photographs and even flat “data” such as chromatograms or TLC plates can all be stuck into a lab book. They should not cover up any information so that photocopying the page shows all information in one go. Anything glued in should lie flat and not be folded.

### **CONTENT**

Generally, lab books will contain:

- title and date of experiment
- notes on the objectives of the experiment
- notes on the method, including all details (eg temperatures, volumes, settings of pieces of equipment) with justification where necessary
- estimates of the uncertainty of measurements
- sketches of how equipment has been set up can be helpful. Photographs pasted in are also acceptable
- data and observations input to tables (or similar) while carrying out the experiment
- calculations – annotated to show thinking
- graphs and charts
- summary, discussions and conclusions
- cross-references to earlier data and references to external information.

This list and its order are not prescriptive. Many experiments change as they are set up and trials run. Often a method will be given, then some data, then a brief mention of changes that were necessary, then more data and so on.

# REQUIRED PRACTICAL ACTIVITIES

The OxfordAQA Exams required practicals have been designed to give students a range of practical experience. Carrying out the required practicals will mean that students will have experienced the use of most of the standard pieces of equipment and techniques expected when students move to further study in the subject at university. Teachers are encouraged to develop students' abilities by inclusion of other opportunities for skills development, as exemplified in the schemes of work for each subject.

Teachers are encouraged to vary their approach to the required practical activities. Some are more suitable for highly structured approaches that develop key techniques. Others allow opportunities for students to develop investigative approaches.

This list is not designed to limit the practical activities carried out by students. A rich practical experience for students will include more than the required practical activities. The explicit teaching of practical skills builds students' competence. Many teachers will also use practical approaches to the introduction of content knowledge in the course of their normal teaching.

## STUDENTS WHO MISS A REQUIRED PRACTICAL ACTIVITY

The required practical activities are part of the specification. As such, exam papers could contain questions about the activities and assume that students understand those activities. A student who misses a particular practical activity may be at a disadvantage when answering questions in the exams.

It will often be difficult to set up a practical a second time for students to catch up, although if at all possible an attempt should be made. Teachers will need to decide on a case by case basis whether they feel it is important for the student to carry out that particular practical. This is no different from when teachers make decisions about whether to re-teach a particular topic if a student is away from class when it is first taught.

## BIOLOGY REQUIRED PRACTICAL ACTIVITIES

AS practical activities	A2 practical activities
<p>Students must carry out the practical activities below. The AS written papers test knowledge and understanding of procedures, as well as evaluation of the techniques adopted. They may require students to interpret specimen results.</p> <p><b>Practical activity</b></p> <ol style="list-style-type: none"> <li>1 Investigation into the effect of temperature, pH or substrate concentration on the rate of an enzyme-controlled reaction.</li> <li>2 Investigation of the effect of solute concentration on the uptake or loss of water from plant tissue.</li> <li>3 Use of chromatography to investigate the pigments present in leaves.</li> <li>4 Preparation of stained squashes of root tips and examination of these with a microscope. Observation of the stages of mitosis and calculation of a mitotic index.</li> <li>5 Investigation into the effect of a specific variable on human heart rate or pulse rate.</li> <li>6 Investigation of the rate of water uptake by means of a simple potometer.</li> </ol>	<p>Students must carry out the practical activities below. The A2 written papers test knowledge and understanding of the procedures involved, as well as evaluation of the techniques adopted. They may require students to interpret specimen results.</p> <p><b>Practical activity</b></p> <ol style="list-style-type: none"> <li>7 Investigation of the effect of a specific limiting factor such as light intensity on the rate of photosynthesis.</li> <li>8 Investigation of a specific variable such as substrate or temperature on the rate of respiration of a suitable organism such as yeast or locust.</li> <li>9 A laboratory based investigation of the effect of competition on seedling growth.</li> <li>10 Investigation of the effect of a suitable variable on the direction of growth of a root or a shoot.</li> </ol>

# TABULATING DATA

It is important to keep a record of data while carrying out practical work. Tables should have clear headings with units indicated using a forward slash (solidus) before the unit.

Temperature/°C	Length/mm
10.0	53
20.0	25
30.0	12

Although using a forward slash is the standard format for post-16 studies, other formats are generally acceptable. For example:

Volume in cm <sup>3</sup>	Time taken in s
15	23
25	45
35	56

Time (hours)	Number of cells
0	1
6	45
12	304

It is good practice to draw a table before an experiment commences and then enter data straight into the table on collection. This can sometimes lead to data points being in the wrong order. For example, when investigating the temperature at which an enzyme works best, a student may do a number of experiments at 25, 30, 35, 40 and 45 °C, and then investigate the range between 30 and 40 further by adding readings at 31, 32, 33, 34, 36, 37, 38 and 39 °C. Whilst this is perfectly acceptable, it is generally a good idea to make a copy of the table in ascending order of temperature to enable patterns to be spotted more easily. Reordered tables should follow the original data if using a lab book.

It is also expected that the independent variable is the left hand column in a table, with the following columns showing the dependent variables. These should be headed in similar ways to measured variables. The body of the table should not contain units.

## TABULATING LOGARITHMIC VALUES

When the logarithm is taken of a physical quantity, the resulting value has no unit. However, it is important to be clear about which unit the quantity had to start with. The logarithm of a time in seconds will be very different from the logarithm of the same time in minutes.

These should be included in tables in the following way:

Reading number	time/s	log (time/s)
1	2.3	0.36
2	3.5	0.54
3	5.6	0.75

# SIGNIFICANT FIGURES

Data should be written in tables to the same number of significant figures. This number should be determined by the resolution of the device being used to measure the data or the uncertainty in measurement. For example, a sample labelled as “1 mol dm<sup>-3</sup> acid” should not be recorded in a table of results as 1.0 mol dm<sup>-3</sup> acid.

There is sometimes confusion over the number of significant figures when readings cross multiples of 10. Changing the number of decimal places across a power of ten retains the number of significant figures **but changes the precision**. The same number of decimal places should therefore generally be used, as illustrated below.

0.97	99.7
0.98	99.8
0.99	99.9
1.00	100.0
1.10	101.0

It is good practice to write down all digits showing on a digital meter.

Calculated quantities should be shown to the number of significant figures of the data with the least number of significant figures.

Example:

Calculate the size of an object if the magnification of a photo is  $\times 25$  and it is measured to be 24.6 mm on the photo.

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

$$\text{size of real object} = \frac{24.6 \times 10^{-3}}{25}$$

$$\text{size of real object} = 9.8 \times 10^{-4}$$

Note that the size of the real object can only be quoted to two significant figures as the magnification is only quoted to two significant figures.

Equipment measuring to half a unit (eg a thermometer measuring to 0.5 °C) should have measurements recorded to one decimal place (eg 1.0 °C, 2.5 °C). The uncertainty in these measurements would be  $\pm 0.25$ , but this would be rounded to the same number of decimal places (giving measurements quoted with uncertainty of  $(1.0 \pm 0.3)$  °C etc).

# UNCERTAINTIES

## SOURCES OF UNCERTAINTIES

Students should know that every measurement has some inherent uncertainty.

The important question to ask is whether an experimenter can be confident that the true value lies in the range that is predicted by the uncertainty that is quoted. Good experimental design will attempt to reduce the uncertainty in the outcome of an experiment. The experimenter will design experiments and procedures that produce the least uncertainty and to provide a realistic uncertainty for the outcome.

In assessing uncertainty, there are a number of issues that have to be considered. These include:

- the resolution of the instrument used
- the manufacturer's tolerance on instruments
- the judgments that are made by the experimenter
- the procedures adopted (eg repeated readings)
- the size of increments available (eg the size of drops from a pipette).

Numerical questions will look at a number of these factors. Often, the resolution will be the guiding factor in assessing a numerical uncertainty. There may be further questions that would require candidates to evaluate arrangements and procedures. Students could be asked how particular procedures would affect uncertainties and how they could be reduced by different apparatus design or procedure.

A combination of the above factors means that there can be no hard and fast rules about the actual uncertainty in a measurement. What we can assess from an instrument's resolution is the **minimum** possible uncertainty. Only the experimenter can assess the other factors, based on the arrangement and use of the apparatus. A rigorous experimenter would draw attention to these factors and take them into account.

## READINGS AND MEASUREMENTS

It is useful, when discussing uncertainties, to separate measurements into two forms:

- Readings: the values found from a single judgement when using a piece of equipment.
- Measurements: the values taken as the difference between the judgements of two values.

### Examples:

When using a thermometer, a student only needs to make one judgement (the height of the liquid). This is a reading. It can be assumed that the zero value has been correctly set.

For stop watches and rulers, both the starting point and the end point of the measurement must be judged, leading to two uncertainties.

The following list is not exhaustive, and the way that the instrument is used will determine whether the student is taking a reading or a measurement.

Reading (one judgement only)	Measurement (two judgements required)
thermometer	ruler
pH meter	protractor
top pan balance	stopwatch
measuring cylinder	analogue meter
volumetric flask	

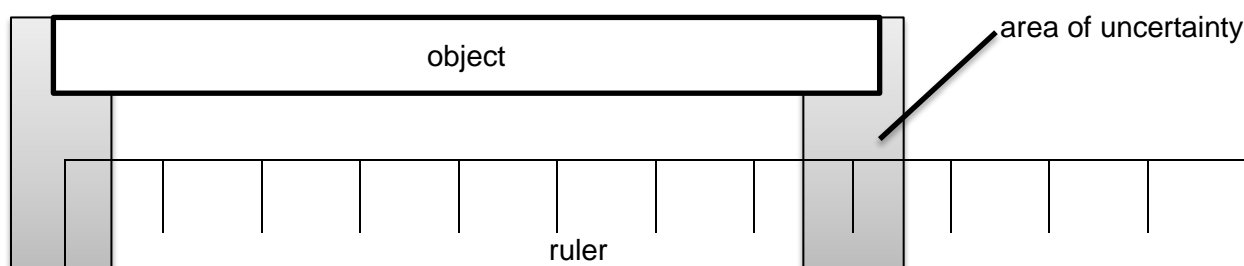
The uncertainty in a **reading** when using a particular instrument is **no smaller** than plus or minus half of the smallest division or greater. For example, a temperature measured with a thermometer is likely to have an uncertainty of  $\pm 0.5$  °C if the graduations are 1 °C apart.

Students should be aware that readings are often written with the uncertainty. An example of this would be to write a voltage as  $(2.40 \pm 0.01)$  V. It is usual for the uncertainty quoted to be the same number of decimal places as the value. Unless there are good reasons otherwise (eg an advanced statistical analysis), students at this level should quote the uncertainty in a measurement to the same number of decimal places as the value.

### Measurement example: length

When measuring length, **two** uncertainties must be included: the uncertainty of the placement of the zero of the ruler and the uncertainty of the point the measurement is taken from.

As both ends of the ruler have a  $\pm 0.5$  scale division uncertainty, the measurement will have an uncertainty of  $\pm 1$  division.



For most rulers, this will mean that the uncertainty in a measurement of length will be  $\pm 1$  mm.

This “initial value uncertainty” will apply to any instrument where the user can set the zero (incorrectly), but would not apply to equipment such as balances or thermometers where the zero is set at the point of manufacture.

In summary

- The uncertainty of a reading (one judgement) is at least  $\pm 0.5$  of the smallest scale reading.
- The uncertainty of a measurement (two judgements) is at least  $\pm 1$  of the smallest scale reading.

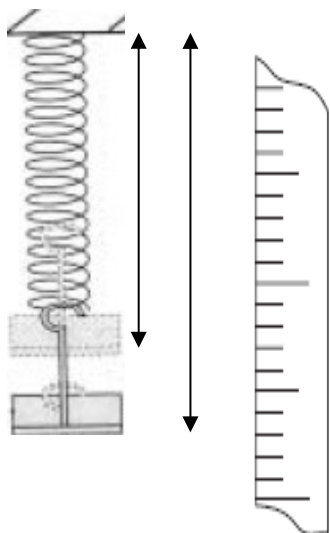
The way measurements are taken can also affect the uncertainty.



### Measurement example: the extension of a spring

Measuring the extension of a spring using a metre ruler can be achieved in two ways

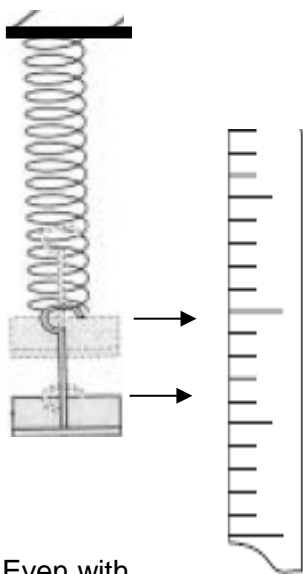
1. Measuring the total length unloaded and then loaded.



Four readings must be taken for this: the start and end point of the unloaded spring's length and the start and end point of the loaded spring's length.

The minimum uncertainty in each measured length is  $\pm 1$  mm using a meter ruler with 1 mm divisions (the actual uncertainty is likely to be larger due to parallax in this instance). The extension would be the difference between the two readings, so the minimum uncertainty would be  $\pm 2$  mm.

2. Fixing one end and taking a scale reading of the lower end.



Two readings must be taken for this: the end point of the unloaded spring's length and the end point of the loaded spring's length. The start point is assumed to have zero uncertainty, as it is fixed.

The minimum uncertainty in each reading would be  $\pm 0.5$  mm, so the minimum extension uncertainty would be  $\pm 1$  mm.

Even with other practical uncertainties this second approach would be better.

Realistically, the uncertainty would be larger than this and an uncertainty in each reading of 1 mm or would be more sensible. This depends on factors such as how close the ruler can be mounted to the point as at which the reading is to be taken.

## OTHER FACTORS

There are some occasions where the resolution of the instrument is not the limiting factor in the uncertainty in a measurement.

Best practice is to write down the full reading and then to write to fewer significant figures when the uncertainty has been estimated.

### Examples:

A stopwatch has a resolution of hundredths of a second, but the uncertainty in the measurement is more likely to be due to the reaction time of the experimenter. Here, the student should write the full reading on the stopwatch (eg 12.20 s), carry the significant figures through for all repeats, and reduce this to a more appropriate number of significant figures after an averaging process later.

If a student measures the length of a piece of wire, it is very difficult to hold the wire completely straight against the ruler. The uncertainty in the measurement is likely to be higher than the  $\pm 1$  mm uncertainty of the ruler. Depending on the number of “kinks” in the wire, the uncertainty could be reasonably judged to be nearer  $\pm 2$  or 3 mm.

## UNCERTAINTIES IN GIVEN VALUES

Often exam papers contain values. In all such cases assume the uncertainty to be  $\pm 1$  in the last significant digit. For example, if an exam stated “a person excreted 1660 mg of creatinine in 24 hours”, uncertainty would be assumed to be  $\pm 10$  mg of creatinine. The uncertainty may be lower than this but without knowing the details of the experiment and procedure that lead to this value there is no evidence to assume otherwise.

## MULTIPLE INSTANCES OF MEASUREMENTS

Some methods of measuring involve the use of multiple instances in order to reduce the uncertainty. For example, measuring the thickness of several leaves together, rather than just one leaf. The uncertainty of each measurement will be the uncertainty of the whole measurement divided by the number of leaves. This method works because the percentage uncertainty of the thickness of a single leaf is the same as the percentage uncertainty for the thickness of multiple leaves.

### Example:

Thickness of 10 leaves:  $(3.10 \pm 0.1)$  mm

Mean thickness of one leaf:  $(0.31 \pm 0.01)$  mm

## REPEATED MEASUREMENTS

Repeating a measurement is a method for reducing the uncertainty.

With many readings one can also identify those that are exceptional (that are far away from a significant number of other measurements). Sometimes it will be appropriate to remove outliers from measurements before calculating a mean. On other occasions, particularly in Biology, outliers are important to include. For example, it is important to know that a particular drug produces side effects in one person in a thousand.

If measurements are repeated, the uncertainty can be calculated by finding half the range of the measured values.

**For example:**

Repeat	1	2	3	4
Distance/m	1.23	1.32	1.27	1.22

$1.32 - 1.22 = 0.10$  therefore

Mean distance:  $(1.26 \pm 0.05)$  m

## PERCENTAGE UNCERTAINTIES

The percentage uncertainty in a measurement can be calculated using:

$$\text{percentage uncertainty} = \frac{\text{uncertainty}}{\text{value}} \times 100\%$$

The percentage uncertainty in a repeated measurement can also be calculated using:

$$\text{percentage uncertainty} = \frac{\text{uncertainty}}{\text{mean value}} \times 100\%$$

## UNCERTAINTIES IN EXAMS

Wherever possible, questions in exams will be clear on whether students are being asked to calculate the uncertainty of a reading, a measurement, or given data.

Where there is ambiguity, mark schemes will allow alternative sensible answers and credit clear thinking.

It is important that teachers read the reports on the examination following each series to understand common mistakes to help their students improve in subsequent years.

## UNCERTAINTIES IN PRACTICAL WORK

Students are expected to develop an understanding of uncertainties in measurements through their practical work.

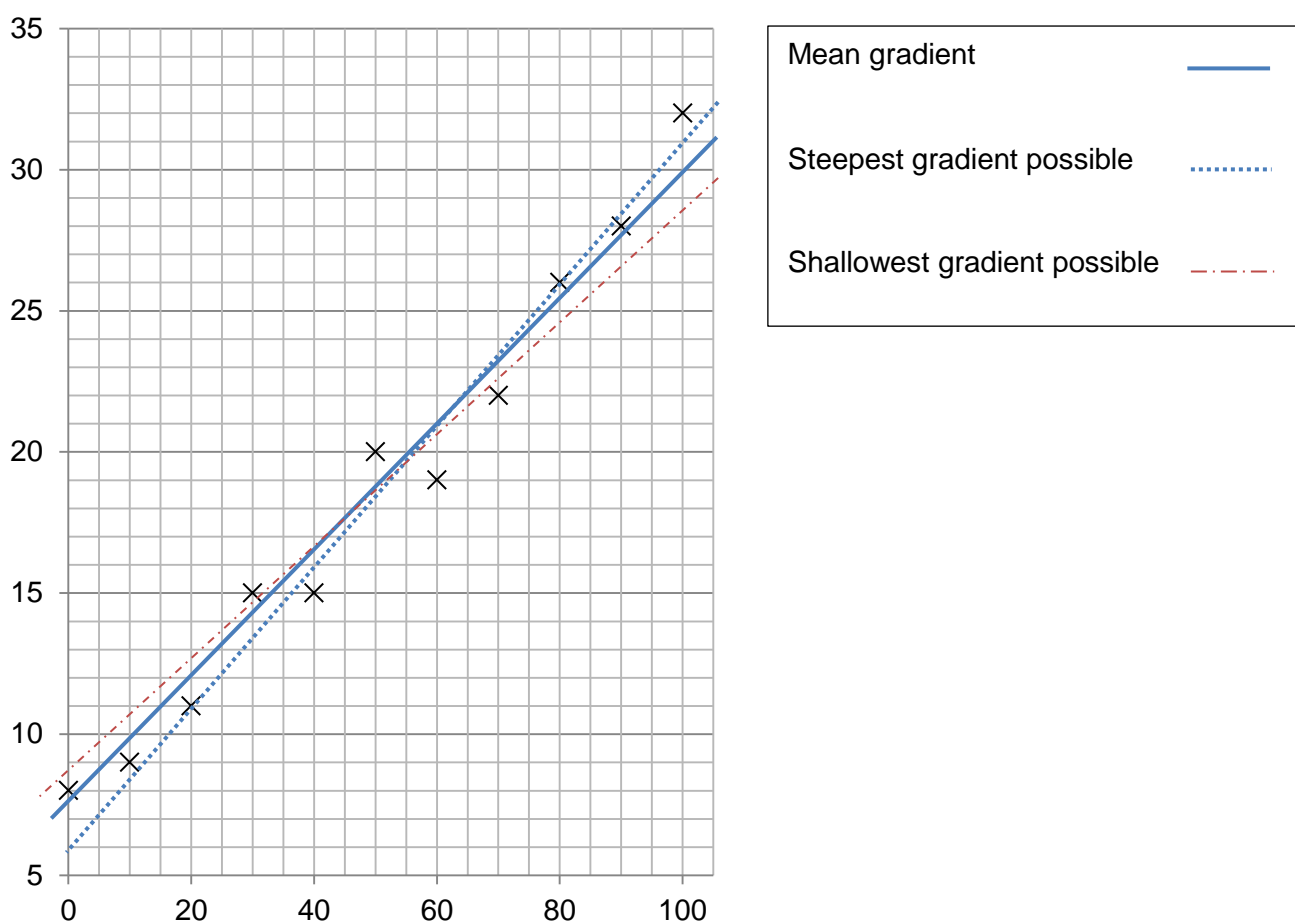
## UNCERTAINTIES FROM GRADIENTS

To find the uncertainty in a gradient, two lines should be drawn on the graph. One should be the “best” line of best fit. The second line should be the steepest or shallowest gradient line of best fit possible from the data. The gradient of each line should then be found.

The uncertainty in the gradient is found by:

$$\text{percentage uncertainty} = \frac{1}{2} \left( \frac{\text{steeper gradient} - \text{shallowest gradient}}{\text{mean gradient}} \right)$$

Note the modulus bars meaning that this percentage will always be positive.



In the same way, the percentage uncertainty in the y-intercept can be found:

$$\text{percentage uncertainty} = \frac{1}{2} \left( \frac{\text{greatest intercept} - \text{smallest intercept}}{\text{mean intercept}} \right)$$

## COMBINING UNCERTAINTIES

Percentage uncertainties should be combined using the following rules:

Combination	Operation	Example
<b>Adding or subtracting values</b> $a = b + c$	Add the absolute uncertainties $\Delta a = \Delta b + \Delta c$	Length of leaf on day 1 = $(5.0 \pm 0.1)$ cm Length of leaf on day 2 = $(7.2 \pm 0.1)$ cm Difference in length = $(2.2 \pm 0.2)$ cm
<b>Multiplying values</b> $a = b \times c$	Add the percentage uncertainties $\epsilon a = \epsilon b + \epsilon c$	Mass = $50.0 \pm 0.1$ g Temperature rise (T) = $10.9 \pm 0.1$ °C Percentage uncertainty in mass = 0.20% Percentage uncertainty in T = 0.92% Heat change = 2278 J Percentage uncertainty in heat change = 1.12% Absolute uncertainty in heat change = $\pm 26$ J (Note – the uncertainty in specific heat is taken to be zero)
<b>Dividing values</b> $a = \frac{b}{c}$	Add the percentage uncertainties $\epsilon a = \epsilon b + \epsilon c$	Mass of salt solution = $(100 \pm 0.1)$ g Mass of salt = $(20.0 \pm 0.5)$ g Percentage uncertainty in mass of solution = 0.1% Percentage uncertainty in mass of salt = 2.5% Percent composition by mass = $\frac{\text{mass of salt}}{\text{mass of solution}} \times 100\% = 20\%$ Percentage uncertainty of percentage = 2.6% Absolute uncertainty = $\pm 0.5\%$
<b>Power rules</b> $a = b^c$	Multiply the percentage uncertainty by the power $\epsilon a = c \times \epsilon b$	Radius of circle = $(6.0 \pm 0.1)$ cm Percentage uncertainty in radius = 1.6% Area of circle = $\pi r^2 = 113.1$ cm <sup>2</sup> Percentage uncertainty in area = 3.2% Absolute uncertainty = $\pm 3.6$ cm <sup>2</sup> (Note – the uncertainty in $\pi$ is taken to be zero)

Note: Absolute uncertainties (denoted by  $\Delta$ ) have the same units as the quantity.

Percentage uncertainties have no units.

Uncertainties in trigonometric and logarithmic functions will not be tested in A-level exams.

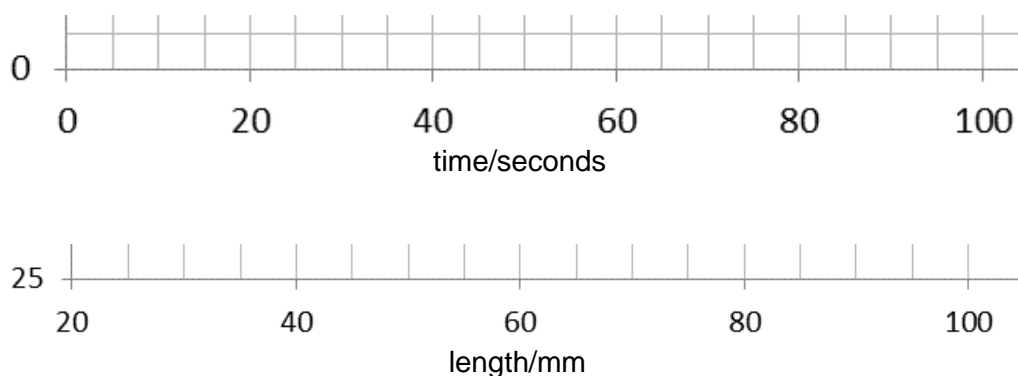
# GRAPHING

Graphing skills can be assessed in the written papers for the International AS and A-level grade. Students should recognise that the type of graph that they draw should be based on an understanding of the type of data they are using and the intended analysis of the data. The rules below are guidelines which will vary according to the specific circumstances.

Please note: The Royal Society of Biology suggests that even straight lines on graphs should be referred to as curve. This convention is not used in the following pages to ensure clarity.

## LABELLING AXES

Axes should always be labelled with the variable being measured and the units. These should be separated with a forward slash (solidus):



Axes should not be labelled with the units on each scale marking.

## DATA POINTS

Data points should be marked with a cross. Both  $\times$  and  $+$  marks are acceptable, but care should be taken that data points can be seen against the grid.

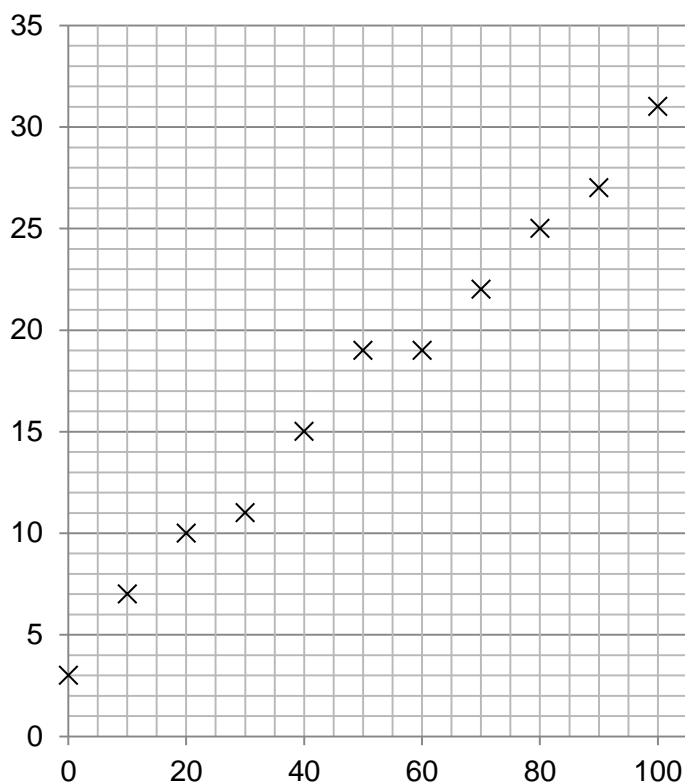
Error bars, standard deviation and ranges can take the place of data points where appropriate.

## SCALES AND ORIGINS

Students should attempt to spread the data points on a graph as far as possible without resorting to scales that are difficult to deal with. Students should consider:

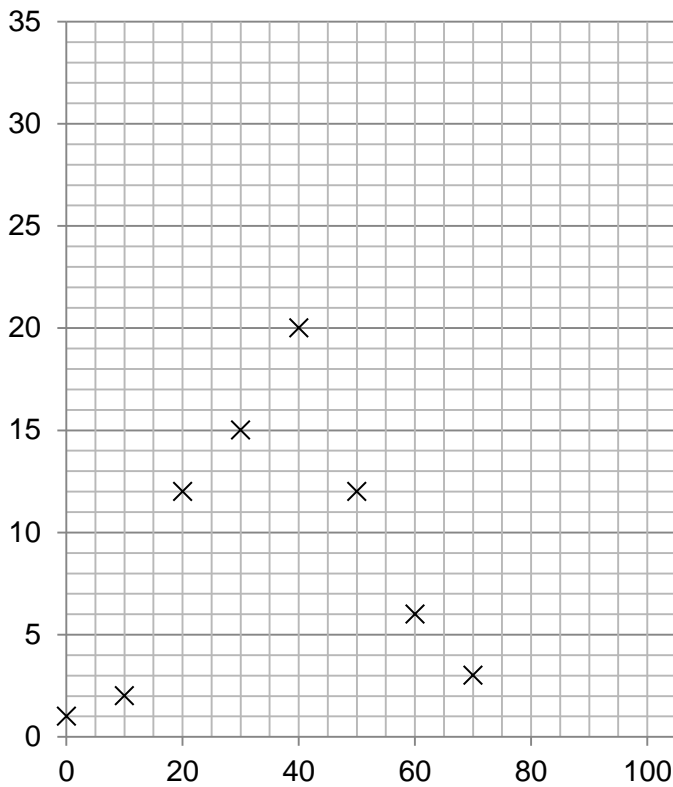
- the maximum and minimum values of each variable
- the size of the graph paper
- whether 0.0 should be included as a data point
- how to draw the axes without using difficult scale markings (eg multiples of 3, 7, 11 etc)
- in exams, the plots should cover **at least half** of the grid supplied for the graph.

Please note that in the Uncertainties and Graphing sections, many generic graphs are used to illustrate the points made. For example, the following three graphs are intended to illustrate the information above relating to the spread of data points on a graph. **Students producing such graphs on the basis of real practical work or in examination questions would be expected to add in axes labels and units.**

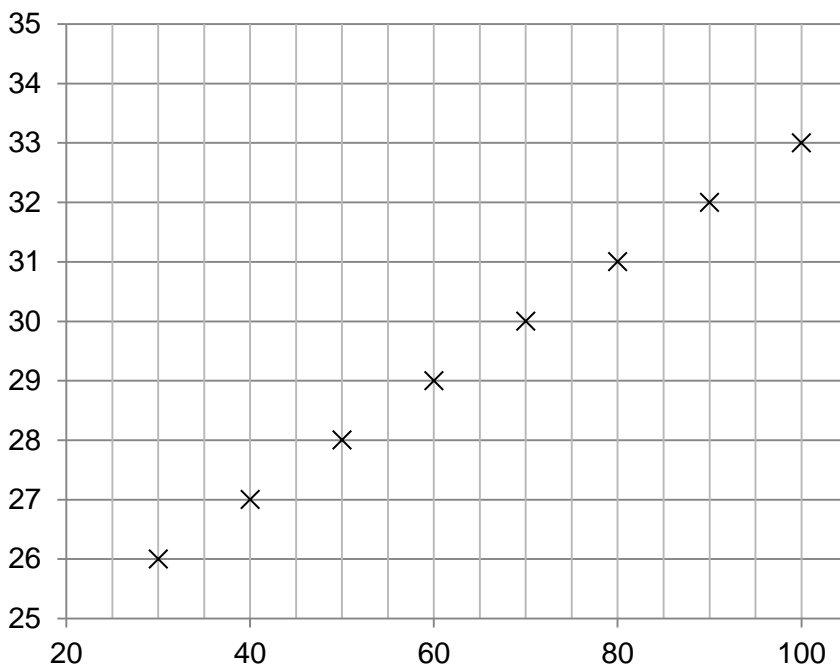


This graph has well-spaced marking points and the data fills the paper.

Each point is marked with a cross (so points can be seen even when a line of best fit is drawn).



This graph is on the limit of acceptability. The points do not quite fill the page, but to spread them further would result in the use of awkward scales.



At first glance, this graph is well drawn and has spread the data out sensibly. However, if the graph were to later be used to calculate the equation of the line, the lack of a y-intercept could cause problems. Increasing the axes to ensure all points are spread out but the y-intercept is also included is a skill that requires practice and may take a couple of attempts.



## LINES OF BEST FIT

Lines of best fit should be drawn when appropriate. Students should consider the following when deciding where to draw a line of best fit:

- Are the data likely to be following an underlying equation (for example, a relationship governed by a physical law)? This will help decide if the line should be straight or curved.
- Are there any anomalous results?
- Are there uncertainties in the measurements? The line of best fit should fall within error bars, if drawn.

There is no definitive way of determining where a line of best fit should be drawn. A good rule of thumb is to make sure that there are as many points on one side of the line as the other. Often the line should pass through, or very close to, the majority of plotted points. Graphing programs can sometimes help, but tend to use algorithms that make assumptions about the data that may not be appropriate.

Lines of best fit should be continuous and drawn as a thin pencil that does not obscure the points below and does not add uncertainty to the measurement of gradient of the line.

Not all lines of best fit go through the origin. Students should ask themselves whether a 0 in the independent variable is likely to produce a 0 in the dependent variable. This can provide an extra and more certain point through which a line must pass. A line of best fit that is expected to pass through (0,0), but does not, would imply some systematic error in the experiment. This would be a good source of discussion in an evaluation.

## DEALING WITH ANOMALOUS RESULTS

At International GCSE, students are often taught automatically to ignore anomalous results. At International AS and A-level, students should think carefully about what could have caused the unexpected result and therefore whether it is anomalous. A student might be able to identify a reason for the unexpected result and so validly regard it as an anomaly. For example, an anomalous result might be explained by a different experimenter making the measurement, a different solution or a different measuring device being used. In the case where the reason for an anomalous result occurring can be identified, the result should be recorded and plotted but may then be ignored. Biology students must be careful in deciding results are anomalous and should always bear in mind that diversity is a feature of biological material.

Anomalous results should also be ignored where results are expected to be the same (for example, when repeat readings of pH are taken of the same sample).

Where there is no obvious error and no expectation that results should be the same, anomalous results should be included. This will reduce the possibility that a key point is being overlooked.

Please note: when recording results it is important that all data are included. Anomalous results should only be ignored at the data analysis stage.

It is best practice whenever an anomalous result is identified for the experiment to be repeated. This highlights the need to tabulate and even graph results as an experiment is carried out.

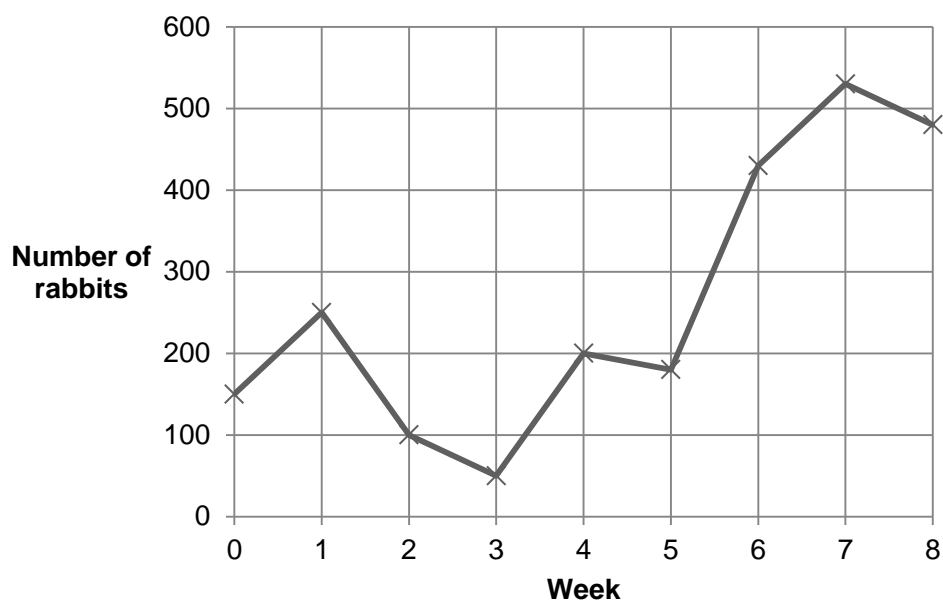
## SCATTER GRAPHS

Often, in Biology, we find a relationship between two continuous variables but cannot infer that the relationship is causal. For example, in the UK, is there a relationship between the number of worms and the number of woodlice? We could plot values for these two continuous variables as a graph but it would not be valid to join the plotted points. We use a scatter graph to investigate correlations. A line of best fit indicates a positive correlation or negative correlation or absence of correlation.



## JAGGED-LINE GRAPHS

In Biology, the Royal Society of Biology recommends that where the interim values of a continuously changing variable are not known, data points should be joined by straight lines.



## HISTOGRAMS

As with a line graph and scatter graph, a histogram is used to show the distribution of a continuous variable. In this case, the data for the dependent variable are arranged into non-overlapping groups. These groups could cover an equal span of data, eg, 0.0 to 4.9, 5.0 to 9.9, 10.0 to 14.9, or an unequal span of data, eg 0 to 0.9, 1 to 3.9, 4 to 7.9, 8 to 8.9.

These groups are arranged on the x-axis with widths scaled to represent each span of data. When the dependent variable is plotted, the area under each rectangle is equal to the frequency of the observations in that interval.

In a histogram, the bars touch.



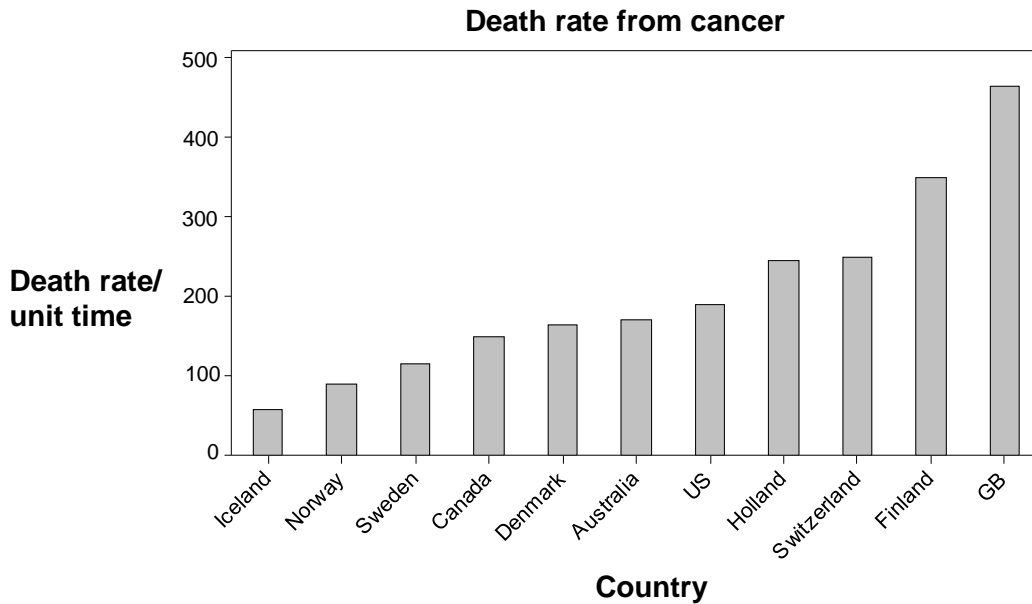
## BAR CHARTS

Line graphs and histograms are used when the data are continuous. In contrast, bar charts are used when the data are discontinuous because they are:

- categoric – only certain values can exist (eg reading at week 1, reading at week 2 etc)
- nominal – there is no ordering of the categories (eg red flowers, pink flowers and white flowers of *Antirrhinum*).

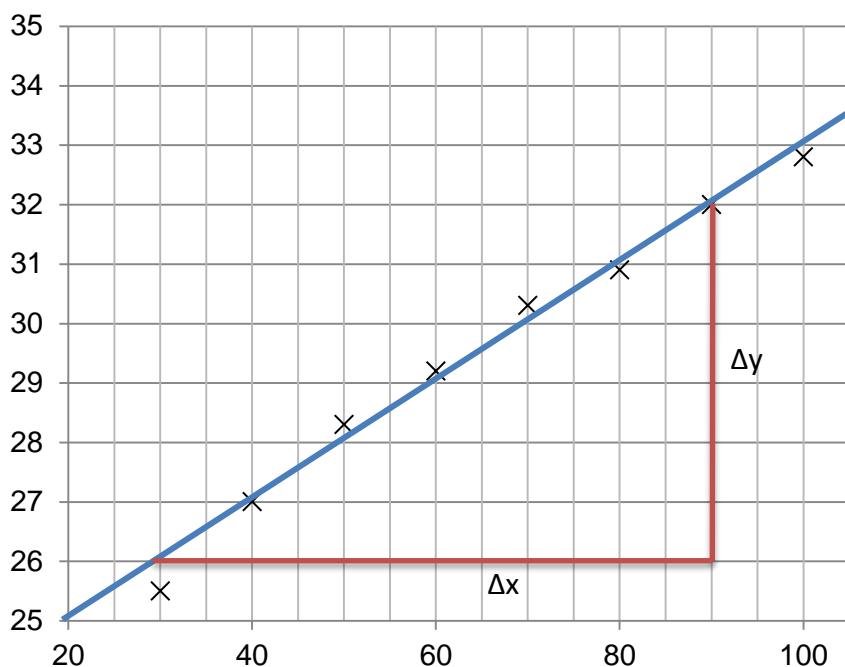
Since these data are not continuous, the intervals on the x-axis should show this and, unlike a histogram, the rectangles must **not** touch.

Unlike histograms, the bars of a bar chart should be of equal width (including a single straight line), so that the height of the bar represents the frequency of each category.



## MEASURING GRADIENTS

When finding the gradient of a line of best fit, students should show their working by drawing a triangle on the line. The hypotenuse of the triangle should be at least half as big as the line of best fit.



The line of best fit here has an equal number of points on both sides. It is not too wide so points can be seen under it.

The gradient triangle has been drawn so the hypotenuse includes more than half of the line.

In addition, it starts and ends on points where the line of best fit crosses grid lines so the points can be read easily (this is not always possible).

$$\text{gradient} = \frac{\Delta y}{\Delta x}$$

When finding the gradient of a curve, eg the rate of reaction at a time that was not sampled, students should draw a tangent to the curve at the relevant value of the independent variable (x-axis).

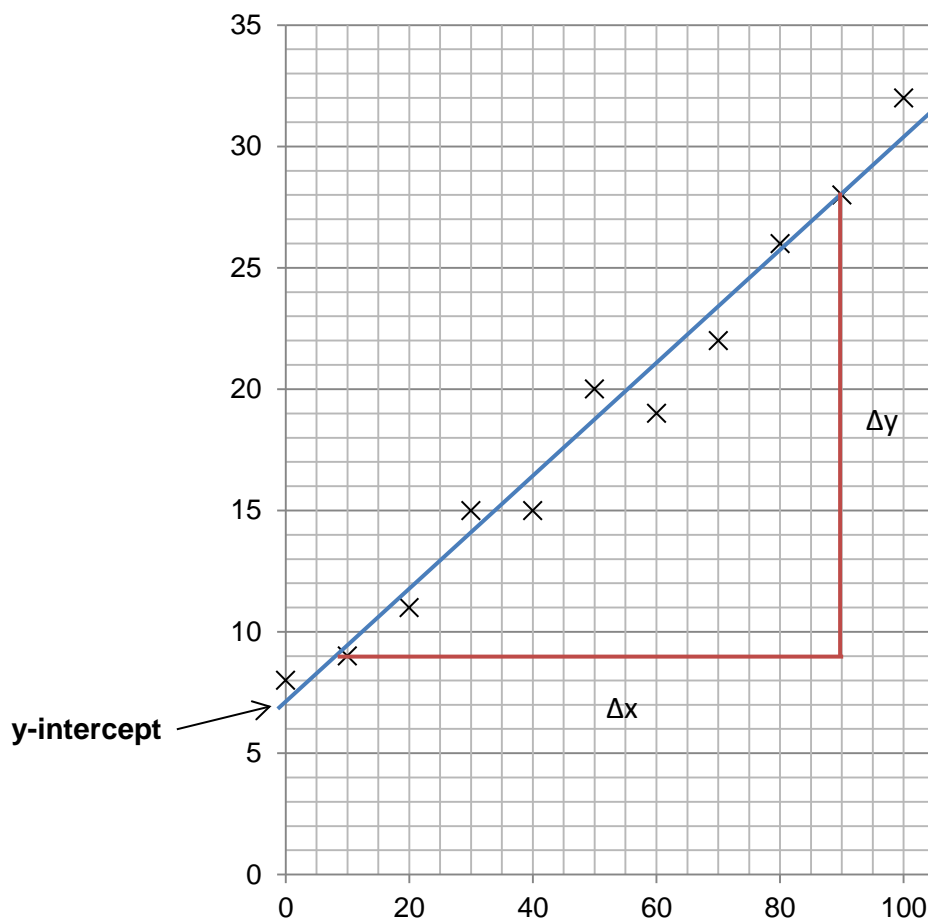
Use of a set square to draw a triangle over this point on the curve can be helpful in drawing an appropriate tangent.

**THE EQUATION OF A STRAIGHT LINE**

Students should be able to translate graphical data into the equation of a straight line.

$$y = mx + c$$

Where  $y$  is the dependent variable,  $m$  is the gradient,  $x$  is the independent variable and  $c$  is the  $y$ -intercept.



$$\begin{aligned}\Delta y &= 28 - 9 = 19 \\ \Delta x &= 90 - 10 = 80 \\ \text{gradient} &= 19 / 80 = 0.24 \text{ (2 sf)} \\ \text{y-intercept} &= 7.0 \\ \\ \text{equation of line:} \\ y &= 0.24x + 7.0\end{aligned}$$

# BIOLOGICAL DRAWING

The purpose of drawing in the teaching of Biology is the development of observational skills. A student must look very closely at a specimen in order to draw it accurately and must have sound knowledge of the component structures in order to choose what to draw and what to omit from the drawing.

Drawings should always be in pencil. Fine detail cannot be represented accurately unless the pencil has a sharp point.

The outlines of any structures should be drawn but there should be no colouring or shading. The relative sizes of the structures drawn should be accurate. Construction lines or frames could be used to solve this problem. If the relative size of any structure has been exaggerated, eg because an actual cell wall was too thin to be able to draw its outline using two pencil lines, a note should be added to the drawing to explain this.

If required, the drawn structures should be labelled, using label lines that do not cross or obscure the drawing, with brief annotations about their functions or interrelationships.

The drawing should have an explanatory title and an indication of the real size of the structures drawn or of the magnification used.

During an AS or A-level Biology course, students are likely to make three types of drawing.

## Cell drawing

The purpose of this drawing is to show accurately the components of individual cells observed using an optical microscope. The drawing should be detailed but should **not** show more than two or three cells.

## Tissue map









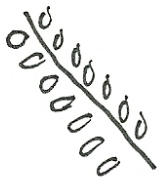



The purpose of a tissue map is to show the location and extent of tissues in an organ or in a whole organism. Cellular detail of any of the tissues should **not** be shown. Instead, the outline of each tissue should be drawn. This often presents a problem, since cell differentiation is seldom discrete. Students must use their background knowledge and understanding to interpret what they see.

## Body plan

Following dissection, a morphological drawing should provide a lifelike representation of the main body parts exposed by the dissection.

### COMMON ERRORS IN BIOLOGICAL DRAWING

The table below shows errors that commonly occur when students begin to practise drawings of biological material. Each would reduce the value of the drawing and result in loss of credit being awarded. Most result from lack of attention or care and are easily solved.

Comment	Incorrect	Correct
Cell outline		
Tissue with too much detail		
Crossing lines		
Hanging lines		
Over casual		
Sketch rather than draw		



# STATISTICAL TESTS IN BIOLOGY

During development of the new specification, there was general agreement amongst examiners, teachers and representatives from higher education and industry that there is no value in students calculating test statistics manually as in most commercial and academic institutions computers are used to carry out the numerical calculations. Consequently, we expect that wherever possible most students will often be encouraged to use electronic devices to calculate test statistics during their classwork.

In written examinations, students might be asked to perform simple calculations such as finding a mean value. In written examinations, students will **not** be asked to perform a calculation using a statistical test. Instead, it is important for students to understand how to select a statistical test that is appropriate for given data and to be able interpret the results of such a statistical test. Students could also be asked in a written exam to explain their choices and interpretation. They should also be able to interpret the probability value calculated from such a statistical test in terms of accepting or rejecting a null hypothesis.

Students taking International AS and A-level Biology should be familiar with the language of statistics and understand the need to devise random sampling procedures that avoid observer bias.

Students will be expected to be familiar with the following types of statistics.

- **Descriptive statistics** that provide an understanding of the data.
- **Inferential statistics** that enable inferences about a population based on the sample of data that has been collected.

Teachers should decide the best method of teaching students the use of statistical tests. In some circumstances, the full numerical analysis may be appropriate so that students understand the information needed for a particular test. In other circumstances, using computers to carry out the analysis followed by discussion of the findings will be sufficient.

## DESCRIPTIVE STATISTICS

At A-level, we usually assume that populations and samples show a normal distribution. This enables students to use a **mean** and **standard deviation of the mean** to describe data.

Students could calculate mean values and their standard deviations during class work but will **not** be asked to calculate a standard deviation in a written examination. They should appreciate the advantage of using standard deviation in preference to the range of values, the latter being overly influenced by outlying values.

When calculating the mean value from a **sample**, the mean is represented as  $\bar{x}$  (pronounced x-bar). It is the sum of all the values, divided by the number of values, ie:

$$\bar{x} = \frac{\Sigma x}{n}$$

The standard deviation (SD) gives an indication of the spread of values around the mean of those values. It is found using the formula

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

In interpreting the values of standard deviations, students should realise that  $\pm 2$  standard deviations from the mean includes over 95% of the data. Whilst not strictly valid, this allows students to use the presence or absence of overlap of the standard deviations of different means as an indication of whether differences in the means are likely to be due to chance.

In addition to the mean, students should be confident in identifying and using the **median** and **mode** values of data.

95% Confidence intervals (95% CI): since students will calculate a standard deviation, teachers could introduce them to the **standard error of the mean** (SE). Calculating SE is **not** a specification requirement but can be used to give an indication of how close the mean of a sample might be to the mean of the population from which the sample was taken or to the mean of another sample from the same population. SE is calculated by dividing the standard deviation of the mean by the square root of the sample size, ie:

$$SE = \frac{SD}{\sqrt{n}}$$

Since the true population mean  $\pm 1.96$  SE will include 95% of the sample means, the standard error enables students to use 95% confidence intervals.

To calculate the 95% confidence interval, we multiply the standard error of each mean by 1.96. Subtracting this value from the mean gives the lower 95% confidence limit and adding it to the sample mean gives the upper 95% confidence limit.

$$95\% CI = \bar{x} \pm SE \times 1.96$$

We can use the 95% confidence interval to state that:

- we are 95% confident that the true mean value of the population from which the sample was taken lies between the upper and lower confidence limits
- if the 95% confidence intervals of two calculated means do not overlap, we are 95% confident that these means are different.

## STATISTICAL TESTS

Students should be aware that statistical tests are used to test a theory, known as a hypothesis. Perhaps, counter-intuitively, the hypothesis is usually that there is **no** difference between the samples being studied, ie is a **null hypothesis**. The table shows how hypotheses can be turned into null hypotheses.

Hypothesis	Null hypothesis
Chickens fed maize supplemented by lipid produce more male offspring than those fed maize alone.	There is no difference between the number of male and female offspring of chickens fed maize supplemented by lipid and those fed maize alone.
There are fewer slugs in dry areas.	There is no difference between the number of slugs found in wet and dry areas.
Tobacco plants exhibit a higher rate of growth when planted in soil rather than peat.	Tobacco plants do not exhibit a higher rate of growth when planted in soil rather than peat.
The incidence of sunburn is associated with the sale of ice cream.	There is no association between the incidence of sunburn and the sale of ice cream.

Once we have a null hypothesis, we design an experiment to try to disprove it. Thus, the result of a statistical test either disproves or fails to disprove (supports or fails to support) that null hypothesis; it can never prove a hypothesis to be true.

## SIGNIFICANCE LEVELS

Given the results of an experiment, we need to know if any difference between the results we predicted from our null hypothesis and those we obtained could be due to chance. If this difference is likely to be due to chance, it is said to be 'non-significant' and the null hypothesis cannot be rejected. On the other hand, if this difference is not likely to be due to chance, it is said to be significant and the null hypothesis can be rejected.

Each statistical test is associated with a table which enables us to calculate a significance level.

For convenience, students can assume that if the probability (P) of the difference in their results being due to chance is equal to, or less than, 1 in 20 ( $P \leq 0.05$ ), the difference **is significant**.

Note that students should **not** refer to their **results** being due to chance (or to their results being significant/insignificant). They should always refer to the differences or associations in their results being due to chance (or differences or associations in their results being significant/insignificant).

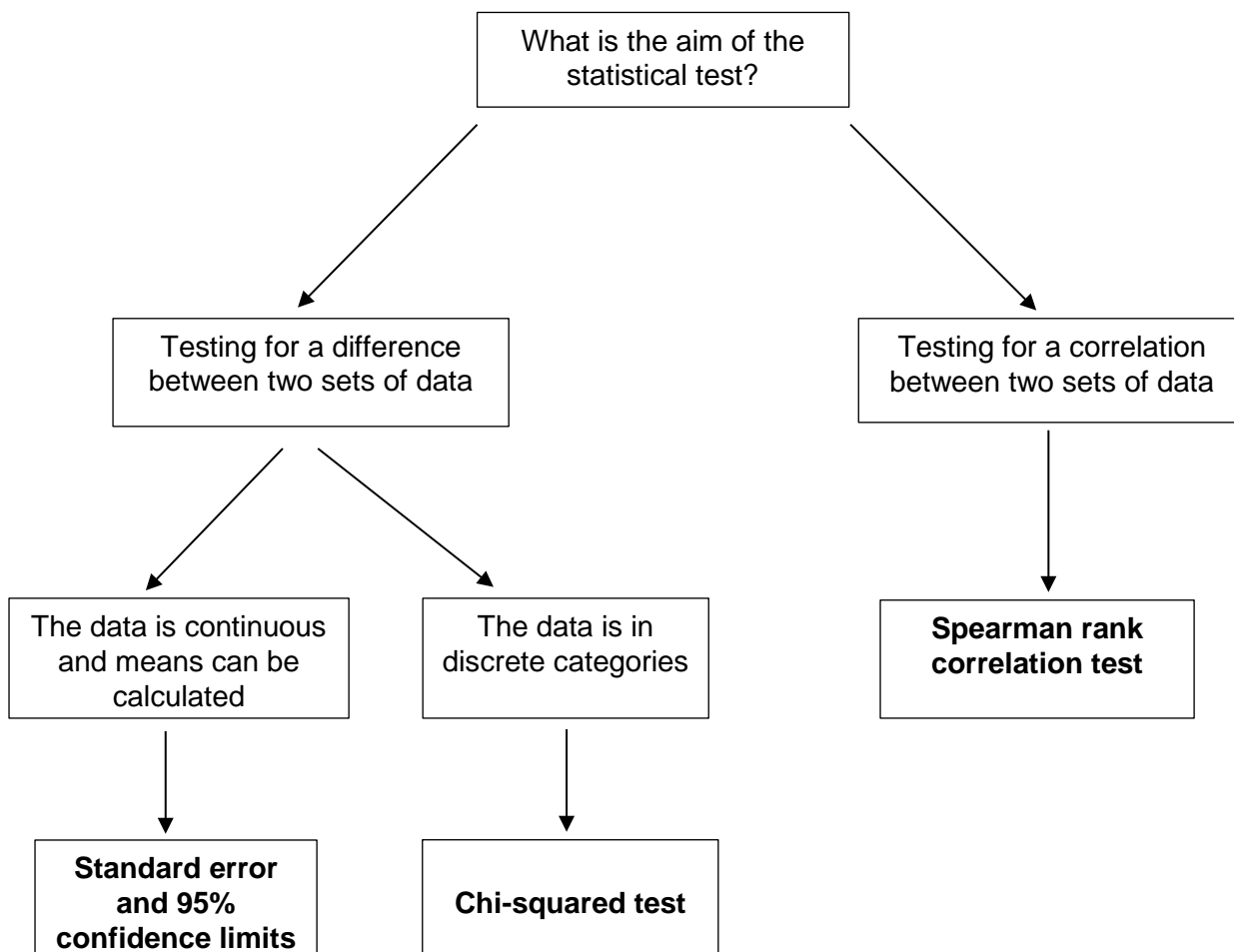
## CHOICE OF STATISTICAL TEST

No single statistical test is suitable for all data. The mathematical requirements of this Biology specification include three statistical tests: the chi-squared ( $\chi^2$ ) test, standard error and 95% confidence limits and Spearman rank correlation test. Although students should use data from their practical work to perform calculations using these tests, they will **not** be asked to do so in a written examination. They can, however, be asked in written examinations to:

- choose which test would be appropriate for given data
- justify their choice of statistical test
- interpret a given probability value in terms of accepting or rejecting a null hypothesis.

The following decision-making flowchart can be used to decide which of the three tests is appropriate for given data. This flowchart will **not** be provided in written examinations.

## FLOWCHART FOR DECIDING WHICH STATISTICAL TEST TO USE



### FURTHER GUIDANCE ON TEACHING STATISTICS IN BIOLOGY

In teaching the OxfordAQA International AS and A-level Biology specifications, students may be made aware of other statistical tests such as the Student's t-test, the Mann-Whitney U-test or the Pearson correlation coefficient. If a written paper requires students to suggest an appropriate test to use with given data, then these tests would be credited if appropriate. However, students would only be expected to understand and interpret data from the three statistical tests that are named in the mathematical requirements of the specification.

In written examinations, students might be asked to perform simple calculations such as finding a mean value. **Students will not be asked to perform a calculation using a statistical test** (or to calculate the standard deviation of a mean). We would expect students to perform such calculations during their class work, however. Whilst teachers might feel there is some value in students performing these calculations manually, we anticipate that most students will use electronic devices. The use of such devices also reflects the general agreement of representatives from higher education and from industry that there is little value in students calculating test statistics manually as in most commercial and academic institutions computers are used to carry out the numerical calculations.

In preparing for written examinations, it will be important for students to understand how to select a statistical test that is appropriate for given data and be able to interpret the results of such a statistical test, in terms of rejection of the null hypothesis if  $P \leq 0.05$ . Students could also be asked to justify their choices and interpretation.

Our papers will expect progression in the understanding of statistical tests in AS and A-level exams.

In AS exams, students could be expected to:	In A-level exams, students could also be expected to:
<p><b>formulate</b> a null hypothesis</p> <ul style="list-style-type: none"> <li>for the experiments they perform during their class work</li> <li>when given appropriate information, for experiments carried out by others</li> </ul>	<p><b>evaluate</b> the null hypothesis of another investigator</p>
<p><b>devise</b> and <b>justify</b> an appropriate table in which to record their raw data</p>	
<p><b>devise</b> and <b>justify</b> an appropriate way to represent their processed data graphically</p>	<p><b>evaluate</b> the way in which another investigator has represented processed data</p>
<p><b>select</b>, and <b>justify</b> the use of, an appropriate statistical test for data they will subsequently collect themselves or data that might be collected by others. The statistical tests are restricted to:</p> <ul style="list-style-type: none"> <li><b>chi-squared test</b> when the data are categoric</li> <li><b>standard error and 95% confidence limits</b> when the data is continuous and the mean values of two sets of data can be calculated</li> <li><b>Spearman rank correlation test</b> when examining a correlation between two sets of data</li> </ul>	<p><b>evaluate</b> the choice of a statistical test made by another investigator</p>
<p><b>interpret</b> a given probability value in terms of the probability of</p> <ul style="list-style-type: none"> <li>the difference between observed data and expected data being due to chance (chi-squared test)</li> <li>the difference between the means of two samples being due to chance (standard error and 95% confidence limits)</li> <li>a correlation between two variables being due to chance (Spearman rank correlation test)</li> </ul>	<p><b>interpret</b> a given probability value in terms of acceptance or rejection of a null hypothesis, using 0.05 as the critical probability value</p> <p><b>evaluate</b> the conclusions from the same data made by another commentator</p> <p><b>show an understanding</b> of 'degrees of freedom' so that, when given appropriate information, a student can use a given result of a statistical test to find the correct probability value from an abridged table of values.</p>

## TEACHING STATISTICS AT AS

There are many opportunities for students to be introduced to statistical concepts during their AS course. The start of every investigative practical presents an opportunity for students to:

- formulate a null hypothesis that is appropriate for the investigation they will perform, eg temperature (the independent variable) has no effect on the rate of an enzyme-catalysed reaction (the dependent variable)
- devise an appropriate way to tabulate the raw data they will collect
- devise an appropriate way to represent their processed data graphically.

The following examples show how the choice and justification of appropriate statistical tests could be included in class work during an AS Biology course. Students could also be encouraged to calculate, and interpret the result of, their chosen statistical test. These are intended only as a guide to areas in which the statistical tests could be used and are **not** specification requirements.

Opportunities for skills development
Students could select and use an appropriate statistical test to find the significance of differences in the rates of reaction following use of a continuous variable (eg pH, temperature, enzyme concentration or substrate concentration) or of a discontinuous variable (eg presence and absence of an enzyme inhibitor).
Students could select and use an appropriate statistical test to find the significance of different mean numbers of a particular organelle (eg mitochondria or chloroplasts) in different types of cells.
Students could select and use an appropriate statistical test to find the significance of differences in the number of cells undergoing mitosis at two close, but different, distances from the root tip.
Students could select and use an appropriate statistical test to find the significance of differences in the number of stomata on the upper and lower surfaces of leaves of a single plant species or on the lower surfaces of leaves of different plant species.
Students could select and use an appropriate statistical test to find the significance of a correlation between data about an environmental variable and data about the incidence of a particular lung disease.
Students could select and use an appropriate statistical test to find the significance of a correlation between data about an environmental variable and data about the incidence of a particular cardiovascular disease.
Students could select and use an appropriate statistical test to find the significance of differences in the effect of different antibiotics on the growth of a species of bacterium or of a single antibiotic on the growth of more than one species of bacterium.
Students could select and use an appropriate statistical test to find the significance of differences in the mean values they have collected or been given.

# SUBJECT SPECIFIC VOCABULARY

## THE LANGUAGE OF MEASUREMENT

The following subject specific vocabulary provides definitions of key terms used in our International AS and A-level science specifications.

### **ACCURACY**

A measurement result is considered accurate if it is judged to be close to the true value.

### **CALIBRATION**

Marking a scale on a measuring instrument.

This involves establishing the relationship between indications of a measuring instrument and standard or reference quantity values, which must be applied.

For example, placing a thermometer in melting ice to see whether it reads 0 °C, in order to check if it has been calibrated correctly.

### **DATA**

Information, either qualitative or quantitative, that has been collected.

### **ERRORS**

See also uncertainties.

#### **Measurement error**

The difference between a measured value and the true value.

#### **Anomalies**

These are values in a set of results which are judged not to be part of the variation caused by random uncertainty.

#### **Random error**

These cause readings to be spread about the true value, due to results varying in an unpredictable way from one measurement to the next.

Random errors are present when any measurement is made, and cannot be corrected. The effect of random errors can be reduced by making more measurements and calculating a new mean.

#### **Systematic error**

These cause readings to differ from the true value by a consistent amount each time a measurement is made.

Sources of systematic error can include the environment, methods of observation or instruments used.

Systematic errors cannot be dealt with by simple repeats. If a systematic error is suspected, the data collection should be repeated using a different technique or a different set of equipment, and the results compared.



### **Zero error**

Any indication that a measuring system gives a false reading when the true value of a measured quantity is zero, eg the needle on an ammeter failing to return to zero when no current flows. A zero error may result in a systematic uncertainty.

### **EVIDENCE**

Data which has been shown to be valid.

### **FAIR TEST**

A fair test is one in which only the independent variable has been allowed to affect the dependent variable.

### **HYPOTHESIS**

A proposal intended to explain certain facts or observations.

### **INTERVAL**

The quantity between readings, eg a set of 11 readings equally spaced over a distance of 1 metre would give an interval of 10 centimetres.

### **PRECISION**

Precise measurements are ones in which there is very little spread about the mean value.

Precision depends only on the extent of random errors – it gives no indication of how close results are to the true value.

### **PREDICTION**

A prediction is a statement suggesting what will happen in the future, based on observation, experience or a hypothesis.

### **RANGE**

The maximum and minimum values of the independent or dependent variables; important in ensuring that any pattern is detected.

For example a range of distances may be quoted as either:

'from 10 cm to 50 cm' or 'from 50 cm to 10 cm'

### **REPEATABLE**

A measurement is repeatable if the original experimenter repeats the investigation using same method and equipment and obtains the same results.

### **REPRODUCIBLE**

A measurement is reproducible if the investigation is repeated by another person, or by using different equipment or techniques, and the same results are obtained.

## RESOLUTION

This is the smallest change in the quantity being measured (input) of a measuring instrument that gives a perceptible change in the reading.

## SKETCH GRAPH

A line graph, not necessarily on a grid, that shows the general shape of the relationship between two variables. It will not have any points plotted and although the axes should be labelled they may not be scaled.

## TRUE VALUE

This is the value that would be obtained in an ideal measurement.

## UNCERTAINTY

The interval within which the true value can be expected to lie, with a given level of confidence or probability, eg “the temperature is  $20\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ , at a level of confidence of 95%.

## VALIDITY

Suitability of the investigative procedure to answer the question being asked. For example, an investigation to find out if the rate of a chemical reaction depended upon the concentration of one of the reactants would not be a valid procedure if the temperature of the reactants was not controlled.

## VALID CONCLUSION

A conclusion supported by valid data, obtained from an appropriate experimental design and based on sound reasoning.

## VARIABLES

These are physical, chemical or biological quantities or characteristics.

### **Categoric variables**

Categoric variables have values that are labels, eg names of plants or types of material.

### **Continuous variables**

Continuous variables can have values (called a quantity) that can be given a magnitude either by counting (as in the case of the number of shrimp) or by measurement (eg light intensity, flow rate etc).

### **Control variables**

A control variable is one which may, in addition to the independent variable, affect the outcome of the investigation and therefore has to be kept constant or at least monitored.

### **Dependent variables**

The dependent variable is the variable of which the value is measured for each and every change in the independent variable.

**Independent variables**

The independent variable is the variable for which values are changed or selected by the investigator.

**Nominal variables**

A nominal variable is a type of categoric variable where there is no ordering of categories (eg red flowers, pink flowers, blue flowers).

# REQUIRED PRACTICAL ACTIVITIES: EXEMPLAR EXPERIMENTS

During the development of our International AS and A-levels in Biology, Chemistry and Physics, we have spoken to hundreds of teachers. Teachers helped us to develop every part of the specification, including its contents and layout, what is examined in which paper and the question types we include. Teachers also helped us to decide which practical activities to include in our required practicals for each subject.

Teachers asked us for full, comprehensive instructions on how to carry out each of the 10 required practicals. In response, we have included a **sample** method for each practical in this section. These have been prepared so that a reasonably equipped school can cover the required activity with their students. It gives **one possible version** of the experiment that teachers could use. They will help inform planning the laboratory time required and help schools to ensure have the right equipment. Many are based on existing tasks as we know that they work well and schools have been using them for a number of years in the current AQA UK specifications.

**This document should only be seen as a starting point. We do not intend to stifle innovation and would encourage teachers to try different methods. Students will not be examined on the specific practical work exemplified within this section but on the skills and understanding they build up through their practical work. Teachers can vary all experiments to suit their and their students' needs.**

## SAFETY

At all times, the teacher is responsible for safety in the classroom. Teachers should intervene whenever they see unsafe working. Risk assessments should be carried out before working, and advice from CLEAPSS and other organisations should be followed.

It is appropriate to give students at International AS and A-level more independence when making decisions about safety. They should be taught how to assess risks and how to write risk assessments when appropriate. They should also understand the appropriate use of safety equipment and how to put measures in place to reduce risks.

**To support teachers further, Mary Philpott, Biology Adviser, previously from CLEAPSS, outlines the difference between identification of major hazards, associated risk and control measures and a full risk assessment:**

The risk assessment should always be complete, as it is this that prevents injury or ill-health.

The risk assessment is fundamentally the **thinking** that has taken place before and during an activity, so that any foreseeable risk is reduced to a minimum. A written record is necessary only to show that the thinking has taken place.

We tend to get caught up in the paperwork that provides evidence for the risk assessment, but the guidance from the Health and Safety Executive is that the written record should be on a **point-of-use document** and there is no particular form etc that needs to be filled in.

The tables/forms etc that many schools use are simply planning documents that the teachers use to provide the point of use risk assessment for each of their lessons. Incidentally, CLEAPSS members must refer to our current advice when preparing their point-of-use documents.

The student is not responsible for their risk assessment. In a large part, therefore, the student's risk assessment will be that they carry through the safety measures that the teacher has put in place. It is therefore fine if the student makes a note on their point-of use document that shows they have thought about how to behave safely, and carried it through. The teacher will also be able to record what they have seen in a practical that shows that the student's risk assessment is effective. For example, the student's written risk assessment could be as simple as making notes on a method

sheet about where they will put on eye protection or how they will arrange any heating equipment so that there is a minimum risk of scalding or burning themselves or the person next to them.

The teacher's observation notes will refer to whether they have carried out their written plans.

It might help the students to think safely if the teacher gives them a little time at the start of each practical to highlight or make notes about the safety aspects, and a class discussion about safety could show up any safety aspects that perhaps the teacher had not considered.

The students may also note where they have reminded other students about any safety issues.

If the students are planning their own practical activities, they could use the safety advice given in the CLEAPSS Student Safety Sheets.

In this case, they could identify hazards, risks and control measures.

In this case, they would make their own point of use document, with the control measures clearly identified.

The teacher would need to check that the risk assessment is adequate before they let the students proceed with the activity.

These are examples of 10 experiments that can be done as part of the International AS/A-level Biology course. The methods are written using commonly used reagents and techniques, although teachers can modify the methods and reagents as desired.

## **TRIALLING**

All practicals should be trialled before use with students.

## **RISK ASSESSMENT AND RISK MANAGEMENT**

Risk assessment and risk management are the responsibility of the centre.

Safety is the responsibility of the teacher and the centre. It is important that students are taught to act safely in the laboratory at all times, including the wearing of goggles at all times and the use of additional safety equipment where appropriate.

## **NOTES FROM CLEAPSS**

Technicians/teachers should follow CLEAPSS guidance, particularly that found on Hazcards and recipe sheets. The worldwide regulations covering the labelling of reagents by suppliers are currently being changed. Details about these changes can be found in leaflet GL101, which is available on the CLEAPSS website. You will need to have a CLEAPSS login.

# INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 1

**Investigation into the effect of temperature, pH or substrate concentration on the rate of an enzyme-controlled reaction:**

**The effect of temperature on the rate of the reaction catalysed by trypsin.**

## TEACHERS' NOTES

This investigation is based on ISA BIO3T/P09

### MATERIALS

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

- 30 cm<sup>3</sup> of 0.5% trypsin solution
- 100 cm<sup>3</sup> of 3% solution of milk powder (fat-free)
- 30 cm<sup>3</sup> of pH 7 buffer solution
- a large beaker to use as a water bath
- test tubes (6 for each temperature they test)
- bungs or cork for test tubes
- test-tube rack
- stopwatch
- marker pen (must be waterproof)
- graduated pipettes or syringes capable of measuring up to 10 cm<sup>3</sup>
- thermometer (to cover range 0 °C to 100 °C)
- large beakers to use as water baths
- access to hot and cold water to set up water baths

In this investigation students will require data from five different temperatures 20 °C, 30 °C, 40 °C, 50 °C, 60 °C. Students could carry out the experiment at each temperature individually or different members of the class could carry out the experiment at different temperatures and pool the data.

A colorimeter could be used to measure progress of the reaction. The following changes would need to be made to the method.

1. Leave all six tubes in the water bath for 10 minutes. While you are waiting set up a colorimeter. Use the trypsin solution as a blank to calibrate the colorimeter to zero absorbance.
2. Add the trypsin and buffer solution from one test tube to the solution of milk powder in another test tube.
3. Put a bung or cork in the test tube and invert about 5 times to mix thoroughly.
4. Put the test tube back into the water bath. Time the reaction for **exactly** 4 minutes. Pour the contents of the tube into a cuvette and measure the absorbance **immediately**.
5. Repeat steps 6, 7 and 8 using the other test tubes you set up.
6. Record the absorbance for each of the three experiments.

**The lower the absorbance reading the more casein has been broken down.**

**This experiment also allows students to do other investigations where they can choose variables such as pH and concentration of trypsin.**

## RISK ASSESSMENT

Risk assessment and risk management are the responsibility of the centre.

## TRIALLING

The practical should be trialled before use with students.

## ADDITIONAL NOTES

- Enzymes, particularly proteases (such as trypsin) can produce allergic reactions in sensitive people. The proteases also break down proteins in the skin and eyes. Care to avoid spillages, eye protection should be worn, and wash off any splashes to skin immediately. Hazcard 33 gives the hazards, risks and control measures for the concentrate and solid and also dilute solutions used by students.
- Water temperatures higher than 50 °C can cause scalding. Take care with hot water baths.
- The enzymes and milk powder should be made immediately before the lesson as trypsin quickly deteriorates when stored.
- If pH 7 buffer solution is purchased instead of being made from tablets then the colourless solution must be used. Some suppliers dye their buffer solutions so check before ordering.
- Mix the solutions gently by inverting the tubes to avoid bubbles and frothing if too vigorously shaken. Bungs or corks should be made available for this.
- Tubes should be inverted about 5 times.
- The time for the cross to become visible is subjective and depends on the person viewing.

## SAMPLE RESULTS

Temperature / °C	Mean time to clear / s	Mean absorbance
25	189	1.71
40	79	0.17
50	108	0.39
60	271	1.10

# INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 1

**Investigation into the effect of temperature, pH or substrate concentration on the rate of an enzyme-controlled reaction:**

**The effect of temperature on the rate of the reaction catalysed by trypsin.**

## STUDENT SHEET

Casein is a protein found in milk. Trypsin is an enzyme that digests casein. When trypsin is added to a dilute solution of milk powder, the casein is digested and the solution goes clear.

### MATERIALS

You are provided with the following:

- 0.5% trypsin solution
- 3% solution of milk powder
- pH 7 buffer solution
- a large beaker to use as a water bath
- test tubes
- bungs or cork for test tubes
- test-tube rack
- stopwatch
- marker pen
- pipettes or syringes
- thermometer

You are required to find the rate of reaction at **five** different temperatures. Your teacher will tell you whether you are going to investigate all the temperatures yourself or whether you will get some results from other students in your class.

### METHOD

You should read these instructions carefully before you start work.

1. Using a marker pen write an 'X' on the glass halfway down one side of each of three test tubes.
2. Add 10 cm<sup>3</sup> of the solution of milk powder to each of these three test tubes.
3. Add 2 cm<sup>3</sup> of trypsin solution to 2 cm<sup>3</sup> of pH 7 buffer in another set of three test tubes.
4. Stand the three test tubes containing the solution of milk powder and the three test tubes containing trypsin and buffer in a water bath at 20 °C.
5. Leave all six tubes in the water bath for 10 minutes.
6. Add the trypsin and buffer solution from one test tube to the solution of milk powder in another test tube.
7. Put a bung or cork in the test tube and invert about 5 times to mix thoroughly.
8. Put the test tube back into the water bath.



9. Repeat steps 6 and 7 using the other test tubes you set up.
10. Time how long it takes for the milk to go clear. Do this by measuring the time taken to first see the 'X' through the solution.
11. Record the time for each of the three experiments.
12. Using the same method, find out how long it takes the trypsin to digest the protein in the solution of milk powder at 30 °C, 40 °C, 50 °C, 60 °C.
13. Record your data in a suitable table.
14. Process your data and draw a graph of your processed data.

## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 2

**Investigation of the effect of solute concentration on the uptake or loss of water from plant tissue:**

**Determining the water potential of potato tuber cells.**

### TEACHERS' NOTES

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

- large potato tuber
- access to a potato chip cutter
- 1 mol dm<sup>-3</sup> sucrose solution
- distilled water
- boiling tube rack
- six boiling tubes
- thermometer
- 10 cm<sup>3</sup> graduated pipette and pipette filler
- White tile
- scalpel or small kitchen knife
- ruler
- paper towels
- timer
- access to a digital balance (3 decimal places if possible, but 2 decimal places will give adequate results)
- forceps
- access to an electric water bath set at 30 °C or large beaker to use as a water bath.

This investigation can be changed to allow students more freedom to select variables for themselves eg the concentrations used, size of potato chip, length of time in solution etc.

The experiment also works with sodium chloride as the solute and other plant material can be used.

10 ml or 20 ml syringes can be used in place of graduated pipettes as some students struggle to use these accurately and with pipette fillers. Small plugs of cotton wool in the top of the pipette can prevent the salt/sugar solution from getting into the pipette filler.

A potato chip cutter is used to prevent wastage and to ensure constant cross-sectional area of chip. The chippers are easily available from hardware stores.

The chips have been left intact to speed up the weighing process. However students could increase surface area by slicing the chips.

A class set of chips can also be easily prepared with a potato chip cutter and the pupils can select chips to work with. If this is done, leave the skin on the potato as this can be the students' responsibility to remove during the measuring process.

Cork borers can also be used to produce the chips. If using cork borers, then size numbers 4, 5 or 6 are most suitable. The teacher should ensure that each pupil only has access to one size of borer to reduce the problems when the chips are made. The teacher can also consider whether scalpels are the most appropriate instrument to cut potatoes, rather than small kitchen knives (if

available). If scalpels are used the teacher should demonstrate safe use and supervise the activity closely. Similar care should be taken when cutting plant tissue using cork borers.

A water bath is used to speed up the diffusion of water in and out of the potato tissue. Good results can be obtained at 30 °C within 20 minutes although if time allows 30 minutes would be better.

### Risk assessment

Risk assessment and risk management are the responsibility of the centre.

### Trialling

The practical should be trialled before use with students.

### SAMPLE RESULTS FOR SUCROSE

Sucrose concentration / mol dm <sup>-3</sup>	Initial mass / g	Final mass / g	Change in mass / g	Percentage change
0.0	6.54	6.81	0.27	4.13
0.2	6.57	6.63	0.06	0.91
0.4	6.46	6.17	-0.35	-5.42
0.6	6.41	5.96	-0.45	-7.02
0.8	6.21	5.57	-0.64	-10.30
1.0	6.33	5.53	-0.80	-12.60

## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 2

**Investigation of the effect of solute concentration on the uptake or loss of water from plant tissue:**

**Determining the water potential of potato tuber cells.**

### STUDENT SHEET

#### MATERIALS

You are provided with the following:

- large potato tuber
- potato chip cutter
- 1 mol dm<sup>-3</sup> sucrose solution
- distilled water
- boiling tube rack
- six boiling tubes,
- marker pen
- thermometer
- 10 cm<sup>3</sup> graduated pipette and pipette filler
- White tile
- scalpel or small kitchen knife
- ruler
- paper towels
- timer
- digital balance
- forceps.

#### PREPARING THE DILUTION SERIES

You should read these instructions carefully before you start work.

1. Label six boiling tubes 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mol dm<sup>-3</sup> sucrose.
2. Use the 1.0 mol dm<sup>-3</sup> sucrose solution and water to make up 20 cm<sup>3</sup> of sucrose solution of each of the following concentrations:
  - 0.2 mol dm<sup>-3</sup>
  - 0.4 mol dm<sup>-3</sup>
  - 0.6 mol dm<sup>-3</sup>
  - 0.8 mol dm<sup>-3</sup>
  - 1.0 mol dm<sup>-3</sup>

Complete **Table 1** to show the volumes of 1.0 mol dm<sup>-3</sup> sucrose solution and water that you used to make up each concentration.

3. Stand the boiling tubes containing the sucrose solutions in a water bath set at 30 °C. Use a thermometer to check the temperatures in all tubes reaches 30 °C.

4. Using the potato chip cutter, cut six chips from your potato tuber. Make sure you remove any peel on the potatoes. Use a ruler, scalpel and tile to cut all of the chips to the same length. Blot the potato chips dry with a paper towel, ie roll each chip until it no longer wets the paper towel and dab each end until dry. **Do not squeeze the chips**. Put each potato chip onto a clean square of paper towel which you have numbered in the same way as the boiling tubes.
5. Weigh each potato chip. Record these initial masses in a suitable table.
6. At the water bath, set the stop clock to zero. Quickly transfer each potato chip from its square of paper towel to its own boiling tube with the same number.
7. After 20 minutes, remove the chips from the boiling tubes. Blot the chips dry, as before. Then reweigh them. Record these final masses in your table.
8. Calculate the change in mass and then calculate the percentage change in mass.
9. Plot a graph of your processed data and use this to determine the concentration of sucrose which has the same water potential as the potato tuber cells.

**Table 1**

Concentration of sucrose solution / mol dm <sup>-3</sup>	<b>0</b>	<b>0.2</b>	<b>0.4</b>	<b>0.6</b>	<b>0.8</b>	<b>1.0</b>
Volume of 1.0 mol dm <sup>-3</sup> sucrose solution / cm <sup>3</sup>	<b>0</b>					<b>20</b>
Volume of water / cm <sup>3</sup>	<b>20</b>					<b>0</b>

## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 3

**Use of chromatography to investigate the pigments present in leaves:  
An investigation of pigments present in leaves eg leaves from shade tolerant and shade intolerant plants or leaves of different colours.**

### TEACHERS' NOTES

#### MATERIALS

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

- boiling-tube rack
- two boiling tubes with bungs
- 10 cm<sup>3</sup> glass measuring cylinder
- 10 cm<sup>3</sup> solvent
- chromatography paper cut to size to fit the boiling tubes as shown in the diagram. The paper must not touch the sides of the tube. Good quality filter paper can be used but chromatography paper gives better results.
- glass rod to crush leaf tissue into the paper
- two leaves, A and B. These can be different colours or from shade-tolerant and shade-intolerant plants. Autumn leaves can be used to give different colours. The best results come from leaves with a thin cuticle.
- cork borer – or a hole punch could be used to produce the leaf discs
- tile on which to use cork borer
- ruler with millimetre measurements
- pencil
- drawing pins
- marker pen
- sticky tape.

#### TECHNICAL INFORMATION

**Solvent:** Propanone: petroleum ether (b.p. 100–120 °C) in ratio of 1:9. This should be supplied to students in a stoppered bottle and labelled 'Solvent'.

- Hazcard 85A relates to propanone, highly flammable and causes serious eye irritation (may cause drowsiness).
- Hazcard 45A relates to Petroleum ether, highly flammable, dangerous to the environment.
- Ensure good ventilation in laboratory, no naked flames and wear eye protection. For disposal see Hazcards.

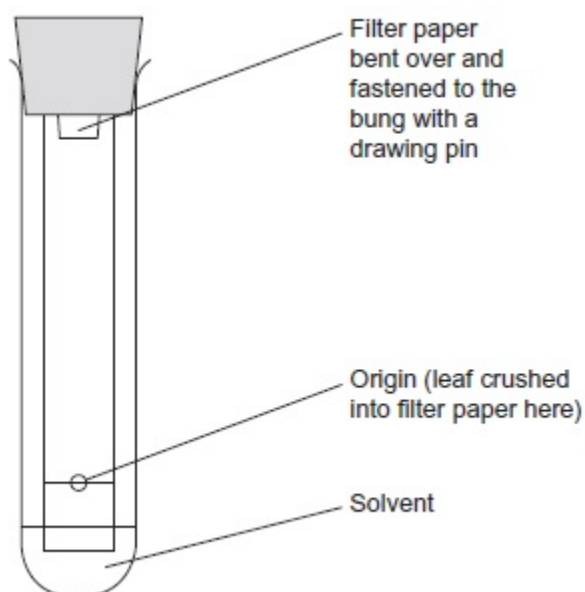
The method given is simple and works well. It avoids the need to grind leaves to extract pigment.

You might want to consider thin layer chromatography instead ([CLEAPSS video](#)). The safety issues with thin layer chromatography are considerably less due to the much smaller scale.

The chromatograms will fade very quickly, particularly in the light. It is advisable to mark the spots immediately to calculate the  $R_f$  values.

Chromatography paper is better provided pre-cut to prevent over-handling by students. It secures well with a drawing pin to either a cork or a rubber bung.

In trials, a red-leaved beet and a spinach leaf were used. These cut well with a number 5 cork borer.



### **RISK ASSESSMENT**

Risk assessment and risk management are the responsibility of the centre.

### **TRIALLING**

The practical should be trialled before use with students.

## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 3

**Use of chromatography to investigate the pigments present in leaves:  
An investigation of pigments present in leaves eg leaves from shade tolerant and shade intolerant plants or leaves of different colours.**

### STUDENT SHEET

In plants, chlorophyll is the main pigment that absorbs light during photosynthesis. Most plants have other photosynthetic pigments as well and these are not green.

You will be using a technique called chromatography to separate chlorophyll and other pigments from two different leaves, A and B.

### MATERIALS

You are provided with the following:

- boiling-tube rack
- two boiling tubes with bungs
- small glass measuring cylinder
- solvent
- chromatography paper
- glass rod
- two leaves, A and B
- cork borer
- tile on which to use cork borer
- ruler
- pencil
- drawing pins
- marker pen
- sticky tape.

### SAFETY

Wear eye protection and work in a well-ventilated room or fume cupboard.

### METHOD

You should read these instructions carefully before you start work.

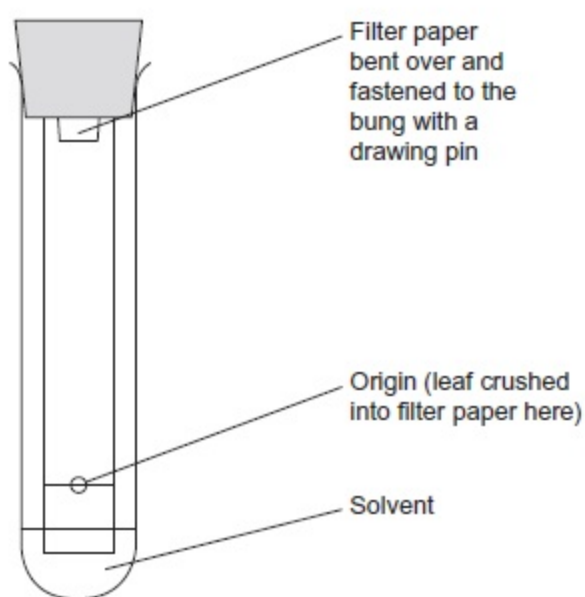
1. Set up two boiling tubes at the start of the investigation. Add 3 cm<sup>3</sup> of solvent to each of the two boiling tubes. Put a bung in the top of each tube and stand them upright in a rack. Label the tubes A and B.
2. Take a piece of chromatography paper that fits into the boiling tube, as shown in the diagram. Rule a pencil line 2 cm from the bottom of the filter paper. This line is called the origin.

Write leaf A at the top of the chromatography paper in pencil.



- Cut a disc from leaf A with a cork borer. Avoid the veins and midrib of the leaf when you do this.
- Place the leaf disc on the chromatography paper at the centre of the line marking the origin. Crush the disc into the paper with the end of a glass rod. The crushed leaf disc should leave a stain on the chromatography paper.
- Pin the chromatography paper to the bung with a drawing pin, and then put the chromatography paper into the tube labelled A as shown in **Figure 1**. Make sure the end of the chromatography paper is in the solvent and that the solvent does not come above the origin. Put the tube carefully back into the rack and do not move it again.

**Figure 1**



- Let the solvent run up the chromatography paper until it almost reaches the top of the paper. Remove the chromatography paper from the tube and immediately draw a pencil line to show how far the solvent moved up the paper. This line marks the solvent front.
- Replace the bung in the tube.
- The filter paper with its coloured spots is called a chromatogram. Let the chromatogram dry. Using a pencil, draw round each coloured spot on the chromatogram.
- Repeat step 2 with the second piece of paper but write B at the top of the chromatography paper.
- Repeat steps 3–8 with leaf B.

Calculate the R<sub>f</sub> value for each of the pigment spots on each chromatogram.

$$\text{Rf value} = \frac{\text{Distance moved by pigment from origin to centre of pigment spot}}{\text{Distance from origin to solvent front}}$$

## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 4

**Preparation of stained squashes of root tips and examination of these with a microscope.  
Observation of the stages of mitosis and calculation of a mitotic index:  
Root tip squash using onion root meristem tissue.**

### TEACHERS' NOTES

#### MATERIALS

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

- 100 ml beaker
- hydrochloric acid ( $5 \text{ mol dm}^{-3}$ )
- microscope slide and cover slip
- toluidene blue stain
- filter paper
- mounted needle
- scalpel
- distilled water
- watch glass
- forceps
- root tip of onion or garlic
- microscope and light source
- piece of white paper
- paper towel.

#### TECHNICAL INFORMATION

##### Reagents:

- 5 M hydrochloric acid (10 ml per student)
- toluidene blue (0.05%) at pH 4; made in McIlvaine buffer; keep in fridge

Buffer formulae

Citric acid  $0.1 \text{ M}$   $21 \text{ g dm}^{-3}$ ;  $61.45 \text{ cm}^3$

$\text{Na}_2\text{HPO}_4$   $0.2 \text{ M}$   $35 \text{ g dm}^{-3}$ ;  $38.55 \text{ cm}^3$

Alternatively use pH buffer tablets to make up buffer.

**Root tip of onion, garlic or shallot** – prepare 1 to 2 weeks in advance. Stand on top of a McCartney bottle full of water in a dark cupboard until roots are about 5 cm long. Prepare plenty as some may not produce many roots.

Organic or home grown garlic, onions or shallots produce the best root systems in a few days. Store bought produce will grow but can take up to 14 days and tend to produce fewer usable roots.

In this investigation each student will need to prepare a microscope slide of the meristem tissue from an onion root. They will add toluidene blue stain to the material which allows them to see the chromosomes. They will look at the slide under the microscope to identify any cells showing stages of mitosis. They will then calculate the mitotic index.

Good quality microscopes with adjustable lighting make it easier to get good mitotic cells in view but any microscope with a  $\times 40$  objective or similar is satisfactory as a good contrast is obtained using the toluidine blue.

### RISK ASSESSMENT

Risk assessment and risk management are the responsibility of the centre.

### TRIALLING

The practical should be trialled before use with students.

### SAFETY

Take care when making the  $5 \text{ mol dm}^{-3}$  hydrochloric acid and consult the Hazcard. Work in a fume cupboard as fumes are released rapidly at dilution when being prepared from concentrated solution.

Hydrochloric acid is corrosive and should be handled with caution.

It is essential to use very concentrated acid to ensure the cells die quickly.

You may wish to dispense the acid to students once they have the beaker on a bench mat to avoid students walking around the room with such concentrated acid.

It is also advisable to collect the beakers of acid directly from the students when they have finished in order to avoid any  $5 \text{ mol dm}^{-3}$  hydrochloric acid being poured down the sinks and not washed away thoroughly. A tray at the front of the room clearly labeled for used beakers of acid should help with the clearing away and make the technicians aware of the acid in the beakers.

### ADDITIONAL NOTES

Do not leave root tips for investigation lying about on the bench top prior to staining. Cut the root tip immediately before you put it into the acid. This will stop any reactions and hopefully some cells will be in the stage of division.

It is a good idea to put the roots into the acid for the students. The reason being that it is very difficult to tell which roots on the onion have already had the tips removed.

**Toluidene blue** is used as the stain in this investigation as it gives reliable results and does not require any heating of the slide to make the chromosomes visible. Other stains are available but you should check if heating is required.

Nucleic material stains blue and the other material lilac. If the stain is fresh then good colour definition is obtained. The stain also stores well in the fridge for several weeks after making.

It would be advisable for students to have seen prepared slides of root tips before carrying out the practical. One problem students might have is preparing a slide which does not contain meristematic cells – they need to know that cells behind the meristem elongate and are no longer dividing. As it is easy to confuse the two ends of the piece of root get students to mount both ends of the piece of root. Once under the microscope it is easy to tell which cells are from the root tip meristem. These cells are small and square with the nucleus in the centre. The other end will have elongated cells with the nucleus off centre.

It would be a good idea to have some pre-prepared slides available as students may not be successful in preparing a slide that contains cells with stages of mitosis and so would not be able to calculate the mitotic index.

Mitotic index = Number of cells in stages of mitosis  $\div$  total number of cells.

## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 4

**Preparation of stained squashes of root tips and examination of these with a microscope.  
Observation of the stages of mitosis and calculation of a mitotic index:  
Root tip squash using onion root meristem tissue.**

### STUDENT SHEET

#### MATERIALS

You are provided with the following:

- 100 ml beaker
- hydrochloric acid ( $5 \text{ mol dm}^{-3}$ )
- microscope slide and cover slip
- toluidene blue stain
- filter paper
- mounted needle
- scalpel
- distilled water
- watch glass
- forceps
- root tip of onion or garlic
- piece of white paper
- paper towel
- microscope and light source.

You are required to prepare a microscope slide of the meristem tissue from an onion root. You will add a stain to the material which allows you to see the chromosomes. You will look at the slide under the microscope to identify any cells showing stages of mitosis. You will then calculate the mitotic index.

#### SAFETY

Hydrochloric acid ( $5 \text{ mol dm}^{-3}$ ) is corrosive and you should handle it with caution. You must wear eye protection.

You must stand the beaker on a bench mat. Do not carry the beaker with acid in it.

**Do not leave root tips for investigation lying about on the bench top prior to staining. Cut your root tip immediately before you put it into the acid. This will stop any reactions and hopefully some cells will be in a stage of division.**

## METHOD

**You should read these instructions carefully before you start work.**

1. Stand the beaker on a bench mat before adding approximately 10 ml of hydrochloric acid ( $5 \text{ mol dm}^{-3}$ ). Put some paper towel on the bench mat and label.
2. Place about 2 cm of root tip in the acid and leave for 15 minutes.
3. Set up your microscope while you are waiting.
4. Rinse the root tip in distilled water in the watch glass.
5. Cut off the root tip (1 mm) and place on a microscope slide.
6. Cover the section with toluidene blue stain and macerate with the mounted needle to separate the cells. Use a piece of white paper to aid colouration of roots.
7. Continue to macerate until the tissue is well broken and the cells are stained dark blue.
8. Add a cover slip and with gentle finger pressure 'spread' the material and blot at the same time by using a folded filter paper between finger and slide.
9. Look carefully at all slides for cells undergoing mitosis. Chromosomes should stain dark blue. Repeat for several fields of view.
10. Record your data in a suitable table.
11. Calculate the mitotic index.

## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 5

**Investigation into the effect of a specific variable on human heart rate or pulse rate:  
The effect of exercise on pulse rate.**

### TEACHERS' NOTES

#### **MATERIALS**

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

- a stop watch or timer.

In this investigation the student is required to count the pulse in the radial artery to determine resting pulse rate, then exercise, then find the pulse rate over the next 5 minutes.

The exercise should be based on repeatedly squatting while holding the edge of a table or laboratory bench. Alternative arrangements should be made for students who are physically unable to carry out this exercise.

This investigation can easily be adapted to so candidates can investigate other variables such as length of exercise or body position.

#### **RISK ASSESSMENT**

Risk assessment and risk management are the responsibility of the centre.

#### **TRIALLING**

The practical should be trialled before use with students.

## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 5

**Investigation into the effect of a specific variable on human heart rate or pulse rate:  
The effect of exercise on pulse rate.**

### STUDENT SHEET

In this investigation, you will investigate the effect of exercise on the pulse rate measured in your wrist.

#### **METHOD**

You are provided with the following:

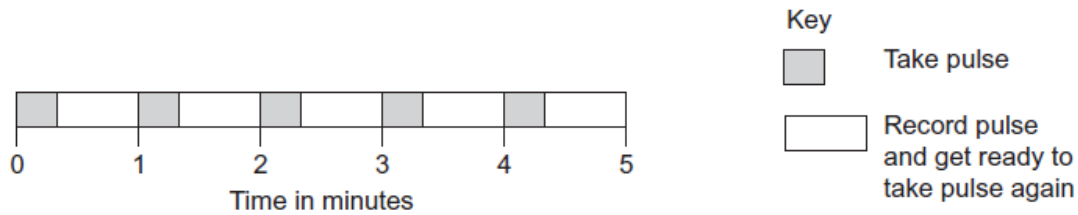
- a stop watch or timer.

You should read these instructions carefully before you start work.

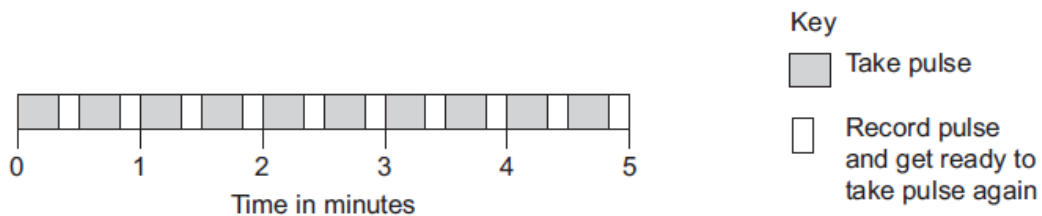
1. Sit down and rest for 10 minutes. You can use this time to draw a table for your results.
2. Remain seated and find the pulse in your wrist by putting the tip of your first finger on the inside of your wrist as shown in the photograph. If you cannot find your pulse, you may ask your teacher for help.



3. Take your pulse for 20 seconds at 1 minute intervals for 5 minutes. The sequence of events is shown in the diagram.



4. Exercise by holding the edge of a table or laboratory bench and repeatedly squatting down then standing up again. You must decide for yourself for how long to continue exercising but it should not be longer than 5 minutes.
5. Immediately after you have stopped exercising, take your pulse for 20 seconds at 30 second intervals for 5 minutes. The sequence of events is shown in the diagram.



6. Record the results of your investigation in a suitable table.
7. Process your data and draw a graph of your processed data.



## INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 6

**Investigation of the rate of water uptake by means of a simple photometer.**

### TEACHERS' NOTES

#### MATERIALS

In addition to access to general laboratory equipment, each pair of students will need access to:

- secateurs
- washing-up bowl
- leafy shoot
- potometer
- 100 cm<sup>3</sup> beaker
- vaseline
- stand, boss and clamp
- paper towels
- stop watch

The potometer used in this experiment is simple to construct and is easier to set up than a Ganong potometer.

Key factors to ensure success are:

- woody shoots with plenty of large leaves (do not use leaves with a waxy cuticle)
- width of shoot should fit tightly into plastic tubing
- tightly fitting tubing around the 3-way tap.

It is recommended that the teacher or technician demonstrates the technique required to set up the potometer.

If there is sufficient time then students could investigate different conditions (for example, using a fan to create air movement).

#### RISK ASSESSMENT

Risk assessment and risk management are the responsibility of the centre.

#### TRIALLING

The practical should be trialled before use with students.

# INTERNATIONAL AS AND A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 6

**Investigation of the rate of water uptake by means of a simple potometer.**

## STUDENT SHEET

### TAKING MEASUREMENTS WITH THE POTOMETER

The potometer is mainly used to measure the rate of water uptake by a cut shoot under different conditions. As the cut shoot takes up water from the glass tube so air is pulled into the capillary tube from its open end. A water meniscus marks the end of the water and the start of the air in the capillary tube. The position of the meniscus is measured at set time intervals, for example every 3 minutes, against the scale. The rate of movement of the meniscus can be measured as cm per minute ( $\text{cm min}^{-1}$ )

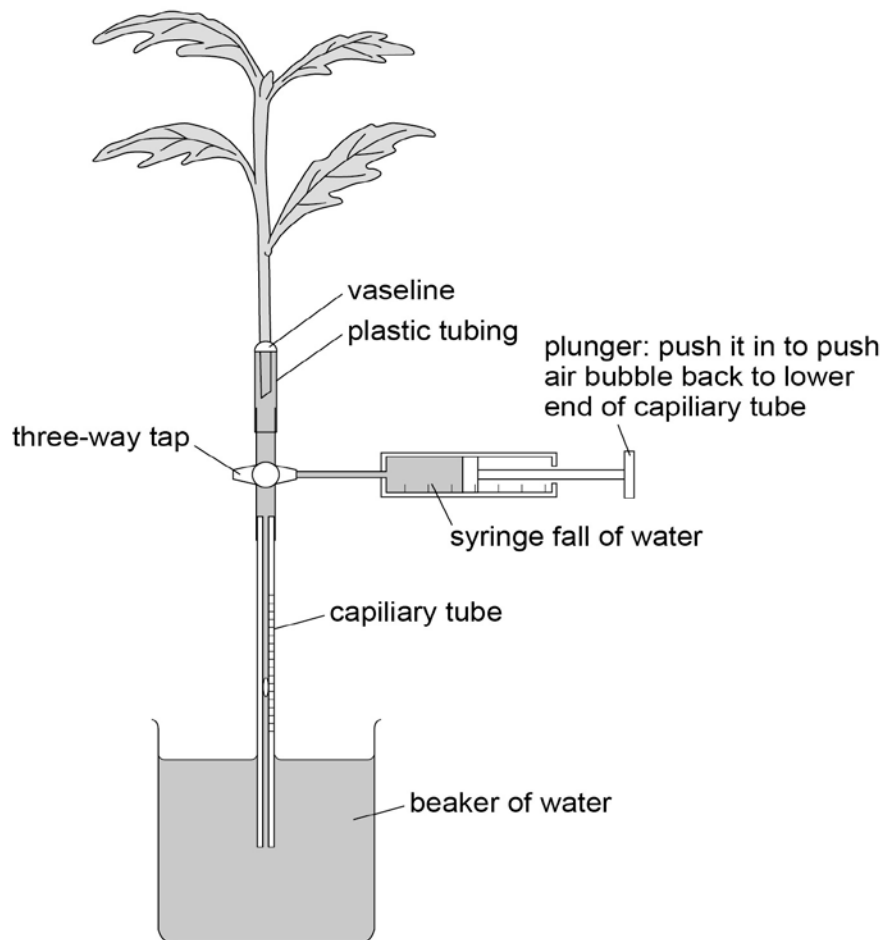
### SAFETY

Take care while using the secateurs when cutting the leafy shoot. Safety goggles will help to prevent eye damage.

### MATERIALS

You are provided with the following:

- secateurs
- washing-up bowl
- leafy shoot
- potometer
- 100  $\text{cm}^3$  beaker
- vaseline
- stand, boss and clamp
- paper towels
- stop watch



## METHOD

1. In the lab, fill a washing up bowl with water.
2. Use secateurs to cut a large leafy shoot. Put the base of the shoot immediately into the bowl of water and then use the secateurs again to make another cut about 5 cm or so above the first, under the water. (This is because the water in the xylem is usually under tension. After the first cut, air would have entered the xylem vessels at the cut. The second cut is to remove the base of the stem where the xylem is filled with air.)
3. Submerge the potometer completely in the water, with the 3-way tap open. Make sure that all air bubbles are removed from the apparatus. You can use the syringe to force water through the apparatus to ensure all air has been displaced. Make sure that the plastic tubing connection for the stem is completely filled with water. Close the 3-way tap to the syringe.
4. (Steps 4 to 6 ideally need 2 people.) **Do not wet the leaves of the shoot.** Use the secateurs to re-cut the base of the stem in water at a point on the stem where it is slightly wider than the rubber tubing. You'll need a shoot with about 6–12 leaves on it.
5. Push the cut stem firmly into the rubber connection of the potometer, under water. Lift the potometer out of the bowl and use a clamp, boss and stand to hold the cut shoot steady. Vaseline the connection between the rubber tubing and the stem.
6. Use a paper towel to gently dry the capillary tubing. Meanwhile check that the meniscus is moving towards the plant.

7. Use a thermometer to record the temperature outside the plant. Record the position of the meniscus at time 0 minutes, as you start the stop clock and then every 3 minutes for (say) 12 minutes altogether. Do not stop the clock after each set of 3 minutes.
8. Record the results of your investigation in a suitable table.
9. Process your data and draw a graph of your processed data.
10. If time allows you could measure the rate of water uptake under a different environmental condition.

## INTERNATIONAL A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 7

**Investigation of the effect of a specific limiting factor on the rate of photosynthesis:  
The effect of light intensity on the rate of photosynthesis.**

### TEACHERS' NOTES

#### MATERIALS

In addition to access to general laboratory equipment, each student will need:

- aquatic plant ( at least 10 cm long)
- 1% sodium hydrogen carbonate solution ( enough to fill a boiling tube)
- boiling tube
- test tube rack
- ruler
- lamp
- stopwatch or timer
- scissors
- glass rod
- forceps

Either Cabomba or Elodea could be used as the aquatic plant in this investigation. It has been found that Cabomba is much more reliable and bubbles more quickly and is therefore recommended. If Elodea is used, it is suggested that the plant is placed in a beaker of dilute sodium hydrogen carbonate in front of a lamp for 2-3 hours before starting the investigation. Cabomba should be kept in a well-lit, aerated tank. In the laboratory its photosynthetic ability decreases with time, so fresh material should be used for the investigation.

Other aquatic plants could be used but should be trialled to ensure results can be obtained in the time available.

You should trial the type of lamp being used as some energy saving bulbs do not give particularly good results.

#### RISK ASSESSMENT

Risk assessment and risk management are the responsibility of the centre.

#### TRIALLING

The practical should be trialled before use with students.

# INTERNATIONAL A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 7

**Investigation of the effect of a specific limiting factor on the rate of photosynthesis:  
The effect of light intensity on the rate of photosynthesis.**

## STUDENT SHEET

### INTRODUCTION

When placed in water and exposed to light of an appropriate intensity, an aquatic plant produces bubbles of gas. The rate of bubbling can be used as a measure of the rate of photosynthesis. You will investigate photosynthesis of an aquatic plant and find out if light intensity and rate of photosynthesis are correlated. Light intensity decreases as the distance between the lamp and plant increases.

### MATERIALS

You are provided with the following:

- aquatic plant
- 1% sodium hydrogen carbonate solution
- boiling tube
- test tube rack
- ruler
- lamp
- stopwatch or timer
- scissors
- glass rod
- forceps

### METHOD

You should read these instructions carefully before you start work.

1. Fill the boiling tube to the top with 1% sodium hydrogen carbonate solution.
2. Take a piece of the aquatic plant about 5 cm long and push it into the tube until the cut end is just below the surface of the sodium hydrogen carbonate solution. Use the scissors to cut the stem at an angle. Make sure that the stem stays below the surface of the solution (**Figure 1**).

Figure 1

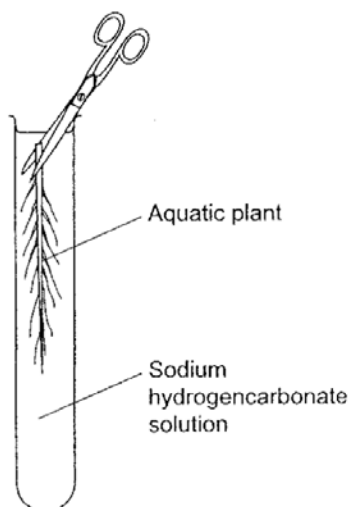
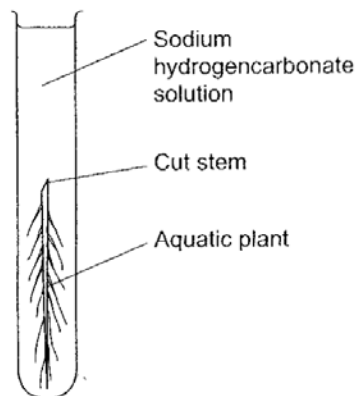


Figure 2



- Use the glass rod to push the plant gently into the tube. The cut end of the stem should be at the top and about half way down the tube as shown in **Figure 2**.
- Place the tube in the test tube rack 10 cm away from the bulb of the lamp.
- Leave the plant for 5 minutes and then count the number of bubbles rising from the cut end of the stem in 1 minute.
- Repeat your counts as many times as necessary.
- Take additional readings at appropriate distances from the lamp to find out if there is a correlation between light intensity and the rate of photosynthesis. The distance of the plant from the bulb should be within the range 5 to 30 cm.
- The distances from the lamp can be converted into a measure of light intensity by calculating  $\frac{1}{d^2}$  where  $d$  = distance from plant to lamp.
- Record the results of your investigation in a suitable table.
- Process your data and use a suitable statistical test to investigate the correlation between light intensity and rate of photosynthesis.

## INTERNATIONAL A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 8

**Investigation of a specific variable such as substrate or temperature on the rate of respiration of a suitable organism such as yeast or locust:  
An investigation of the effect of temperature on respiration in yeast.**

### TEACHERS' NOTES

This investigation is based on BIO6T/Q12

### MATERIALS

In addition to access to general laboratory equipment, each student will need:

- yeast and glucose mixture
- methylene blue
- test tubes
- test-tube rack
- beaker to act as water bath
- a way of changing the temperature of the water bath eg Bunsen burner or supplies of hot or cold water. The experiment needs to be in a glass beaker so colour change can be observed so an electric water bath is not really suitable.
- 2 cm<sup>3</sup> graduated pipettes or syringes
- marker pen
- thermometer
- timer.

### TECHNICAL INFORMATION

Make up a solution of 1 g glucose in 100 cm<sup>3</sup> water. Just before use, raise the temperature of this solution to 30 °C and add 5 g dried yeast. Shake to suspend the yeast in the glucose solution. Use an open-topped flask or beaker as bubbling will occur (if bubbling does not occur, check that the yeast used is not old).

The yeast must be checked as it needs to be active. Freshly-purchased dried yeast was used in trials as yeast that was already in stock did not give good results. The yeast needs to be fresh and well fermenting at the start of the experiment.



### **METHYLENE BLUE SOLUTION**

Make a stock solution of 1 g methylene blue and 0.6 g sodium chloride dissolved in 100 cm<sup>3</sup> water. For the solution to be used in the investigation, take 0.1 cm<sup>3</sup> of the stock solution and add it to 100 cm<sup>3</sup> water.

Decolourisation of the methylene blue should occur within approximately 5 minutes when the experiment is conducted at 35 °C. If it is taking longer than this the methylene blue solution can be diluted further. In some trials, the methylene blue had to be diluted further (1:1) in order to get results within 5 minutes.

The stock solution of methylene blue should be prepared in advance as the concentration means the methylene blue takes a while to fully dissolve. It can be diluted to the working concentration on the day of use.

### **ADDITIONAL INFORMATION**

An alternative method includes a different recipe for the yeast. To 250 cm<sup>3</sup> of tap water, add 25 g of yeast and 12.5 g of glucose and leave this for 24 hours in a 25 °C water bath. The methylene blue used in this method should be at 0.005% and 5 cm<sup>3</sup> yeast and 1 cm<sup>3</sup> methylene blue should be added to each test tube.

Other suggested alternatives are to use Janus green (diazine green) in place of methylene blue. The indicator reduces to a pink colour when all the oxygen is used up instead of decolourising.

The amount of indicator needed is less critical. All that is needed is to make initial yeast solution a noticeable blue/green colour.

### **RISK ASSESSMENT**

Risk assessment and risk management are the responsibility of the centre.

### **TRIALLING**

The practical should be trialled before use with students.

It is advised to trial this experiment using the batch of yeast and methylene blue that will be used by students as yeast activity can vary considerably. Trials need to establish the safest range of temperatures that is effective. It is not necessary to use very high temperatures to get results as it is not the aim of the experiment to find the optimum temperature.

This method for investigating rate of respiration in single-celled organisms is simple and needs little apparatus. Rate of anaerobic respiration can be investigated using a respirometer if this apparatus is available.

### SAMPLE RESULTS

Mean decolorisation times during trials were:

Temperature / °C	Mean decolourisation time / s
20	275
35	128
45	145

## INTERNATIONAL A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 8

**Investigation of a specific variable such as substrate or temperature on the rate of respiration of a suitable organism such as yeast or locust:  
An investigation of the effect of temperature on respiration in yeast.**

### STUDENT SHEET

Yeast is a single-celled fungus. It can respire aerobically and anaerobically. During aerobic respiration, the transport of electrons is linked to the synthesis of ATP. In this investigation, these electrons will be taken up by a substance called methylene blue. When methylene blue is reduced, it changes from blue to colourless.

#### MATERIALS

You are provided with the following:

- yeast and glucose mixture
- methylene blue
- test tubes
- test-tube rack
- beaker to act as water bath
- a way of changing the temperature of the water bath
- graduated pipettes or syringes
- marker pen
- thermometer
- timer.

#### METHOD

You should read these instructions carefully before you start your investigation.

1. Use the beaker to set up a water bath at 35 °C.
2. Label five test tubes 1 to 5.
3. Shake the yeast and glucose mixture.
4. Add 2 cm<sup>3</sup> of the yeast and glucose mixture to all five tubes.
5. Place all five tubes in the water bath and leave them until their contents reach 35 °C. Make sure the water bath stays at 35 °C
6. Add 2 cm<sup>3</sup> methylene blue to test tube 1.
7. Immediately shake this tube for 10 seconds and replace the tube in the water bath. Note the time and do not shake this tube again.
8. Record how long it takes for the blue colour to disappear in the tube.
9. Repeat steps 6 to 8 for the other four tubes.
10. Your teacher will tell you which other temperatures to use. Repeat steps 1 to 9 at each temperature.

## INTERNATIONAL A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 9

**A laboratory based investigation of the effect of competition on seedling growth.**

### TEACHERS' NOTES

This investigation is looking at intraspecific competition between seedlings for space and water.

### MATERIALS

In addition to access to general laboratory equipment, each student will require the following:

- 5 Petri dishes
- black marker pen/ chinagraph pencil and ruler
- filter papers to fit base of Petri dishes (these should fit the base of exactly)
- seeds – lettuce, cress or radish or other suitably fast germinating species.
- boiled water
- forceps

This is a simple experiment but problems can occur due to moulds growing on the seeds and the dishes drying out.

To avoid moulds the Petri dishes should be clean/sterile. The seeds can be rinsed in a very dilute bleach solution before being given to the students. It is suggested that boiled water is used to reduce contaminants.

It should be stressed to students not to handle the seeds and there should be minimal handling of the inside of Petri dishes and filter papers.

The Petri dishes can be stacked and placed in a dark cupboard. This eliminates competition for light. Ideally the temperature should be constant and not too high to prevent dehydration.

Centres should trial this experiment as there can be a wide variation in seeds, size of Petri dishes, volume of water etc.

### RISK ASSESSMENT

Risk assessment and risk management are the responsibility of the centre.

### TRIALLING

The practical should be trialled before use with students.

## INTERNATIONAL A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 9

**A laboratory based investigation of the effect of competition on seedling growth.**

### STUDENT SHEET

#### MATERIALS

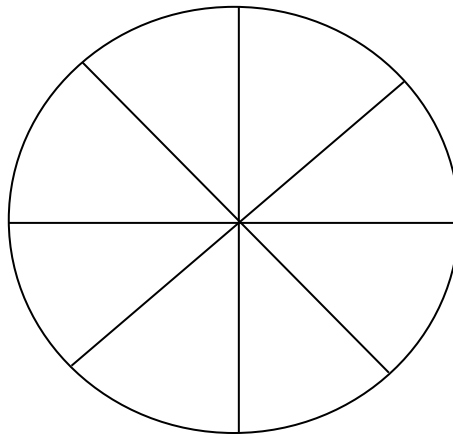
You are provided with the following:

- 5 Petri dishes
- black marker pen and ruler
- filter papers to fit base of Petri dishes
- seeds
- boiled water
- forceps

#### METHOD

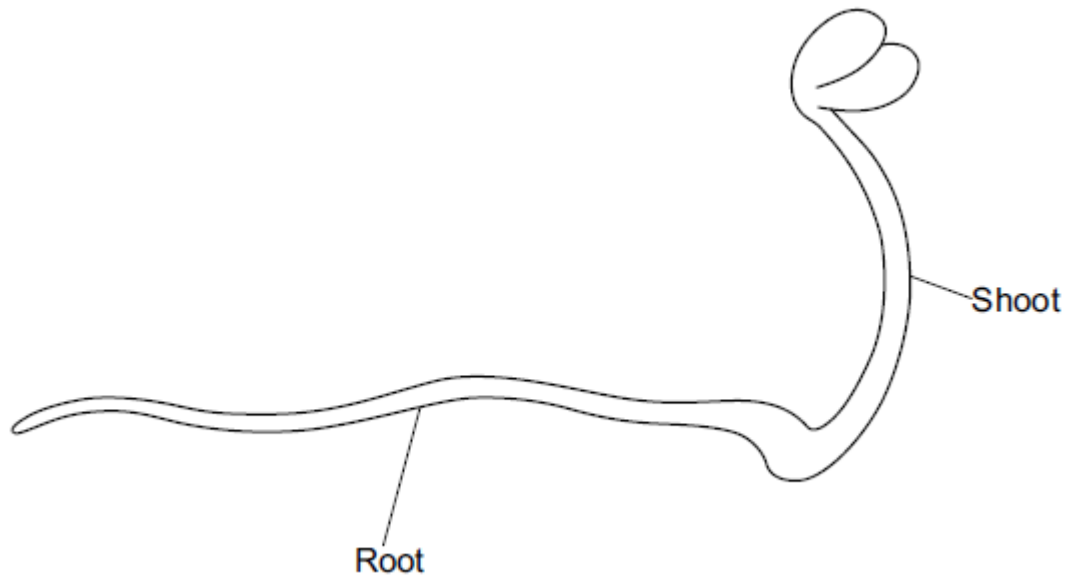
1. Turn the base of the Petri dish over and on the outside of the dish divide the area into segments using the marker pen (**Figure 1**). Do this for each dish.

**Figure 1**



2. Place a filter paper in each Petri dish base. Add the same amount of water to each dish. This should be enough to ensure the filter paper is wet but there should be no surface water.
3. In the first Petri dish place one seed in the middle of each segment. In the second Petri dish place two seeds in each segment. Continue to place seeds in the remaining dishes increasing the number of seeds in each segment each time, so the last dish has 5 seeds in each segment.
4. Place the lids on each Petri dish. Put all the dishes together in a dark box or cupboard.
5. Observe the seeds at the same time each day and record the number of seeds that have germinated.

6. After about 3 days remove the seedlings and measure the growth of shoot and root.



7. Record your results in a suitable table.
8. Process your data and carry out a statistical test to see if there is a significant difference in germination and initial growth in the seedlings at different densities of sowing.

## INTERNATIONAL A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 10

**Investigation of the effect of a suitable variable on the direction of growth of a root or a shoot:**

**Investigation into the effect of gravity on direction of root growth.**

### TEACHERS' NOTES

#### MATERIALS

In addition to access to general laboratory equipment, each student will require the following:

- Blotting paper, cut to fit the plastic panes
- Seeds – radish, lettuce, cress or other fast germinating species should be suitable.
- 6 CD 'jewel' cases ( alternatively you could use panes of glass from photo frames, clear plastic sheet protectors, or plastic bags)
- Rubber bands
- permanent marker pen
- modelling clay or plasticine or blu-tack
- shallow pie plates or similar shallow dish
- water in dropper bottle or wash bottle
- ruler and protractor

Seed sandwiches 1 and 2, and 3 and 4 act as controls. Sandwiches 5 and 6 are rotated 90 degrees clockwise every 2 days to see the effect of gravity on direction of root growth.

If you use panes of glass to make the sandwiches loop rubber bands top to bottom and right to left around the panes to hold them together. If using plastic sheet protectors, trim the edges with scissors and use paper clips or staples to hold it together. To keep the plastic sheets up right pin or tape them to a vertical surface inside a cardboard box or to the wall of a dark cupboard where they will not be disturbed.

#### RISK ASSESSMENT

Risk assessment and risk management are the responsibility of the centre.

#### TRIALLING

The practical should be trialled before use with students.

## INTERNATIONAL A-LEVEL BIOLOGY EXEMPLAR FOR REQUIRED PRACTICAL 10

**Investigation of the effect of a suitable variable on the direction of growth of a root or a shoot:**

**Investigation into the effect of gravity on direction of root growth.**

### STUDENT SHEET

#### MATERIALS

You are provided with the following:

- Blotting paper, cut to fit the plastic CD jewel cases
- Seeds
- 6 CD 'jewel' cases
- Rubber bands
- permanent marker pen
- modelling clay
- shallow dishes
- water
- ruler and protractor

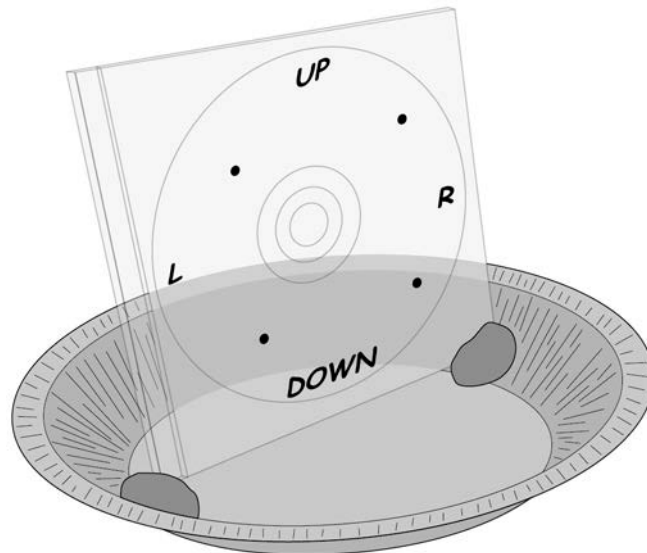
#### METHOD

1. Cut the blotting paper to fit into the CD jewel cases and place in the cases.
2. Moisten the blotting paper so that it is wet but there is no surface water.
3. Place four seeds in the middle of the moist paper, evenly spaced from each other. None of the seeds should be close to the sides of the paper. Close the CD case. Repeat for each CD case.
4. Label the backs of all six seed 'sandwiches' 1, 2, 3, 4, 5, and 6 with a permanent marker. Mark the four edges on the front side of each 'sandwich': Up, Down, Left and Right.



- Using the modelling clay to hold them in place, set two 'sandwiches' (1 and 2) upright, each in their own shallow dish, with Up on the top edge (**Figure 1**).

**Figure 1**



Seed sandwiches made in CD cases should look similar to this one. The modelling clay is used to keep the seed sandwich upright.

- Place two different sandwiches (3 and 4) horizontally (flat) in two more shallow dishes. These sandwiches lie flat so that the roots cannot grow in the direction of gravity. Use modelling clay under the sandwiches to lift them off the surface of the dish, so any water that is added can drip into the dish to dry.
- The final two seed sandwiches (5 and 6) should be set vertically in the modelling clay on two more shallow dishes with the label Up at the top. You will rotate these sandwiches 90° clockwise every 2 days to see how this changes the direction of the root growth.
- Put all of your seed sandwiches inside a dark cupboard or large, light-proof cardboard box where they will not be disturbed. This prevents the effect of light on developing plants from interfering with your observations on the effect of gravity.
- Check the seed sandwiches each day. Keep the seeds moist by adding water using a dropper, taking care not to dislodge the seeds.
- Once the roots start to grow measure the angle of growth daily with a protractor. Use the direction directly toward the bottom edge, 'Down' as the 0°; to the left as 90°; directly up as 180°; and to the right as 270°.
- Record your results in a suitable table.

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E: [science@oxfordaqaexams.org.uk](mailto:science@oxfordaqaexams.org.uk)



**OXFORD INTERNATIONAL AQA EXAMINATIONS**  
GREAT CLARENDON STREET, OXFORD, OX2 6DP  
UNITED KINGDOM

[enquiries@oxfordaqaexams.org.uk](mailto:enquiries@oxfordaqaexams.org.uk)  
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