

Practical Booklet 7
Measurement of gas exchange using a respirometer

Cambridge International AS & A Level Biology 9700



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# Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

- plan experiments and investigations
- 2. collect, record and present observations, measurements and estimates
- 3. analyse and interpret data to reach conclusions
- 4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

# Guidance for teachers

#### Aim

To determine the volume of oxygen consumed and the volume of carbon dioxide released by respiration. To use these volumes to calculate RQ for the organism tested. The procedure for measuring oxygen uptake is used as a model to develop a plan to find the optimum temperature for respiration.

#### **Outcomes**

Syllabus sections 12.1 (h) and 12.2 (m)

## Skills included in the practical

A Level skills	How learners develop the skills
Planning	Identify hazards and produce a simple risk assessment Modify a method to investigate a different independent variable
Analysis	Calculate volume using the appropriate formula Calculate RQ value Calculate rate of oxygen consumption
Evaluation	Evaluate the methods used and their effect on the accuracy and reliability of the results
Conclusions	Use scientific theory and their results to determine which substrate was used

This practical provides an opportunity to build on essential skills introduced at AS Level.

AS Level skills	How students develop the skills
MMO collection	Measure distance moved by dye
PDO recording	Record quantitative results appropriately in a table

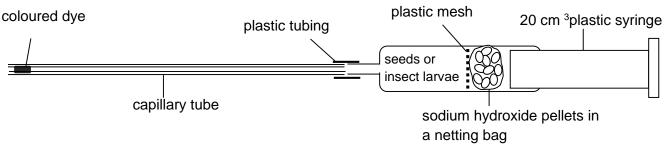
# Guidance for teachers, continued

#### Method

### Safety glasses must be worn when preparing the slide.

- Respiration is a process that consumes oxygen and releases carbon dioxide. The volume of oxygen consumed and carbon dioxide released can be measured using a respirometer.
- Learners need to understand how a respirometer works so a preliminary discussion is needed
  to encourage learners to think of a respirometer as a closed air-tight system from which the test
  organisms take oxygen and release carbon dioxide. The carbon dioxide is absorbed by the
  sodium hydroxide, causing the dye to move. The potential hazards of this apparatus should
  also form part of the discussion so learners can choose suitable safety precautions. This could
  take the form of a question and answer session or demonstration of the apparatus.
- Learners should be provided with a respirometer and asked to set up and use it by following the learner worksheet. This task gives learners the opportunity of re-visiting AS skills in a more complex procedure. The learners should work in pairs and each measure the gas exchange of a different organism, either germinating seeds or insect larvae. This gives an opportunity for learners to compare gas exchange in different organisms. The procedure for learners takes approximately one hour to complete. Learners may need help in setting up and making sure the respirometer is air-tight.

Diagram of a simple respirometer set up ready to use:



#### Results

- Learners record their results in a table, making use of the skills developed during the AS Level
  course to produce a suitable table. The table will be more complex than those used for AS and
  will help to develop A Level skills in interpreting complex tables.
  - They can be reminded of the expected form of tables from AS.
  - They can be prompted to think about how to present two sets of measurements taken over time.
- 2. A further table of total volume of oxygen and total volume of carbon dioxide should be generated by pooling the results of all the learners.
- 3. Learners will then process their results by calculating the total volume of oxygen consumed and the total volume of carbon dioxide produced. This provides an opportunity for learners to practice mathematical skills that are an integral part of A Level. Learners can work out the volume of a 10 mm length of the capillary tubing and use this to work out the total volume, or they can use the total distance measured to work out the volume. This activity provides an opportunity to revisit the idea of significant figures and sensible rounding of numbers generated by calculators.

# Guidance for teachers, continued

### Interpretation and evaluation

- Learners will then carry out a further calculation to find the RQ value for the organisms tested.
   At this point the idea of RQ and how it relates to respiratory substrate can be introduced. Each
   pair of learners can compare their results from germinating seeds or from insect larvae and
   draw conclusions about the substrate being used for respiration.
- 2. Learners will then describe the pattern shown by their results, noting if there is any change in the distance moved as time increases.
- 3. The idea of using a respirometer as a means of estimating the rate respiration can be introduced, as learners then carry out a further calculation to work out the rate of oxygen consumption in mm<sup>3</sup>g<sup>-1</sup> s<sup>-1</sup>. This provides learners with an opportunity to manipulate a sequence of calculations.
- 4. The experiment provides an opportunity for discussion about the reliability of the results in relation to the method used. The main considerations are the lack of replication of the measurements and the absence of a control. Learners could then suggest that at least three complete sets of measurements should be taken for both oxygen consumption and carbon dioxide release from which a mean can be calculated. They should also suggest a suitable control, such as replacing the test organism with an inert material such as beads or stones and be able to explain how the results of the control should be used.
- 5. Learners should then be asked to modify the method they have used in order to produce an experimental method to find the optimum temperature for respiration. This method should be suitable for another learner to use and ideally should be trialled. This gives another opportunity for learners to use a respirometer again and to follow this up by deciding how to use the results to find the optimum temperature.

# Information for technicians

#### Each learner will require:

- 1 x respirometer
- 10 cm<sup>3</sup> coloured solution (water and blue or red food dye)
- 20 Mung bean seeds (Vigna radiata) previously soaked for 24 hours in tap water or 15 below fly larvae, approximately 2cm in length
- 1 x sheet of 2 mm graph paper
- 10-15 sodium hydroxide pellets in a mesh bag made from fabric (nylon, cotton or polyester)
   [H] [C]
- 1 x 10 cm x 10 cm square of cotton, polyester or nylon mesh fabric. Muslin or window curtain netting can be used.
- 30 cm length of cotton suitable for trying seeds inside a fabric bag
- 1 x clamp stand, boss and clamp or other means of support for capillary tubing
- 1 x pair of forceps for handling sodium hydroxide
- 1 x 15 cm or 30 cm ruler, marked in mm
- access to top pan balance or scale weighing to 0.00 g
- 1 x pair of safety glasses
- 1 x pair of gloves plastic or vinyl

#### For follow-on investigation into effect of temperature

- 5 x containers suitable to use as water-baths
- 1 x thermometer
- Access to a supply of hot and cold water

# Information for technicians, continued

#### **Additional instructions**

To make a simple respirometer:

20 cm<sup>3</sup> syringe with tight fitting plunger that does not leak when immersed in water

20 cm length of capillary tubing of 1 mm bore

3 cm length of plastic tubing

The capillary tubing is attached to the syringe by plastic or rubber tubing. The tubing must be tight enough to prevent air leakage. This can be tested by immersing the joint in water.

Centres that have commercially produced simple respirometers of similar design could substitute these. U-tube manometers are not suitable substitutes.

Housefly larvae can be used instead of blow fly larvae, but approximately 25 will be needed to give results in the time. Fishing suppliers often have blowfly and housefly larvae (maggots) which can be kept in a refrigerator for several days before use and allowed to warm up 2-3 hours before the practical.

### **Hazard symbols**

 $\mathbf{C}$  = corrosive substance  $\mathbf{F}$  = highly flammable substance

**H** = harmful or irritating substance **O** = oxidising substance

N =harmful to the environment T =toxic substance

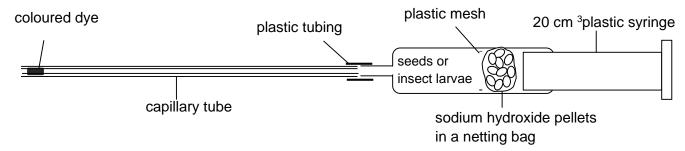
# Worksheet

#### Aim

To determine the volume of oxygen consumed and the volume of carbon dioxide released by respiration. To use these volumes to calculate RQ for the organism tested. The procedure for measuring oxygen uptake is used as a model to develop a plan to find the optimum temperature for respiration.

## **Apparatus**

Simple respirometer set up ready to use.



#### Method

### Safety glasses must be worn when preparing the slide.

- 1. Identify hazards and appropriate safety precautions for this investigation.
- 2. Remove the plunger from a 20 cm<sup>3</sup> syringe that is attached to a capillary tube.
- 3. Place 20 soaked mung bean seeds or 15 blowfly larvae into a piece of netting.
- 4. Fold the netting around the seeds and tie the open end with cotton to make a bag.
- 5. Find and record the mass of the seeds or insect larvae in their netting bag.
- 6. Place the bag of seeds or insect larvae into the syringe.
- 7. Insert a piece of plastic mesh to keep the bag of seeds or insect larvae at the bottom of the syringe, making sure it does not touch the bag containing the organisms.
- 8. Place a netting bag containing sodium hydroxide pellets into the open syringe so that it lies next to the plastic mesh.
- 9. Replace the plunger of the syringe and leave the assembled respirometer for 5 minutes.
- 10. Prepare a scale using the graph paper provided so it can be attached to the capillary tube.
- 11. Place the end of the capillary tube into the dye solution and use the syringe plunger to pull in a 1 cm length of the dye.
- 12. Attach the graph paper scale and use a support to keep the capillary tube horizontal.
- 13. Note the time.
- 14. Measure the distance moved by the dye at 1 minute intervals for 10 minutes.
- 15. If the dye reaches the end of the capillary scale before 10 minutes, reset the respirometer by pushing the plunger until the dye reaches the beginning of the scale.
- 16. Remove the syringe plunger and allow fresh air to enter the respirometer.
- 17. Remove the bag of sodium hydroxide pellets and replace them with a bag containing beads or small stones of the same mass.
- 18. Replace the syringe plunger and repeat steps 10 to 15.

# Worksheet, continued

#### Results

- 1. Record the raw results in a table.
- 2. Use the formula for volume of a cylinder to:
  - Calculate the oxygen consumption after 10 minutes in mm<sup>3</sup>
  - Calculate the carbon dioxide production after 10 minutes in mm<sup>3</sup>.
- 3. To calculate the RQ value use the formula

4. Calculate the rate of respiration from the oxygen consumption in mm<sup>3</sup> g<sup>-1</sup> s<sup>-1</sup>.

## Interpretation and evaluation

- 1. Summarise how a respirometer works, emphasising which steps in the procedure are essential to obtain reliable results.
- 2. Analyse the raw data and comment on the pattern including:
  - i. whether the rate of oxygen uptake is constant or changes during the 10 minute period
  - ii. the volume of carbon dioxide produced in comparison to the oxygen consumed
  - iii. what the RQ value indicates about the substrate being used for respiration.
- 3. Comment on the reliability of the results including:
  - i. use of apparatus
  - ii. the control
  - iii. the number of replicates.
- 4. Plan an investigation using a respirometer to find the optimum temperature for respiration germinating seeds of insect larvae. This plan should be detailed enough for another person follow.