MEASURING THE RATE OF OXYGEN UPTAKE

Purpose

- To investigate the uptake of oxygen in respiration.
- To measure the rate at which an organism respires.
- To develop practical skills.

SAFETY
Wear eye protection and disposable gloves when handling soda lime.
Soda lime is corrosive. Do not handle directly; use a spatula. See CLEAPSS Student Safety Sheet 31 for further details.
Write a risk assessment including any safety precautions. Discuss this with your teacher before starting.
Wash your hands thoroughly after handling living organisms.

YOU NEED	
• Respirometer (see Figures 1 and 2) • 5 g of actively respiring organisms • Soda lime • Coloured liquid • Dropping pipette	• Fine permanent marker pen (as lines must be very thin) • Solvent (to remove the marker) • Cotton wool • Stopclock

Respirometers

Respirometers range from relatively simple pieces of equipment used in school science labs with seeds or invertebrates, to elaborate devices the size of a room used to measure respiration rates in humans living near-normal lives over a period of several days. In this practical you will be using a very simple respirometer, while considering the advantages of some of the slightly more complex ones.

1 Scientific questions and information research

Before you start the experiment you should:

- Research relevant information about the design and use of respirometers.

Look at the respirometer in Figure 1. Annotate the diagram to show what each component does or make a list of the components shown for this respirometer and suggest their functions.

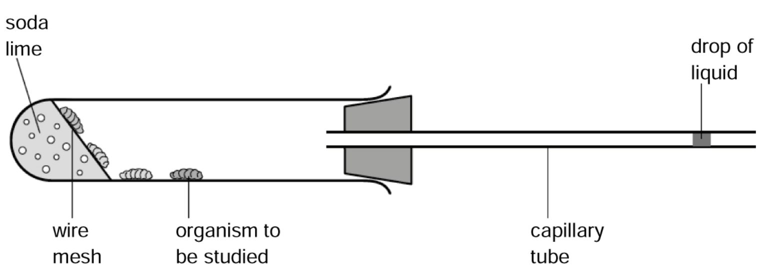

Figure 1 A very simple respirometer.

Study Figure 2 on page 2, and Figure 7.31 on page 149 of Student Book 2 that show two other types of respirometer. Make a table to compare the advantages and disadvantages of the three types of respirometer to help you think about the type of apparatus you will use.

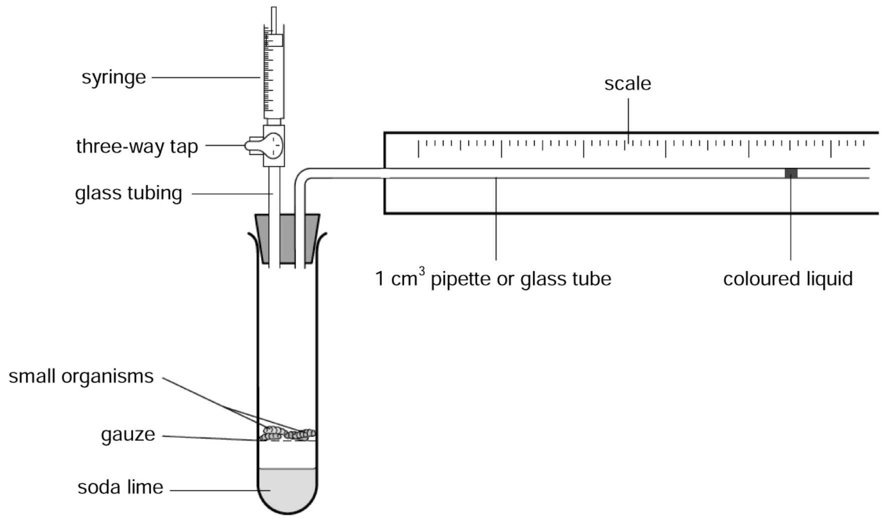

Figure 2 A simple respirometer using a boiling tube.

- Research relevant information about factors that will affect the rate of respiration.
- State what you are going to investigate. You should express this as a question to answer, a problem to investigate or a hypothesis to test.

2 Planning and experimental design

1 Find out what living organisms you will be using and investigate the temperature range of their normal environment. This will allow you to consider temperature as a possible variable to investigate or to control.

2 Find out the dimensions of the apparatus that you will be using, and the size and mass of the organisms; this will allow you to consider the mass or numbers of organisms as possible variables to investigate or to control.

3 Find out the dimensions of the capillary tube, pipette or other glass tube that your apparatus uses. Check if it has a scale associated with it, and what units of length and volume you will be working with.

4 Read through the steps in the given procedure. This procedure uses the type of respirometer shown in Figure 2. If you use a different design you may need to modify the procedure.

- **a** State what you expect to happen to the drop of liquid and why. In your answer, explain in detail what happens to the oxygen molecules, the carbon dioxide molecules and the pressure in the tube containing the liquid.
- **b** For the variable you will be investigating decide how you will modify the procedure to test your hypothesis. Predict what you expect to happen to the oxygen uptake as the independent variable is changed.
- **c** Decide if all the other variables have been identified and, where possible, controlled or allowed for.
- **d** Write a risk assessment for the practical work and make sure you consider how you will handle the living organisms to avoid stress (even rough handling of germinating seeds could affect their respiration) and what will happen to organisms, such as woodlice, after the investigation.
- **e** Draw up a table for your results and consider the most appropriate graph to present your likely data.

Procedure

1 Assemble the apparatus as shown in Figure 2.

2 Place 5 g of maggots, woodlice, or germinating peas or seeds into the boiling tube and replace the bung. Handle live animals with care to avoid harming them.

3 Introduce a drop of marker fluid into the pipette using a dropping pipette. Open the connection (three-way tap) to the syringe and move the fluid to a convenient place on the pipette if needed (i.e. towards the end of the scale that is furthest from the test tube).

4 Mark the starting position of the fluid on the pipette with a fine permanent pen.

5 Isolate the respirometer by closing the connection to the syringe and the atmosphere, and immediately start the stopclock. Mark the position of the fluid on the pipette at 1 minute intervals for 5 minutes.

6 At the end of 5 minutes open the connection to the outside air.

7 Measure the distance travelled by the liquid during each minute (the distance from one mark to the next on your pipette).

8 If your tube does not have volumes marked onto it you will need to convert the distance moved into volume of oxygen used. (Remember the volume used = πr^2 × distance moved, where r = the radius of the hole in the pipette.)

9 Record your results in a suitable table.

10 Calculate the mean rate of oxygen uptake during the 5 minutes.

11 Collect mean rate of oxygen uptake results from other groups in the class. Note the range of data recorded for mean oxygen uptake.

4 Analysis and interpretation of data

- Draw a graph that shows the mean rate of oxygen taken up against the variable you have investigated, for example the mass (or numbers) of organisms or temperature. Take care to use appropriate units. A scatter diagram may be the best choice to present your data; you should be able to justify why this might be the best choice.
- Decide if any correlation, positive or negative, is evident between your two variables and use a statistical test to determine if the correlation is significant.

5 Conclusion and evaluation

- Explain any correlation evident from the analysis using your biological knowledge.
- State a clear conclusion and comment on the validity of your conclusion. In your discussion, you may wish to suggest:
  - why a range of mean oxygen uptake results were obtained if more than one group used the same mass or number of organisms, or temperature
  - why a mean was calculated and the significance of any variation within the data from which the mean was calculated
  - what could be done to reduce the variation in class results and individual group results.

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SAFETY

Review students' risk assessments and discuss any safety considerations.

Ensure students wear eye protection and disposable gloves when handling soda lime.

Soda lime is corrosive. Do not handle directly; use a spatula. See CLEAPSS Student Safety Sheet 31 for further details.

Ensure students wash their hands thoroughly after handling living organisms.

Notes on the procedure

The respirometer shown in Figure 1 on the Student Sheet is a very simple one; more complex ones (for example, U-tubes) can be used if available.

The choice of what respiring organisms to put into the tubes is left to you. Germinating peas, maggots or woodlice are commonly used.

The choice of which independent variable to use is also left to you. Investigating the variation in temperature on the rate of oxygen uptake is likely to provide more interesting biological explanations than change in mass/number, though the latter could provide good discussion points about the effects of surface area to volume ratios. If temperature is used as the independent variable then the range 5–35 °C is suggested as suitable, with 5 °C increments and ideally 3 repeats at each temperature.

If you are using pipettes there is no need to do any volume calculations; students just read the change in volume off the scale on the pipettes. If you are using thick-walled capillary tubing it is worth reminding students of the formula for working out the volume of oxygen used:

volume of air used = $\pi r^2 \times$ distance moved

where r = the radius of the hole in the pipette

Three-way taps can cause confusion with some students. A diagram of how the three-way tap works is shown in Figure 1 on page 4 of this sheet. Projecting this diagram during the practical can help.

1 Scientific questions and information research

Students might include the following in their response to explaining the components of the respirometer in Figure 1.

- Soda lime – absorbs carbon dioxide released in respiration from the living organisms being studied.
- Wire mesh – prevents organisms being studied coming into contact with the soda lime, which is both corrosive and an irritant.
- Organisms – take up oxygen in respiration.
- Capillary tube – allows small changes in the movement of the drop of liquid to be measured and volume changes to be calculated.
- Drop of liquid – allows changes in volume to be seen.

A comparison of the three types of respirometer might include some of the following points:

Type of respirometer	Advantage(s)	Disadvantage(s)
Basic respirometer as shown in Figure 1 of Student Sheet	Very simple to set up Minimal number of connections make a good seal easier to obtain	Does not allow you to reset Needs control tube alongside it No scale, so measurements likely to be less accurate
Respirometer as shown in Figure 2 of Student Sheet	Fairly simple to set up Can be reset using the syringe Scale allows more accurate measurements	Needs control tube alongside it
Respirometer with control tube as shown in Figure 7.31 on page 149 of Student Book 2	Does not need an additional control as the second tube balances out the effects of any changes in temperature or atmospheric pressure Syringe allows you to move the liquid in the U-tube to reset the apparatus	Tendency for the connections to leak in elderly school/college models (making the equipment useless) More expensive than the simpler models Bulk may make use in a small water bath or ice bath problematic

2 Planning and experimental design

1–3 Students are asked to find out information about the apparatus and living organisms that they are going to be using. It might be helpful to have at least one set of apparatus ready for them to explore how the set up works and also the living organisms that they are going to be using.

4 **a** Simple answer: Oxygen molecules are absorbed by the organism and used in respiration. The same number of carbon dioxide molecules are released, but these are absorbed by the soda lime. This reduces the pressure inside the test tube (fewer molecules = lower pressure). Atmospheric pressure pushes the liquid along the tube, until the pressure inside and outside the tube is equal.

Detailed respiration review answer: As above, but should include reference to the role of oxygen as the final electron acceptor and the fact that it eventually combines with hydrogen to make water. The carbon dioxide comes from the link reaction and the Krebs cycle, as the carbohydrate is broken down.

b How much choice you give your students over the independent variable may depend upon your class size and the time that you have. You do need as much data as possible for a scatter diagram, so it could work best to have one independent variable and different groups investigating different values of the same variable. If using temperature, obviously it is sensible and responsible to keep within the possible environmental range of the living organisms that you are using.

c Possible other variables and their control:

- **Environmental temperature** – could be controlled via a water bath or monitored to check for any change.
- **Number of organisms** – controlled by careful counting/double-checking numbers.
- **Mass of organisms** – controlled by weighing. To keep both number and mass the same, using organisms of similar size will be helpful.
- **Mass of soda lime** – this should be weighed out.
- **Activity and age of organisms** – partly controlled by choosing organisms of the same age (days germinated/same length of maggot, etc.)
- **Species** – there may be variation in metabolic rates in organisms of different species, so the same species organisms should be used. If woodlice are collected from school/college grounds, they may well be different species.

d Students should consider how they handle the living organisms to reduce stress to both themselves and the organisms. Small, plastic spoons and soft brushes can work well for both maggots and woodlice; germinating seeds need no special measures apart from gentle handling, though disposable gloves may be used.

After the investigation, effective hand-washing procedures should be used. Living animals should be returned to the environment that they were taken from.

e Tables – if students are going to share data, it might help if they use similar tables for recording their data.

Graphs – students are asked to consider the best way to present their data graphically. It might help to remind them about the various types of graphs and the reasons for choosing particular graphs, including scatter diagrams.

5 Conclusion and evaluation

- Students are asked to comment on the validity of their conclusion. It is suggested that they discuss:
  - why a range of mean oxygen uptake results were obtained if more than one group used the same mass or number of organisms, or temperature

    This may be due to a number of factors that cannot be controlled, for example, organisms having different metabolic activities; fluctuations in environmental temperature (whether or not this is the independent variable); variation in equilibration times; variation in mass, as it is difficult to get exact masses of living organisms, etc.
  - why a mean was calculated and the significance of any variation within the data from which the mean was calculated

    Answers might include that taking several readings over five minutes is equivalent to carrying out repeated measurements and that using a mean value is more accurate than individual values. Each reading within the five minutes may be different; this could be due to the organism still acclimatising or the apparatus still equilibrating, as well as changes in activity of the organism, etc.
  - what could be done to reduce the variation in class results and individual group results.

    Answers might refer to points within the list of variables given above, such as selecting organisms of similar size and thus, hopefully, of similar age and activity. Other acceptable answers could mention consistency of timing and measuring, with perhaps the same person carrying out the same tasks, to minimise random error; re-using the same apparatus to control any systematic error.

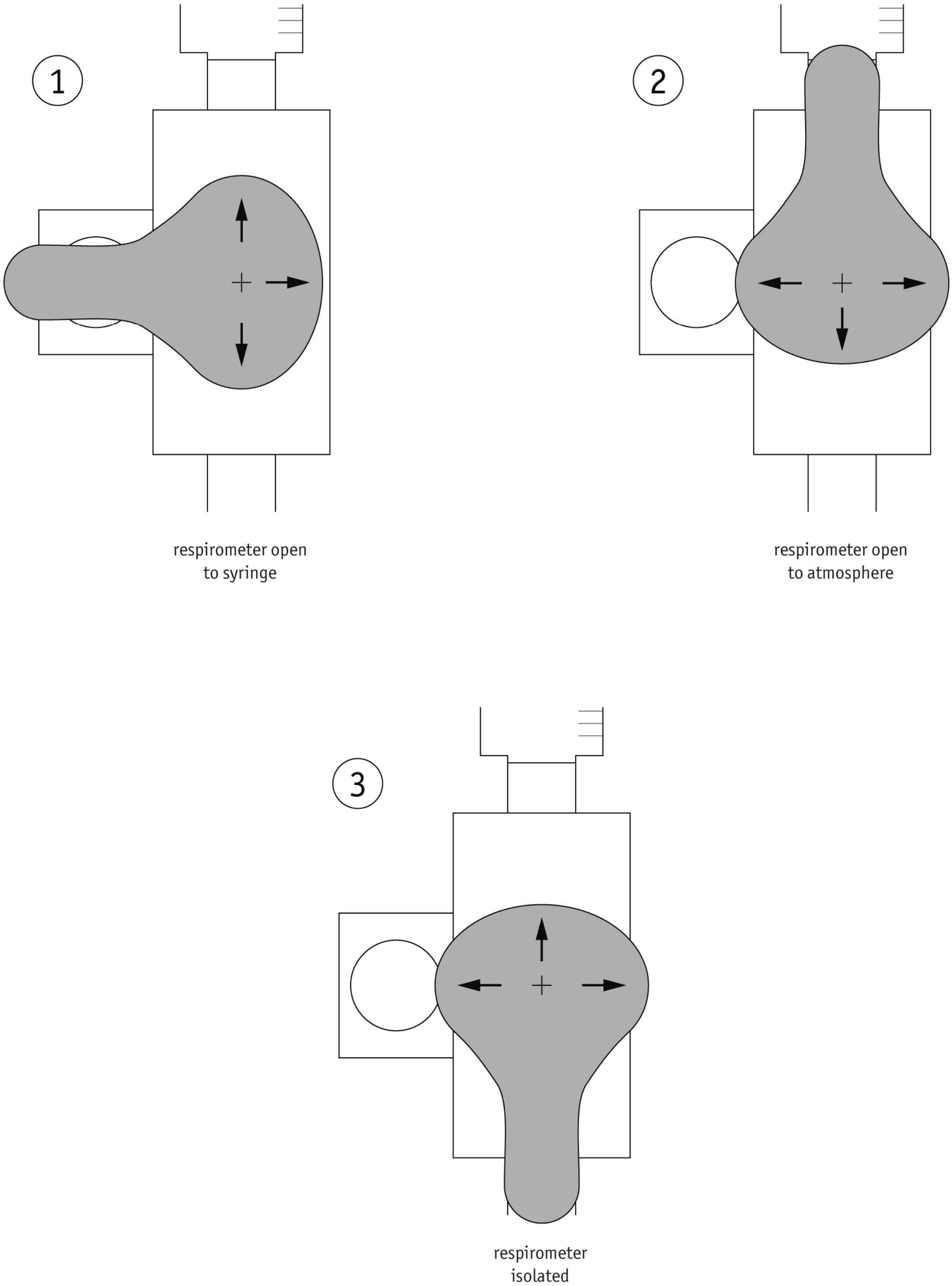

Figure 1 Tap positions for a three-way tap (viewed from the side).

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SAFETY

Soda lime is corrosive, but much less of a hazard than solutions of potassium or sodium hydroxide. Even so, eye protection is needed when handling the soda lime. Do not handle directly; use a spatula and wear disposable gloves. Avoid exposing invertebrates to corrosive soda lime dust.

Respirometers

Requirements per student or group of students	Notes
Respirometer	See Figure 2 on the Student Sheet. If a pipette is used, the scale shown on Figure 2 will not be needed. Ideally, the syringes are attached with a three-way tap. If these are not available, a rubber tube and clip can be used. See also section 15.10 Respirometers in the CLEAPSS *Laboratory Handbook* for details of other (bulk) suppliers. U-tube respirometers would be even better, if there is a class set available.
5 g of actively respiring organisms	Use actively germinating peas, beans or other seeds, or maggots or woodlice.
Roughly a tablespoon of soda lime	To absorb the carbon dioxide.
About 2 cm^3 of coloured liquid	e.g. water and food colouring or equivalent.
Dropping pipette	
Permanent marker, or chinagraph pencils (fine, to make lines as thin as possible)	For marking the position of the coloured liquid.
Solvent to remove the marker	
Small amount of cotton wool to wipe pipette	
Stopclock	
Eye protection	
Disposable teaspoon or fine brush/disposable gloves	For handling organisms.
Soap/water	For hand-washing after handling organisms.

A respirometer is shown in Figure 1 on the Student Sheet. Many schools and colleges have at least one U-tube respirometer (Figure 7.31 on page 149 of Student Book 2). These can be used, but can be a lot more fiddly and the connections often leak. Ensure rubber bungs and connecting tubing are not perished. A thin layer of petroleum jelly can be used in emergencies to try to seal leaky equipment, but may cause the rubber to perish more quickly when removed thoroughly afterwards. If the apparatus works, the respiring organisms use up the oxygen and give off CO_2. The CO_2 is absorbed by the soda lime. This means there is less air in the test tube so the liquid moves towards the tube with the organisms in it. If it does not work there is usually an air leak somewhere, or possibly the organisms are too cold, or dead.

Safety checked, but not trialled by CLEAPSS. Users may need to adapt the risk assessment information to local circumstances.