INVESTIGATING BREATHING: SHEET A

Purpose

- To investigate tidal volume, vital capacity, rate of breathing, respiratory minute ventilation and oxygen consumption.
- To interpret data from spirometer traces.

Using a spirometer

- Q1 Use the interactive tutorial that accompanies this activity to:
 - label the diagram of a spirometer in Figure 1 below a
 - write a paragraph explaining how a spirometer can be calibrated, then used to measure b breathing rate and volume. Write a second paragraph to explain how these measurements can be used to calculate respiratory minute ventilation and oxygen consumption.



Figure 1 A spirometer.

- **Q2** Explain the purpose of:
 - wearing a nose clip while using the spirometer a
 - b the soda lime canister.

Analysis and interpretation of a spirometer trace

The spirometer chart in Figure 2 shows two traces; one at rest (lower trace) and one after exercise (upper trace).



Figure 2 Spirometer chart: top trace = after exercise; bottom trace = before exercise.

The three dots above the upper trace are the calibration dots: the first dot, the lowest of the three, is the baseline level recorded before any oxygen is added, the second dot is after adding 1 dm³ oxygen to the chamber within the spirometer and the third dot is after adding another 1 dm³ oxygen. The chart recorder was set at 0.5 cm s⁻¹. (NB 1 dm³ is the same as 1 litre; 1 dm³ = 1000 cm³.)

Q3 a Use the trace in Figure 2 to find the effect that exercise had on:

- i breathing rate
- ii tidal volume
- iii respiratory minute ventilation
- iv oxygen consumption.
- **b** Suggest an explanation for your findings.
- **c** Sketch what the after exercise spirometer trace would look like if the person was asked to take a single deep breath to allow the measurement of vital capacity.
- Q4 If you were asked to investigate other changes to the body during exercise, what other factors could you measure easily?
- Q5 Spirometers are used to calculate a subject's basal metabolic rate (BMR) from the volume of oxygen consumed in a given time. Explain why it would be difficult to measure your own BMR in this way during a biology lesson.

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INVESTIGATING BREATHING: SHEET B

Purpose

- To investigate tidal volume, vital capacity, rate of breathing, respiratory minute ventilation and oxygen consumption.
- To interpret data from spirometer traces.

SAFETY

Use eye protection when handling soda lime.

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

A spirometer should only be used with teacher supervision. If you have breathing or circulation (heart) problems or suffer from epilepsy you should not use the spirometer. Read the manufacturer's instructions and safety notes before using the equipment.

Stop using the spirometer at once if you experience any unusual breathing problems, or feel dizzy or uncomfortable.

(Asthmatics may use a spirometer if they are otherwise in good health.)

A trained member of staff should use an oxygen cylinder to fill the spirometer.

YOU NEED

- Spirometer
- Kymograph, chart recorder, datalogger or computer
- Soda lime (for the spirometer canister)
- Disinfectant solution

Using a spirometer

The apparatus shown in Figure 1 is a spirometer. Spirometers allow us to study both breathing and respiration. In this activity you will learn how a spirometer works and how to interpret the spirometer trace that is produced.



Figure 1 A spirometer.

The general principle behind a spirometer is simple. It is effectively a tank of water with an air-filled chamber suspended in the water. It is set up so that adding air to the chamber makes the lid of the chamber rise in the water and removing air makes it fall. Movements of the chamber are recorded using a kymograph (pen writing on a rotating drum), a chart recorder, computer or datalogger.

Tubes run from the chamber to a mouthpiece and back again. Breathing in and out through the tubes makes the lid of the chamber fall and rise. The volume of air the person inhales and exhales can be calculated from the distance the lid goes up and down.

The apparatus can be calibrated so that the movement of the lid corresponds to a given volume. A canister containing soda lime is inserted between the mouthpiece and the floating chamber. This absorbs the CO_2 that the subject exhales.

Q1 In what direction will the pen move when the subject inhales?

Carry out practical work safely and ethically

Using students as subjects for an investigation raises ethical issues. It is important that you are well informed about the procedure and that you give your consent to taking part. You should read the information sheet at the end of this activity and read the procedure below carefully.

Procedure

Calibration

In order to interpret the spirometer trace you need to know what both the vertical and the horizontal scales represent.

Finding the vertical scale

The vertical scale measures the volume of air in the chamber. The spirometer's floating-chamber lid has markings on it showing how much gas it contains.

- 1 First, empty the chamber completely and make a mark on the kymograph paper, while it is stationary, to show where the pen lies when there is no gas in the tank. Then force a known volume of air into the tank (for example, 500 cm³) and make a second mark on the kymograph trace.
- 2 Repeat this procedure until the chamber has been completely filled with air. If the range on the kymograph is too large or too small, the length of the arm supporting the pen can be adjusted so that the trace fits onto the paper.
- 3 Write the values next to your calibrating marks they will help with interpretation of the trace later. Once the marks have been made on the paper it should be possible to count how many squares on the trace represent 1 dm³. (NB 1 dm³ is the same as 1 litre, 1 dm³ = 1000 cm³).

Finding the horizontal scale

4 On most kymographs there is a switch allowing you to set the speed at which the drum turns. Choose a speed close to 1 mm per second. This is your horizontal scale. Make a note of the speed on your trace, so that the trace can be interpreted once the experiment is complete.

Collecting data on breathing

- 5 After calibration, the spirometer is filled with medical grade oxygen. A disinfected mouthpiece is attached to the tube, with the tap positioned so that the mouthpiece is connected to the outside air. The subject to be tested puts a nose clip on, places the mouthpiece in their mouth and breathes the outside air until they are comfortable with breathing through the tube.
- 6 Switch on the kymograph, and at the end of an exhaled breath turn the tap so that the mouthpiece is connected to the spirometer chamber. The trace will move down as the person breathes in. After breathing normally the subject should take as deep a breath as possible and then exhale as much air as possible before returning to normal breathing.

Analysis and interpretation of data

A diagram of a spirometer trace is shown in Figure 2. In this example the subject has breathed in and out normally three times, then taken as deep a breath in as possible, then forced the air from their lungs. Several pieces of information about the subject's breathing can be read off this kind of trace, or worked out from it.



Figure 2 A sketch of a trace showing normal breathing and one forced breath in and out.

- The tidal volume is the volume of air breathed in and out in one breath at rest. The tidal volume for most adults is only about 0.5 dm³.
- Vital capacity is the maximum volume of air that can be breathed in or out of the lungs in one forced breath.
- Breathing rate is the number of breaths taken per minute.
- Minute ventilation is the volume of air breathed into (and out of) the lungs in one minute. Minute ventilation = tidal volume × rate of breathing (measured in number of breaths per minute).

Some air (about 1 dm³) always remains in the lungs as residual air and cannot be breathed out. Residual air prevents the walls of the bronchioles and alveoli from sticking together. Any air breathed in mixes with this residual air.

- Q2 Using the trace produced in class, or one provided by your teacher/lecturer, find the following values:
 - a tidal volume
 - **b** vital capacity
 - **c** breathing rate
 - d minute ventilation.

Ensure you quote figures and their correct units.

Collecting data on rate of respiration

Each time we take a breath, some oxygen is absorbed from the air in the lungs into our blood. An equal volume of carbon dioxide is released back into the lungs from the blood.

When we use the spirometer, each returning breath passes through soda lime, which absorbs the carbon dioxide, so less gas is breathed back into the spirometer chamber than was breathed in.

If we breathe into and out of the spirometer for (say) 1 minute, a steady fall in the spirometer trace can be seen. The gradient of the fall is a measure of the rate of oxygen consumption and so is a measure of the rate of respiration by the body.

- Q3 Use the trace produced in class, or one provided by your teacher/lecturer, to work out the rate of oxygen consumption in someone at rest.
- Q4 What differences would you expect if the subject had been exercising before a trace was taken?
- **Q5** Describe how you could use the apparatus to measure changes in breathing and respiration rates due to exercise. State what exercise would be appropriate and any hazards involved.
- Q6 Sketch the shape of the trace you would expect before and after exercise.

Investigating Breathing: Using a Spirometer to Investigate Human Lung Function

Information Sheet

The purpose of this practical is to investigate human lung function, to measure tidal and respiratory minute volumes, breathing rate and oxygen consumption. Variation between individuals may also be considered.

You and your classmates will be the humans that we investigate.

SAFETY: If you have any condition that might affect you breathing into a spirometer, please make sure your teacher knows before you start the investigation.

In the investigation you are going to measure and record how much air you breathe in and out. The results will tell us something about the lung capacity of people in your group and you may see how your lung capacity compares with others. When the information is recorded, there will be no record of which trace belongs to which person.

- You are participating in a scientific process of observation and data collection.
- The activity is not a competition.
- The results will not show up any health problems, because we are measuring lung capacity on only one day, in a classroom laboratory.
- If your lung function details are very different from others in your group, don't worry. There is a lot of variation within the healthy range of lung function.
- If you are worried about anything at the end of the practical, please stay to talk to your teacher about it.
- You do not have to take part in the exercise.
- You can stop at any time.
- You don't have to put your results into the class set.
- There will be no long-term effects.

This sheet has been modified from a Nuffield Foundation Practical Biology sheet, see weblinks.

INVESTIGATING BREATHING

Purpose

- To investigate tidal volume, vital capacity, rate of breathing, respiratory minute ventilation and oxygen consumption.
- To interpret data from spirometer traces.

Using a spirometer

Two Student Sheets are provided. Sheet A is to be used with the interactive tutorial that accompanies this activity and Sheet B is for use when undertaking a spirometer practical. A copy of a spirometer trace is provided at the end of these notes for use when there is no access to a spirometer or as extra data to interpret. Note that the specification requires students to 'Investigate the effects of exercise on tidal volume, breathing rate, respiratory minute ventilation and oxygen consumption using data from spirometer traces'. The focus appears to be on interpreting data.

The interactive tutorial and Sheet B describe the use of a spirometer with a kymograph. Alternatively, a datalogger can be used with a traditional water-filled spirometer or with an airflow spirometer. A range of different types of sensors can be used and some alternatives are described in the section, on page 4, on using a spirometer with a datalogger. Note that if only a few breaths are to be recorded, filling the spirometer with atmospheric air, rather than oxygen, is acceptable.

If you do not have access to a spirometer, measurement of vital capacity and tidal volume can be carried out using breath volume bags. These are a fraction of the cost of a spirometer. However, they cannot be used for measuring oxygen consumption.

It is not possible to use spirometers of the type most likely to be used in schools while exercising. Recordings should be made immediately after exercise has stopped.

It may be possible to arrange a visit to a local university Physiology or Sports Science department where they will have exercise-compatible spirometers. These pieces of kit are often on show during open days, so tell your students to look out for them!

Student Sheet A

The interactive tutorial and Student Sheet A associated with this activity can be used if a spirometer is not available. It can also be used in preparation for, or as a follow up to, a spirometer practical. The animation shows how the spirometer works, how it is calibrated and how to interpret a spirometer trace.

Answers





- b When the air tank is filled with oxygen and the water tank filled with water, you can breather the oxygen through the breathing tubes. The lid of the air tank goes up and down as you breathe in and out, making a trace on the chart recorder. The speed of the chart recorder can be set, so the relationship between distance and time can be calculated on the trace. The volume readings can be calibrated by making marks on the chart with the pen before and after a known volume of oxygen is added to the air tank. Breathing rate is calculated by counting the number of breaths in a given time. The volume of air breathed in and out – the tidal volume – is calculated from the vertical movements of the trace. Respiratory minute ventilation, also known as minute volume, is the volume of gas inhaled or exhaled from a person's lungs per minute. If both tidal volume and breathing rate are known then respiratory minute ventilation can be calculated by multiplying the two together. Oxygen consumption can be found by drawing a line through the lowest points of the trace for a set time. The change in volume (change in calibrated vertical height) for the set time can then be measured and the oxygen consumption calculated per minute.
- Q2 a The nose clip makes sure that all breathing is only through the air tubes of the spirometer, so that the trace reflects the true volume of gas breathed in and out.
 - **b** The soda lime absorbs CO_2 in the exhaled air. This ensures that the CO_2 levels in the inhaled air do not change during the experiment, making uptake of oxygen easier to determine. The removal of CO_2 is also necessary for safety reasons.
- Q3 a i Breathing rate before exercise: 4 breaths in 26–27 seconds; 4 × 60/26 (or 27) = 9 breaths per minute.
 Breathing rate after exercise: 11 breaths in 34 seconds; 11 × 60/34 = 19 breaths per minute.
 Breathing rate increases by about 10 breaths per minute; it more than doubles (increasing by a factor of 2.1).

- Tidal volume before exercise: average vertical height of trace = 1.3 cm. ii From calibration 2.5 cm = 1 dm³. 1 dm³ \div 2.5 cm \times 1.3 cm = 0.52 dm³. Tidal volume after exercise: average vertical height of trace = 2.4 cm. $1 \text{ dm}^3 \div 2.5 \text{ cm} \times 2.4 \text{ cm} = 0.96 \text{ dm}^3$. Tidal volume increases by 0.44 dm³ after exercise; it nearly doubles (increasing by a factor of 1.8).
- Respiratory minute ventilation = breathing rate \times tidal volume iii Before exercise = 9×0.52 dm³ = 4.68 dm³ min⁻¹ After exercise = $19 \times 0.96 \text{ dm}^3 = 18.24 \text{ dm}^3 \text{ min}^{-1}$ Exercise causes respiratory minute ventilation to increase by 13.56 dm³ min⁻¹; it also causes it to approximately quadruple.
- Oxygen consumption is the change in volume shown per minute. iv

Volume changes are shown on Figure 2. A line of best fit is drawn through the low points of the recordings and extended for 30 small squares. As two small squares represent one second, this line covers 30 s so the change in volume needs to be multiplied by two to give the change per 60 s.

Before exercise, the volume change is shown as 0.4 cm on the trace.

From calibration, 2.5 cm represents 1 dm^3 so 0.4 cm represents 160 cm^3 .

Oxygen consumption over 1 minute must be $2 \times 160 = 320 \text{ cm}^3 \text{ min}^{-1}$.

After exercise, the volume change is shown as 1.1 cm on the trace.

From calibration, 1.1 cm represents 440 cm³.

Oxygen consumption over 1 minute must be $2 \times 440 = 880 \text{ cm}^3 \text{ min}^{-1}$.

Exercise has caused oxygen consumption to increase by 560 cm³ min⁻¹, a 2.75 fold increase.



Figure 2 Spirometer chart: top trace = after exercise; bottom trace = before exercise.

- b The changes in breathing rate, tidal volume and respiratory minute ventilation reflect increased gas exchange during exercise due to the increased demand for energy in skeletal muscles. The supply of oxygen to the tissues is increased where more oxygen is needed for aerobic respiration. The increased oxygen consumption reflects the increased rate of respiration in the tissues. More energy is made available for muscle contraction during exercise.
- С The trace would show a single maximum breath in and out, looking similar to the one in Figure 2 on page 5 of the Student Sheet.
- Temperature, sweating rate, pulse rate. **Q4**
- **Q5** BMR can only be measured when the subject is horizontal and completely at rest, and has not eaten for at least 12 hours. This is unlikely in a classroom situation.

Student Sheet B

SAFETY

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

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



A spirometer should only be used with supervision. Check to make sure no students have breathing or circulation (heart) problems or suffer from epilepsy as they should not use the spirometer. Read the manufacturer's instructions and safety notes before using the equipment.

If the students are allowed to breathe through the spirometer for too long they can lose consciousness from lack of oxygen. Limiting the time spent breathing through the spirometer and carefully observing each student should prevent problems.

When using oxygen and absorbing CO_2 : maximum time 5 minutes; in any other situation: maximum time 1 minute. If a student becomes less alert or has any feeling of suffocation they should stop immediately. Because the levels of CO_2 are kept low by the soda lime, the students will not be aware that they are running out of oxygen until it is potentially too late.

Stop using the spirometer at once if the student experiences any unusual breathing problems or feels dizzy or uncomfortable.

(Asthmatics may use a spirometer if they are otherwise in good health.)

A trained member of staff should use an oxygen cylinder to fill the spirometer.

The subject will feel some resistance to breathing when using a spirometer. Because of this they should not use the spirometer while exercising. To investigate the effect of exercise, readings should be taken immediately after exercise. If resistance to breathing suddenly increases it may be due to valves in the spirometer sticking. If this occurs the valves may need to be replaced.

Either replace the consumable cardboard mouthpiece between each student or devise a cleaning procedure if the respirator has a permanent mouthpiece.

Explain the information form prior to starting the practical.

Carrying out practical work safely and ethically

Using students as subjects for an investigation raises ethical issues. Any student considering taking part in the practical work should be given the information sheet. They should only take part once they have shown that they understand the information.

An example of an information sheet is given at the end of the Student Sheet. This may be modified to suit circumstances, but the basic principle is that students should have a full understanding of what they are being asked to do before they take part.

Notes on the procedure

The Student Sheet describes how a spirometer works and how to interpret the spirometer trace that is produced. Effects of exercise on breathing rate and volumes can be investigated using the spirometer. The spirometer should not be used while exercising. To investigate the effect of exercise take readings immediately after exercise. Although this is not detailed on the activity sheet, students do need to be able to describe how to investigate the effects of exercise on tidal volume and breathing rate.

Using a spirometer with a datalogger

One advantage of collecting the data electronically is that the data can be moved from the datalogging software to a spreadsheet or a word processing program. The data collected can then be made available to students involved in the investigation.

Sensors, such as motion sensors, can be used to record the up and down motion of the air-filled spirometer lid.

A motion sensor can be mounted above the large top surface of the moving lid (see Figure 1A on page 5). As the lid moves up and down the change in distance can be recorded. Make sure the limits of the

motion sensor are known and the minimum recordable distance is available when the lid is at its closest to the sensor.

If a known series of gas volumes are introduced into the spirometer, and the distance between the motion sensor and the surface of the lid are recorded, it is possible to produce a calibration curve of distance versus volume. Most datalogging software can convert distance measurements recorded by the sensor to volume data.

In a similar way, a linear motion sensor (for example, rotary motion or position sensor) can be attached to the spirometer, as shown in Figure 1B below. With a position sensor, a string weighted with a small counterweight can be run from the end of the lid over the sensor's pulley wheel. When the lid moves up and down, the string moves over the pulley. The sensor records a changing distance or angle. Calibration relating the volume of gas in the spirometer tank to the sensor's reading will be required.

Carbon dioxide and oxygen levels in the spirometer lid can be datalogged. Monitoring these levels gives another safety check that the CO_2 absorber is working and that O_2 levels are not falling dangerously low.

Alternatively, many of the datalogging companies now produce an airflow spirometer which measures volume by integrating the flow data over time. These devices are very lightweight, do not need carbon dioxide absorbers and do not need cylinders of medical oxygen (they use the oxygen from the atmosphere). Note that students need to be able to describe how to investigate the effects of exercise on tidal volume, breathing rate, respiratory minute ventilation and oxygen consumption using data from spirometer traces.



Figure 1 Motion sensors fitted to a spirometer.

Answers

Q1 Down.

- **Q2** The values here are for the trace provided at the end of these notes (Figure 2). Obviously if a student's own trace is used the answers may be different.
 - **a** Tidal volume between 0.5 and 0.8 dm³. Encourage students to take an average over several breaths.
 - **b** Vital capacity = 2.55 dm^3 averaged over the two breaths.
 - **c** Breathing rate = between 20 and 22 breaths per minute (depending on the section of the graph used).
 - **d** Minute ventilation = 21 breaths per minute $\times 0.65$ dm³ = 14 dm³ min⁻¹.

- Q3 The rate of oxygen consumption determined from the trace in Figure 2 is approximately $1.0 \text{ dm}^3 \text{ min}^{-1}$ for the first 30 seconds.
- Q4 If the subject had been exercising, the rate of oxygen consumption would be higher, so the slope would be steeper. The trace would also show that the subject was breathing faster and more deeply.
- Q5 This question allows students to think through the practicalities of using a spirometer in an investigation. They should think about the type of exercise; it is easier to use a spirometer immediately after running up and down stairs than after swimming or paragliding. They may also consider individual variation and sample sizes. They should also think about the limits imposed by the volume of oxygen available in the tank. Comments on the effect of the spirometer on the subject are also worth encouraging -a spirometer often causes breathing to become slightly more laboured. (Older models which use a smaller diameter tubing, are usually the culprits here.)
- Q6 The graph should show that breaths become deeper and more frequent after exercise, with a greater rate of oxygen consumption.





Figure 2 Spirometer trace for spirometer used with soda lime and filled to 9 dm³ with oxygen.

INVESTIGATING BREATHING

Purpose

- To investigate tidal volume, vital capacity, rate of breathing, respiratory minute ventilation and oxygen consumption.
- To interpret data from spirometry traces.

In this activity students are shown how a spirometer works and how to interpret the spirometer trace that is produced. Student Sheet A and the interactive tutorial that accompanies this activity describe the use of a spirometer with a kymograph. Alternatively, a datalogger can be used with a traditional water-filled spirometer or with an airflow spirometer. A range of different types of sensors can be used and some alternatives are described on the Teacher Sheet.

A spirometer can be used to investigate the effect of exercise on breathing rate, lung volumes and oxygen consumption. There is an accompanying interactive tutorial with an animation of a spirometer.

If you do not have access to a spirometer, measurement of vital capacity and tidal volume can be carried out using breath volume bags. These are a fraction of the cost of a spirometer. However, students do need to be able to interpret data from spirometer traces.

SAFETY

The safety guidelines in Sections 14.5 and 14.5.1 in the CLEAPSS Handbook should be followed when using a spirometer.

This investigation is potentially hazardous. Students should be closely supervised at all times when using a spirometer. If the students are allowed to breathe through the spirometer for too long they can lose consciousness from lack of oxygen. Limiting the time spent breathing through the spirometer and carefully observing each student should prevent problems. When using oxygen and absorbing CO_2 : maximum time allowed is 5 minutes; in any other situation: maximum time allowed is 1 minute. If a student becomes less alert or has any feeling of suffocation they should stop immediately. Since the levels of CO_2 are kept low by the soda lime, the students will not be aware that they are running out of oxygen until it is potentially too late.

A trained member of staff should use an oxygen cylinder to fill the spirometer.

Use eye protection when handling soda lime. Soda lime is corrosive. Do not handle it directly; use a spatula. Use a type of soda lime that changes colour when it is saturated and replace it when it changes colour. Use 5–10 mesh particle size. Follow CLEAPSS guidelines on removing dust for spirometer use. A layer of polymer wool can be put at the inflow and outflow of the soda lime canister chamber to prevent dust getting into the chamber. Ensure that the soda lime canister is fitted so that air is breathed out through the canister.

The subject will feel some resistance to breathing when using a spirometer. Because of this they should not use the spirometer while exercising. To investigate the effect of exercise, readings should be taken immediately after exercise. If resistance to breathing suddenly increases it may be due to valves in the spirometer sticking. If this occurs the valves may need to be replaced.

Do not use oil, grease or glycerine on any part of the spirometer tubing as these may make explosive compounds with oxygen. Water with a little liquid detergent can be used to aid connection of tubes, etc. Keep all flames away from the spirometer.

It is essential to follow good hygiene practice with regard to cleaning and disinfecting the mouthpiece, and removing condensation and saliva from the tubes.

Care should also be taken to choose the subject, avoiding any students with asthma or other breathing or circulatory problems. (Asthmatics may use a spirometer if they are otherwise in good health.)

Additional guidance on health and safety may be found in the Nuffield Practical Biology sheet on 'Using a spirometer to investigate human lung function': http://www.nuffieldfoundation.org/practical-biology/using-spirometer-investigate-human-lung-function

Read the manufacturer's instructions carefully before using a spirometer.

Subjects should be given an information sheet to read and should sign a consent form before taking part in the investigation.

Requirements per student or group of students	Notes
Spirometer	If the spirometer has a pen it is worth checking that it is still working – they fail quite quickly. Always keep the ink reservoir-type with its piece of wire in place. Some new spirometers can be set up to feed the data into a computer. See notes on Teacher Sheet about using spirometers with dataloggers. Safety <i>See notes on page 1.</i>
Kymograph	See CLEAPSS folder Section 15.1 for advice on speeds, etc. A chart recorder, computer or datalogger can be used in place of a kymograph.
Kymograph paper	Be aware that it matters which way up you attach the paper. If it is the right way up the pen moves smoothly over the join in the paper. If it is the wrong way up it catches on the join.
Soda lime for the spirometer canister	Safety See notes on page 1.
Disinfectant solution, 70% ethanol or fresh 0.1% hypochlorite followed by rinsing with water	For rinsing mouthpiece and breathing tubes before and after use. To minimise an unpleasant taste on the mouthpiece, Milton [®] can be used at the manufacturer's dose. This, however, takes 30 minutes to achieve disinfection.
Eye protection	
Information sheet and consent form	2 copies required per participant

Notes

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