Using a respirometer

Practical skills A **respirometer** can be used to investigate factors affecting the rate of aerobic or anaerobic respiration of organisms.

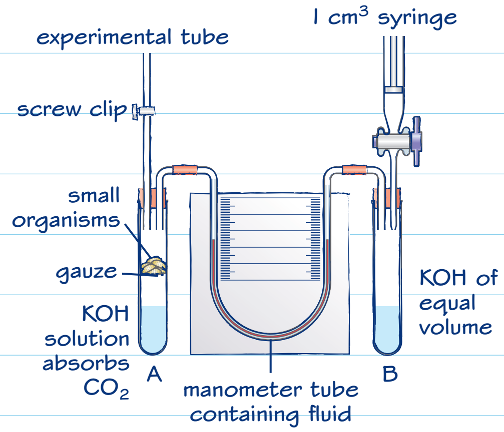
Using a respirometer

A respirometer allows measurement of changes in gas volumes as organisms respire in a sealed chamber.

As they respire, organisms consume O_2 and produce CO_2, which is absorbed by potassium hydroxide (KOH) solution. The volume of gas in the tube will fall, reducing pressure and allowing the liquid in the capillary tube to be pushed towards tube A.

Measurements can be taken after a known time by using the syringe to return the liquid to its original position and recording the change in volume in the syringe.

Tube B helps to control variables, particularly temperature and pressure. It will be affected in the same way as tube A, so changes in these variables do not affect the position of the liquid.



The apparatus shown here is a common example of a respirometer that can be used in a school laboratory. Potassium hydroxide could be replaced with soda lime as both absorb CO_2.

Design issues in this practical

The apparatus can be used to investigate a number of IVs, such as:

- organism used
- temperature.

The DV is the change in volume of gas in the tube. You can assume this to be a measure of the rate of respiration.

CVs include:

- mass of tissue used
- temperature
- pressure.

Skills tested in this practical

- ✓ Use appropriate apparatus to record a range of quantitative measurements.
- ✓ Safely and ethically use organisms to measure plant or animal responses and physiological functions.
- ✓ Use ICT such as computer modelling or a data logger to collect data, or use software to process data.

The main issue with the use of organisms is avoiding contact with potassium hydroxide or soda lime as they are harmful substances.

Worked example

In an experiment with simpler apparatus to that shown above, measurements were made of the distance moved by the liquid in the capillary tube.

Organism	Maggots	Barley seeds
Distance in 30 s / mm	150	300
Mass of organism / g	50	150
Volume of O_2 used / $mm^3\,min^{-1}\,g^{-1}$	18.8	

The diameter of the cylindrical capillary tube was 2 mm. Calculate the volume of O_2 used by barley seeds in $mm^3\,min^{-1}\,g^{-1}$. **(3 marks)**

Volume of gas used is $\pi \times 1 \times 300$ (using $\pi r^2 h$ for volume of a cylinder) $= 942\,mm^3$ in 30 s per 150 g

which is $942 \times 2 = 1884\,mm^3\,min^{-1}$ per 150 g

which is $\frac{1884}{150} = 12.6\,mm^3\,min^{-1}\,g^{-1}$

Maths skills You are expected to be able to convert between units and to calculate volumes. You should give your answer to a sensible degree of precision and always show correct units.

Now try this

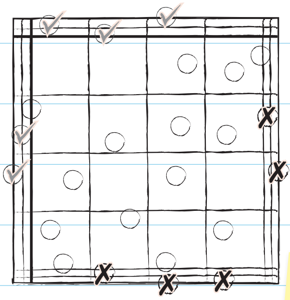
In another experiment, no potassium hydroxide solution was used and the conditions within the tubes were anaerobic. Give reasons why the liquid moved in the opposite direction for barley seeds but not at all for maggots. **(3 marks)**

Growth of bacteria

The **exponential growth rate constant** of a culture allows calculations of population size at any time during the log phase since the culture started growing.

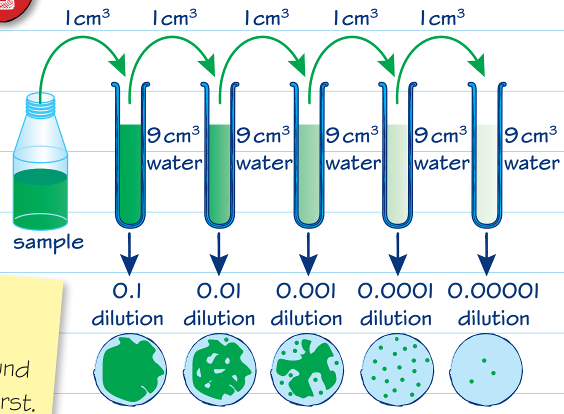
Methods for counting bacteria

1 Direct cell counts using a haemocytometer



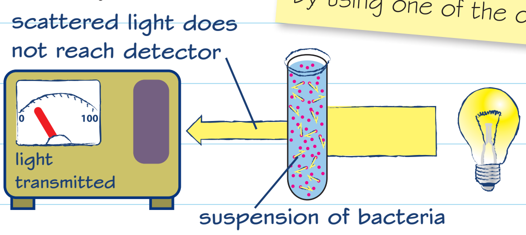
A microscopic grid with known depth of sample allows counts in a known volume using a microscope.

2 Dilution plating



A culture is diluted until the number of cultures can be counted when spread on a plate.

3 Turbidity

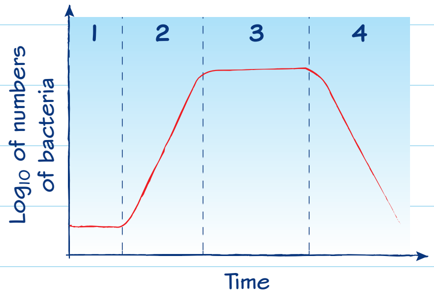


The amount of light scattered by bacteria will depend on the number present. This needs known values found by using one of the other methods first.

4 Dry mass

A known volume of culture can be dried and weighed to find the dry mass of bacterial cells.

Changes in population size in a bacterial culture



1. **Lag phase.** Slow growth as bacteria prepare to use the nutrients available, which may involve activating genes or producing enzymes.
2. **Log phase.** Growth is exponential with rapid doubling of numbers. The *y*-axis of the graph is logarithmic so the change appears as a straight line.
3. **Stationary phase.** Death rate equals division rate due to a build-up of waste and lack of nutrients.
4. **Death phase.** Population of live cells falls because deaths exceed new cell production as conditions continue to deteriorate.

Maths skills You need to be able to manipulate numbers in standard form.

Calculating population size

The number of bacteria in a population at time t can be found from:

$$N_t = N_0 \times 2^{kt}$$

N_t = number of organisms at time t

N_0 = number at the beginning of growth

k = exponential growth rate constant given by

$$k = \frac{\log_{10} N_t - \log_{10} N_0}{\log_{10} 2 \times t}$$

An example of how to use these equations is given on page 87.

Worked example

Five dilutions of a bacterial culture halved the density each time: 36 colonies were found in 0.1 cm³ of the final dilution. Calculate the number of bacteria in 1 cm³ of the original culture, expressed in standard form. **(3 marks)**

Five dilutions will give 2^5 × number of bacteria (2 is the dilution factor and 5 the number of dilutions).

So $2^5 \times 36$ is the number in 0.1 cm³ of undiluted culture = 1152

So in 1 cm³: $1152 \times 10 = 11\,520 = 1.152 \times 10^4$

Now try this

Explain why a bacterial culture can grow exponentially for only a limited time. **(3 marks)**

Dihybrid inheritance

Two non-interacting **unlinked genes** will be inherited **independently** of each other. The expected ratios of offspring with each characteristic will be the same as if the genes were considered separately.

Independent assortment

Mendel's second law

This states that the inheritance of one characteristic will have no effect on the inheritance of another. It has become clear that this only applies when genes are not linked on the same chromosome (see page 112).

Dihybrid crosses look at the pattern of inheritance when two genes are considered at the same time.

Dihybrid genetic diagrams

Diagrams for dihybrid crosses should be set out similarly to those of monohybrid crosses (see page 110). This example uses the same flower colour gene (P = purple, p = white) and includes a gene for height of plant (T = tall, t = short).

The main difference is in showing the genotype for both genes at the same time:

	Monohybrid		Dihybrid
Parent genotypes	PP × pp	TT × tt	PPTT × pptt
Possible gametes	P p	T t	PT pt
F_1 genotypes	Pp	Tt	PpTt

Don't confuse the issue by writing the same gamete four times for the dihybrid cross – a homozygote can only pass on one type of allele for the gene. Check you have one of each letter in every gamete.

The next generation shows all possible combinations of inheritance for the two genes:

F_1 genotypes	PpTt	PpTt
Possible gametes	PT Pt pT pt	PT Pt pT pt

F_2 genotypes

	PT	Pt	pT	pt
PT	PPTT	PPTt	PpTT	PpTt
Pt	PPTt	PPtt	PpTt	Pptt
pT	PpTT	PpTt	ppTT	ppTt
pt	PpTt	Pptt	ppTt	pptt

This works out as a **9:3:3:1 ratio**:

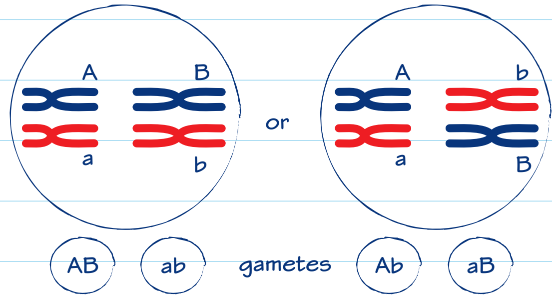
9	3	3	1
purple tall	purple short	white tall	white short

Notice the pattern for each of the two genes still works out independently as the expected 3:1 ratio.

- For the colour gene, the 3:1 ratio is shown by repeated genotypes in each quarter of the grid.
- For the height gene, the 3:1 ratio repeats in each quarter of the grid.

Explaining the 9:3:3:1 ratio

1 The four different types of gamete arise because the chromosomes arrange randomly during meiosis.



2 The ratio is all combinations of two 3:1 ratios:

$$(3P + 1p)(3T + 1t) = (9PT + 3Pt + 3pT + pt)$$

3 The probability of purple **and** tall is given by the probability of purple times the probability of tall:

$$\frac{3}{4} \times \frac{3}{4} = \frac{9}{16}$$

Worked example

Maths skills

A plant has purple and white alleles for flower colour and tall and short alleles for height. All of the offspring from a cross between tall purple plants and short white plants were tall and purple. Calculate the probability that a plant produced from a cross between one of these offspring and a short white plant will be tall. **(5 marks)**

Genotypes	PpTt	pptt
Possible gametes	PT Pt pT pt	pt

Offspring

	PT	Pt	pT	pt
pt	PpTt	Pptt	ppTt	pptt

Phenotypes	purple tall	purple short	white tall	white short
Probability	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$

Probability of tall is $\frac{1}{4} + \frac{1}{4} = \frac{1}{2}$ (0.5)

Now try this

Explain why a single meiotic division in an organism heterozygous for two unlinked genes can only form two types of gamete, but four types of gamete are shown in a genetic diagram. **(4 marks)**

Reaction rates

The rate of an enzyme-catalysed reaction slows down as substrate is used up, so to get a true measure of reaction rate, the **inital rate** should be measured.

Practical skills — Investigating rates

In one possible experiment, protease is used to break down a protein solution, which is cloudy.

Protease is used to break down a cloudy protein solution

↓

Decrease in cloudiness is measured with a colorimeter, taking an absorbance reading every few seconds

↓

Results can be plotted as absorbance (y) against time (x)

↓

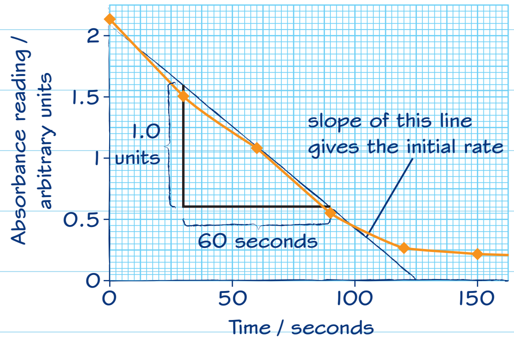
The gradient of the straight line graph is calculated

↓

From this, the initial rate is calculated

Maths skills — Calculating rate

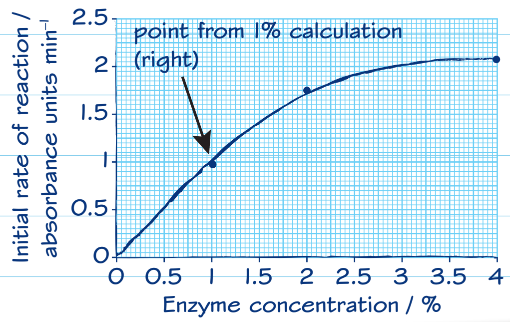
The results below were gained at enzyme concentration 1%.



Rate is change in absorbance over time.
Change over 60 seconds is 1.0 arbitrary unit.
So, rate is 1 unit min^{-1} at 1% enzyme concentration.

The effect of enzyme concentration

Measure the initial rate for each enzyme concentration. Plotting these results gives a graph showing the effect of enzyme concentration on initial reaction rate.



Effect of enzyme concentration on initial reaction rate

Practical skills — Time and rate

✗ A time taken is not a rate!

✗ $\frac{1}{time}$ gives an indication of rate, but it is still not a rate without knowing how much substrate is used or how much product is produced.

✗ The experiment on this page does not actually give a true rate as the units are arbitrary (AU). To convert to a true rate in, say, mg protein broken down per second, you would need to convert AU to mg.

Worked example

Explain the effect of enzyme concentration on initial reaction rate. **(4 marks)**

As concentration of enzyme increases, so does initial rate of the reaction. A point is reached when the curve levels off. This is because when there is not very much enzyme and lots of substrate, every active site is full. Add more enzyme and there are more active sites and lots of substrate, so the rate goes up. Eventually adding more enzyme achieves nothing as there is not enough substrate to fill all the active sites.

Very often, when you are asked to explain something there will need to be some description as well.

Now try this

Explain why it is necessary to measure the initial rate of reaction when investigating the effect of enzyme concentration on the rate of reaction. **(3 marks)**

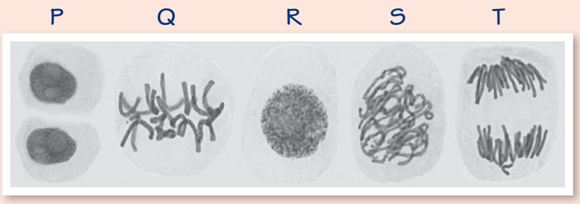
Had a look ☐ Nearly there ☐ Nailed it! ☐

Exam skills

This exam-style question uses knowledge and skills you have already revised. Have a look at pages 33 and 34 for reminders about **mitosis** and how to examine mitotic cells.

Worked example

(a) The illustration shows the main stages in the process of mitosis.



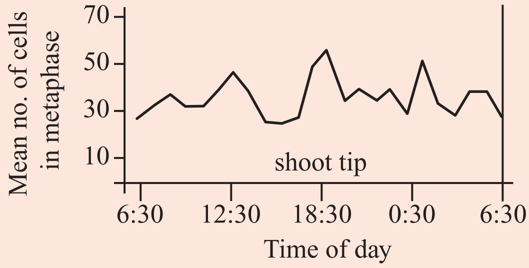
What is the correct order in which they occur? **(1 mark)**

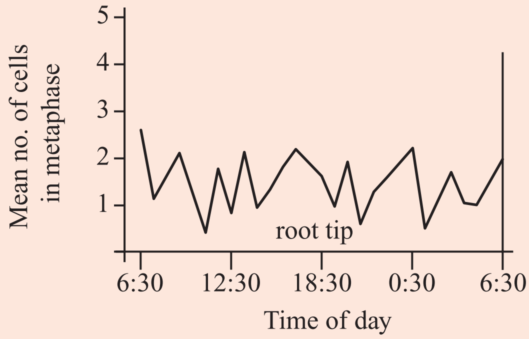
☐ **A** R → S → T → Q → P
☒ **B** R → S → Q → T → P
☐ **C** S → R → T → Q → P
☐ **D** P → S → Q → T → R

(b) Explain the procedure that was carried out to enable the cells to be viewed as shown. You should start with fresh root tips from an onion. **(5 marks)**

The root tips were fixed in ethanol to preserve the structure of the cells. A 2–3 mm piece was removed from the tip because this is where dividing cells are located. This was placed in concentrated HCl to macerate the tissue, that is, to dissolve the 'glue' between the cells. The tip was then placed on a slide and ethanoic orcein was added to stain the DNA. Finally, a cover slip was added and pressed firmly to separate the cells from each other.

(c) A student decided to investigate daily rhythms in mitosis in a grass. The following data were obtained.





Compare and contrast the pattern shown for shoot tips and root tips. **(4 marks)**

Both show a change in the number of cells undergoing mitosis throughout the day. Mitotic activity in the shoot tip is much higher than that in the root tip. Shoot tips show three distinct peaks at 12:30, 18:30 and 1:30, roughly every 6 hours. Although the root tip shows change in numbers through the day it is much less regular than in the shoot.

Multiple Choice Questions (MCQs)

MCQs are often considered to be easy, but this is by no means true.

You should approach them by eliminating the three answers you think are wrong. Then, as a double check, make sure the one that is left is correct.

In this case, you can eliminate D, as the end product should have two cells (P). The first two steps involve the chromosomes becoming more distinct, so that suggests R and S – and they are more distinct in S than R, so that eliminates C. It is now just a question of which way round are T and Q. In Q the chromosomes are lined up in the centre, in T they are separating, so the order is QT.

Practical skills

This refers to a Core Practical (CP3), so you should know how it is done. In addition, the question asks you to **explain**. In explanations of practicals, or ones where you have to say how you would modify a practical, you need to say **why** you did what you did to get the marks.

Command word: Compare and contrast

If you are asked to do this you must point out at least one similarity and one difference. The total number of marks indicates the number of points you should make.

The cell theory

Cell theory states that new cells are formed from other existing cells and that the cell is a fundamental unit of structure, function and organisation in all living organisms. Cell theory is now accepted as a unifying concept in biology.

Cells are tiny!

The fact that living things are made from cells was not discovered until relatively recently. This is because cells are so small they cannot be seen unaided.

Robert Hooke invented one of the earliest microscopes in 1665 and saw cells for the first time, in tiny slivers of cork.

The first picture of cells in cork, from Hooke's Micrographia (1665).

An exception to the rule?

If all living things are made of cells, then what about viruses? They are not made of cells, so perhaps the rule is wrong.

The alternative is that viruses are not living organisms.

There is more about viruses on pages 29 and 30.

The three architects of cell theory

 +  +  = cell theory

Matthias Schleiden (1804-1881)
the botanist:
plants are made of cells

Theodore Schwann (1810-1882)
the physiologist:
animals are made of cells

Rudolf Virchow (1821-1902)
the doctor:
cells come from pre-existing cells

The three sets of conclusions of these three scientists came together as the **cell theory**.

Cells, tissues and organs

In complex organisms, that is animals and plants, cells are organised into tissues, tissues into organs, and organs into organ systems.

muscle cell	muscle	heart	circulatory system	human
specialised cell →	tissue →	organ →	system →	organism
xylem vessel	xylem	vascular bundle	transport system	bush

Worked example

Plants are complex organisms that contain different tissues and organs. Explain what the term 'tissue' means. **(2 marks)**

A tissue is a group of cells working together to carry out a particular function.

Always look at the number of marks and make the correct number of points. Here you might think 'a tissue is a group of cells' is enough, but this is just one point.

Now try this

Describe one way in which tissues and organs are similar, and one way in which they are different. **(2 marks)**

Had a look ☐ Nearly there ☐ Nailed it! ☐

Biodiversity

The variety in the vast array of life on Earth is its diversity, or **biodiversity**.

What is biodiversity?

- Number of different ecosystems.
- Number of species.
- Number of individuals within each species.
- Genetic variation within each species present in an area.

Maths skills: Measurement within a habitat

An **index of diversity** is the most widely used formula for measuring biodiversity of an area at the species level.

$$D = \frac{N(N-1)}{\Sigma n(n-1)}$$

Where:

N = total number of organisms of all species

n = total number of organisms of each individual species

Σ = sum

D = index of diversity

This takes into account species richness (number of species) and abundance of each species.

The higher the value of D, the greater the biodiversity of the area.

Why conserve biodiversity?

Economic reasons (ecosystem services):

- purification of air and water
- waste decomposition
- stabilising the atmosphere and the world climate
- nutrient recycling in ecosystems
- provides genetic diversity for the production of crops and medicines.

It would cost money for machinery to do all these things, even if it were possible.

Ethical reasons:

- spiritual enrichment
- cognitive development
- reflection
- recreation
- aesthetic experiences.

Measurement within a species

The cause of variation within species is the great variety of **alleles** found in the **gene pool**. All organisms of the same species have the same genes but, often, different forms of them (alleles). The bigger the gene (allele) pool, the more genetically diverse the species.

A species with a small gene pool: the cheetah, *Acinonyx jubatus*, where all individuals are almost genetically identical.

A species with a large gene pool: *Brassica oleracea*, has been selected to give crops such as cabbage, cauliflower, Brussel sprouts and broccoli. This has been possible because of the large gene pool.

Worked example

Explain why extinction will reduce biodiversity.

(3 marks)

Biodiversity depends on the number of species. Extinction will reduce the number of species as well as ones that depend on them.

This question is worth 3 marks so you need to make three points.

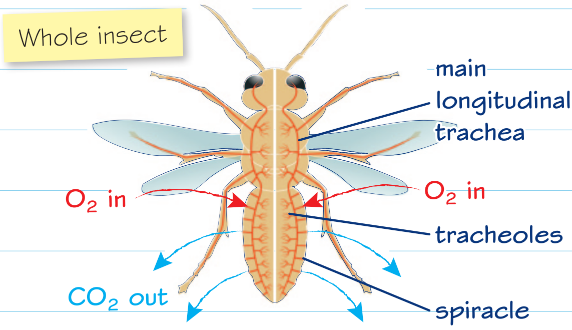
Now try this

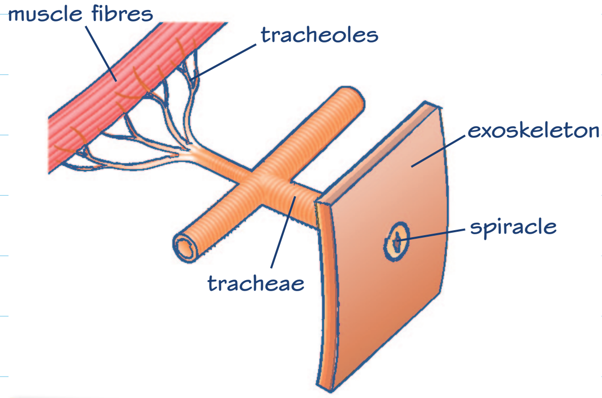
What further data, apart from species richness, is needed to compare the biodiversity of two sites?

(4 marks)

Gas exchange in insects

Insects are invertebrates. They are complex animals, with relatively high oxygen demands. They have a specialised gas exchange system, very different from that of vertebrates.





Detail of one spiracle

Insect bodies have a branching system of tubes (the tracheae and smaller tracheoles) running through them. Tracheae are lined with spirals of chitin (a structural polysaccharide), which keeps them open, but is also impermeable to gases. Smaller tracheoles spread through insect tissues and even into individual cells. They have no chitin, so are freely permeable to gases.

Tracheae open to the outside by pores called spiracles. These are found along the thorax and abdomen, and in many insects can be opened and closed by sphincters.

The tracheal system of an insect

Oxygen diffuses into the tracheae through the spiracles and then into the tracheoles. It then travels to the cells of the organs. Carbon dioxide leaves in the opposite way. Most of the gas exchange occurs in the tracheoles.

In large, very active insects, such as bees and flies, the abdomen can be pumped in and out to draw in more air. This is called mechanical ventilation.

The insect gas exchange system therefore provides a large surface area, with its network of thousands of tiny tracheoles, which have very thin surfaces. In very small insects, the gradient of O_2 and CO_2 between inside and outside is maintained by its use within the body, keeping the concentration of O_2 lower than in the air and that of CO_2 its higher.

Worked example

Discuss the safety and ethical issues that need to be taken into account when dissecting an insect.

(4 marks)

An insect has a simple nervous system, so reduced awareness. If a suitable species is chosen, e.g. *Locusta*, it is abundant in nature so there is no threat to it or its dependent species by using it – locusts are bred for this purpose. Take care to avoid cuts from sharp dissection tools. Keep these implements in an ordered fashion in a tray when not in use. Wash hands with soap after handling insects. Inform the teacher if you suspect you may be allergic to locusts. Methylene blue is low hazard when diluted. Wear eye protection when using the stain and wash with water if there is any contact with skin.

Practical skills — Insect dissection

An insect can be dissected to show the structure of the gas exchange system.

You will need fine dissecting instruments such as scissors and scalpel, as well as forceps. The dissection will need to be done, or at least viewed, under water so that the air-filled tracheae and tracheoles can be easily seen, as they will be silvery. This is best done in a dissecting dish lined with wax on its base so the animal can be easily pinned in place.

A dissecting microscope or hand lens is best for looking at what you are doing when you dissect. You will need a more powerful compound microscope to view the tracheae and tracheoles.

You should record your observations in labelled diagrams and you may wish to take photographs.

Now try this

Describe the route taken by an O_2 molecule from the air outside to a muscle cell in an insect's leg.

(4 marks)