# IB Biology Investigations

## Volume 2 (Higher Level)

# COPY MASTERS

*(For use with the IB Diploma programme)*

**(Fourth edition)**

**Author: Paul Billiet**

**Series editor: David Greig**

Produced by: 'Science Teaching And Resources' ('S.T.A.R.')
Published by: IBID Press, Box 9, Camberwell, 3124, Victoria. Australia

Library Catalogue: Billiet Paul
1. Biology Investigations
2. International Baccalaureate
Series Title: Investigations for IB Sciences

First published in 1999. Fourth edition published 2014

ISBN: 978-1-921784-41-5

The contents of the volumes in this series are continually being improved and are subject to change without notice. They have been revised and reprinted to incorporate recent IBO syllabus changes (© IBO 2013). All possible endeavours have been made by the publishers to ensure that the contents of this resource are correct and appropriate. However, the Publishers accept no responsibility for any errors made by the authors. The contents and materials are solely the responsibility of the authors.

Although every care has been taken in preparing and trialling these investigations and safety advice has been given, absolutely no responsibility whatsoever can be accepted for any damage or accident which may occur for whatever reason during the conduct of any of these activities.

These Investigations have been developed independently of the International Baccalaureate (IB) Organisation. This publication is in no way connected with, or endorsed by, the IB.

# Publishing Information

This volume of practical Investigations is part of a series of resources for IB Sciences produced by IBID Press.

More information about this series and other IB products may be obtained from:
IBID Press, PO Box 9, Camberwell, Victoria, Australia 3124
Phone: +61 3 9747 0840 Fax: +61 3 9747 0840 URL: www.ibid.com.au

# Copyright Information

## Copying for educational purposes



Cover: Dragonfly Sympetrum sanguinium sunning itself. © 2013 author P Billiet

# Author profile

**Paul Billiet** B.Sc. (Biology), M.Sc. (Ecological Systems), P.G.C.E., C. Biol.

Paul has devoted his career to the teaching of IB Science and Biology in particular. He is Head of Science at *École Active Bilingue Jeannine Manuel* (EABJM) in Paris and he has taught Biology for over 38 years, including 32 years teaching IB Biology.

During his involvement with the IB, Paul has been examiner for various components of IB Biology and Environmental Systems as well as authoring several exam papers. He served as Deputy Chief Examiner for Biology from 1997 to 2002.

Paul has been involved in curriculum reviews for Biology since 1986. He has taken part in the development of internal assessment in both Biology and Sports Exercise and Health Science. As Principal Moderator for Biology, Paul was part of the committee that developed the current Internal Assessment (IA) model.

# Author's acknowledgements

Firstly I would like to thank my School's Administration for its support throughout and my students who, over the years, have trialled these Investigations.

Colleagues, too numerous to cite all by name, at EABJM and around the world, have provided me with useful tips and guidance over the years. Whether in formal workshops or over a coffee, their combined experience was an enormous asset. Special thanks for pointing me in the right direction go to Andrew Allott, Shirley Burchill, Alan Cadogan, Catherine Casalis, Alan Damon, Alison Davis, Guy Décarie, Sandra Deighton, Paul Fairbrother, Karen Frey, Robert Gaurenne, Lonnie Guralnick, Lionel Guerin, Jazcqueline Gout, Deborah James, John Knopp, David Mindorff and Adrian Thompson.

My thanks also go to two young medical students, Mathilde Scarton and Pierre-Antoine Billiet, for guidance on several issues of medical physiology. I must not forget the laboratory technical assistants and laboratory managers at EABJM in acquiring and preparing material and equipment. Without them a lot of the preparation for this collection would have been impossible.

I would also like to thank to my editor David Greig, who encouraged me to write for IBID Press and has reminded me, diplomatically, ever since of the 'deadlines'.

Finally, a very special thanks to my wife, Evelyne, who has patiently put up with the impact of those 'deadlines' during the writing of successive editions of 'those books' over the years. Without her support it would never have happened.

*Paul Billiet* *June 2014*

# Editor profile

**David Greig** (B.Sc., Dip. T(Sec)., Dip. Ed., Grad. Cert. Sc. Ed.)

David has been a teacher of senior Biology for 40 years or so in South Australian schools. For the last 15 years or so he has worked as a consultant on a wide variety of writing, editing and publishing projects, producing Science materials for use in Australian Schools, and also internationally, for use with the International Baccalaureate Programmes.

# Editor's acknowledgements

David wishes to acknowledge the author Paul's very high level of expertise, enthusiasm and the excellent work done by him over the years on the successive editions of these Biology Practical Investigations. It has not always been 'a walk in the park' on a warm and sunny autumn afternoon in Paris!

In addition David wishes to acknowledge the invaluable support, friendship and encouragement offered by technical guru Colin Flashman, publisher Rory McAuliffe, proof reader Jenna Crierie, wife Mary and other friends and family. A project like this does not happen by accident!

# Foreword

This collection of Investigations/Activities is part of a series which has been written specifically to support the teaching of the practical component of current International Baccalaureate Science courses (IBO). Volumes have been written for Biology, Chemistry and Physics at both Standard (SL) and Higher level (HL). These Investigations and Activities have been written by an experienced IB teacher with very close reference to the syllabus, which is current at the time of writing. It is vital that staff consult syllabus and assessment guidelines which are current at the time they use this material because these syllabus documents are subject to review and modification.

These copy Masters and accompanying Teaching Notes may be used by anyone in the purchasing school but may not be passed on to another school. The Investigations and Activities in this volume will fit comfortably into a Practical Scheme of Work as required (see below), but they are not intended to be exclusive or exhaustive. Syllabus Correlation Tables are provided showing the relevant Skills and Applications for the various Topics and sub-Topics and suggested focus for each Investigation.

This collection of Investigations does not in any way form a proscribed Practical Scheme of Work (PSOW). They are meant primarily to give teachers ideas of the sort of experimental work that is suitable for a PSOW and to assist teachers in designing their own PSOW. In some of the activities the method of analysis is quite clearly laid out but where possible the students must be encouraged to design and draw up their own data tables, decide the best way to analyse the data of a particular experiment and make their own evaluation of an experiment. These are all necessary skills that the students need to acquire if they are to be successful in their assessed Individual Investigation. It is hoped that this set of Investigations will help the students achieve this requirement. Suggestions are made where planned Investigations may be developed from the Investigations in this collection.

For the sake of consistency, the Options have been numbered as follows: Option A will be Chapter 12, Option B will be Chapter 13, Option C will be Chapter 14 and Option D will be Chapter 15.

The author has trialled all of these Activities and he has suggested various safety precautions but will not accept any responsibility whatsoever for any accident that may arise during the conduct of these Investigations and Activities. The publisher (IBID Press) will be pleased to receive any suggestions and comments from staff or students using this material. The ideas for the Practicals appearing in these volumes arise of a variety of sources and multicultural exchanges.

Within this Volume, we hope you will find many new ideas. Many have been redesigned from ideas generated from conversations or observations. We are indebted to those scientists and teachers who designed and developed the original experiments, from which many of these Practicals have been developed, and on behalf of the teachers and students that use them we warmly acknowledge these pioneers. We also acknowledge and thank those that generated ideas and suggestions that helped us.

*Please refer to www.ibid.com.au for current information about our complementary full colour IB Biology Textbook and other publications.*

*P. Billiet, (Author)*

*Paris 2014*

# Health and Safety Symbols

Laboratories can be hazardous places. Often scientists, Science teachers and students handle equipment and materials which can be dangerous to their health and safety. Throughout these Volumes of Investigations you will see a number of symbols and warnings which will represent particular hazards. For each of these we will briefly describe the hazard and indicate what precautions you should take to avoid damage and/or what responses are appropriate. In all cases, of course, you should seek advice and assistance from the teacher or laboratory technician.


A biohazard is any organism or body fluid which could possibly cause illness or disease in your body. This particularly includes micro-organisms.


A flammable substance is one which will readily burn in air. It may be a solid, liquid or gas. If you are using such a substance it is vital that there are no sparks or naked flames which could ignite it. It is vital that you know what to do in the event of fire. This may include the use of fire extinguishers and evacuation procedures.


A radioactive substance is one which emits particles or 'radiation'. This radiation is known to cause damage to cells and may also be cancer causing. If you are using radioactive substances it is vital that you wear protective clothing, use metal tongs and listen carefully to instructions given by your teacher or laboratory technician.


Sharp instruments are often used in Science and particularly in Biology, to cut sections through plant or animal tissue. These instruments, which include scalpels and razor blades are very sharp and obviously will also cut through your tissues. When using these instruments it is essential that you always cut away from your body and preferably onto a cutting board. It is also important to be very careful when carrying these instruments and also ensure they are placed on the workbench in a safe place.


When certain chemicals are mixed together they can become explosive. An explosion is caused by rapid expansion of gas in a confined space and can be very dangerous. Sometimes it is important to ensure that the space is not confined and sometimes it is important to conduct these reactions behind a protective screen.


It is often necessary to protect your hands from heat, chemicals or other hazards and gloves will be made available for these situations. The type of glove needed will depend on the particular hazard and your teacher will provide further advice. In some cases you will be advised to dispose of the gloves after use and in other cases to wash and dry them carefully.


Your eyes are the most vulnerable and easily damaged external part of your body. This is why they must be protected if you are using solids and liquids which could get into them. Whenever you are heating things or using corrosive liquids, and in other cases as instructed by a teacher, you should wear safety goggles. You should also do this if possible even if you wear spectacles to correct your vision. In the event that something gets in your eye you should immediately make use of the eyewash facility in the laboratory as instructed and then notify your teacher.


Some chemicals, which are used in a laboratory, are *corrosive*. This means that they can react with and 'eat away' materials like the bench, your books, clothing and skin. It is essential that you handle these materials, which are usually liquids, with care. Always tip from the container with the label uppermost, never add water to concentrated acid and never have your face anywhere near the container. It is usually advisable to wear both safety goggles and gloves. If protective aprons are available you should also wear one.

As a general rule, 12 or 24 volt *electrical* appliances are unlikely to cause serious injury. However, 'mains' voltage (110V or 240V or higher) can cause serious injury or death. The appliances you use should be regularly tested and certified safe. If you notice sparks or smell insulation burning, turn the power off immediately and notify staff. Be particularly careful not to allow water to get into any appliance as it may cause a short circuit.


Some chemicals are *poisonous* and should not be inhaled or ingested. It will be necessary to use a fume cupboard when using poisonous gases or volatile liquids. They could make you very ill and you may require medical assistance. It is vital that you listen to instructions, follow them carefully and notify your teacher immediately if there is accidental exposure to poisonous or toxic substances.


Lasers are very intense beams of light. They are capable of causing burns to the skin and permanent damage to the eyes. It is essential that these are only ever used under the supervision of a teacher and in a situation where people can not see the beam directly or when it is reflected from a shiny surface. Sunglasses or welding masks do not provide sufficient protection and special 'laser glasses' must be used where there is a risk.


UV light is harmful to skin and especially eyes. Do not expose these areas directly to a UV light source. If it is not avoidable, sunscreen can be applied to the skin and special goggles should be worn.


There are other *dangers* or hazards as well, for example carrying heavy or hot objects. This may also include chemicals which are not poisonous but which may smell unpleasant or irritate the skin. Whenever you see this icon more information will be provided in the adjacent text about the specific danger.


In Science and particularly in Biology, there are situations when ethics and ethical issues need to be considered in experimental work. This is particularly the case when human volunteers are being used, not just for experimental work but also when they are being surveyed to collect personal information. In these cases a consent form should be used to explain the nature of their involvement and to get their approval. Ethics will also be an issue whenever animals are used in experimentation or when they are collected in the field. They should not be exposed to conditions that are outside their natural range of tolerance and wild animals must be released back where they were sampled with the minimum of disturbance.


The environment and environmental issues become important when hazardous substances are used or produced during an experiment. Their disposal must result in minimal impact on the environment. In field work the protocol that is used must reflect practices that minimise the impact of the investigation on the site.


## IMPORTANT NOTE

**Although every care has been taken in preparing and trialling these investigations, absolutely no responsibility or liability whatsoever can be accepted for any damage or accident which may occur for whatever reason during the conduct of any of these activities. The Safety Warnings and Icons are advisory only and are not intended to be exhaustive or exclusive. It is a strict condition of sale that safety in the laboratory is the responsibility of the staff and students doing the laboratory work and not the author, editor or publisher of this work.**

# Scientific Investigations

Each time you design an investigation check that you have done the following.

## Design

- Have I consulted the literature and written a short concise introduction establishing the theoretical background?
- Have I correctly cited my sources?
- Have I stated my aim or objective (research question)? It should contain reference to the independent and dependent variables.
- Does my investigation have a clear purpose?
- Have I identified the origin of the material used and/or the species used by their scientific names?
- If it is valid, have I written my hypothesis (a justified prediction)?
- Have I clearly identified the variables?
- Which variable will I change (the independent or manipulated variable)?
- Which variable will I measure/observe (dependent or measured variable)?
- Which other variables do I need to control (which ones will affect the experiment)?
- How significant are each of these control variables?
- Which variables cannot be controlled but need to be monitored?

## Method

- What equipment and materials will I need?
- Can this be best described using a labelled diagram or photograph of the experimental setup?
- What safety factors should I bear in mind?
- How exactly will I measure the dependent variable?
- What must I do to control all the other variables identified in the controlled variables?
- If I cannot control these variables, then how will I monitor them or do I need a control experiment? If so, have I described it? (Note: controlled variables and a control experiment are not the same thing).
- How can I make my experiment a fair test?
- What range am I setting for the independent variable?
- How often will I measure the independent variable, or at what intervals?
- How many trials do I need to be sure of my results?
- What safety factors should I bear in mind?

## Results/Data

- How should I present the data (e.g. tables, annotated drawings, map)?
- How precise must I be with my data?
- What was the precision of the instruments I used?
- Where are the errors in my measurements and how big are they likely to be?
- Have I set up a data table for my raw results? Try visualising this first and sketching it out roughly by hand.
- Has the table got a clear title ('The results' is insufficient)?
- Are the headers of my table columns clear?
  e.g. the name of the variable, the units, the uncertainties (e.g. Time / s ± 0.1s).
- Have I explained my uncertainties?
- Is the independent variable first in the table (top line or left hand column)?
- Are all my data to the same degree of precision (same number of decimal places)?
- Have I centred my data in each column?
- Are my data to the same precision as my uncertainties?
- Did I see anything else happen during the investigation that needs to be described?
- During the experiment, did I make any significant qualitative observations?(Photographs could help here)

## Processing and Analysis of Data

- Do I calculate a change, a proportion, a percentage, an average of the repeats, and a standard deviation of the repeats?
- Are my calculations easy to follow? For those done in a spreadsheet, a screen shot can be included.
- Are my processed results to the same precision as my raw data?
- Do I present these data as a table, graphically or both?
- If graphically, what sort of graph is best? Line graph, scatter plot, bar chart histogram, pie chart…?
- Try visualising by hand what you want to produce as a graph BEFORE you go to a program like MSExcel.
- Am I respecting the conventions for graphs?
- Do I need to analyse the graph to obtain a result?
- Do I look for intercepts (e.g. where a line cuts an axis)?
- Do I calculate a gradient to establish a rate?
- Do I establish an optimum? Do my graphs have clear titles? What, precisely, do they show and what are the error bars representing?
- Have I presented the results such that the uncertainties are clear? For example have I used error bars or drawn a trend line if one can be drawn?
- Do I need to analyse the data statistically to obtain a result (e.g. t-test, $\chi^2$ test, correlation)? If so, which is the correct one to use?

## Discussion of results

- What do the results show? Are there any trends?
- What can I interpret from the results?
- Can I explain the results in a systematic way and come to a conclusion?
- Are the results consistent with what I expected?
- Do they fit in with the theoretical background (the literature values)?
- Have I correctly cited my sources?
- How consistent are the results?
- Is there a lot of scatter beside the trend line?
- Are the standard deviations particularly large?
- Have any results been justified as outliers and excluded?
- Can I explain any unexpected results?
- What are the sources of error: in my method, in the manipulation, in the analysis?
- How significant were each of the weakness that I have identified?
- What improvements could be made?
- Do I need to suggest a new hypothesis to account for the results?
- How could I take the investigation further?
- Have I added footnotes where there are direct quotes or citations?
- Have I included a bibliography of the references that were consulted?

## Style

- Keep it impersonal (e.g. "The tubes were left for 10 minutes to incubate" instead of "I left the tubes for 10 minutes to incubate").
- Use subheadings to organise your report (Discussion, Conclusion, Evaluation etc).

# Table of Contents

## Standard Level

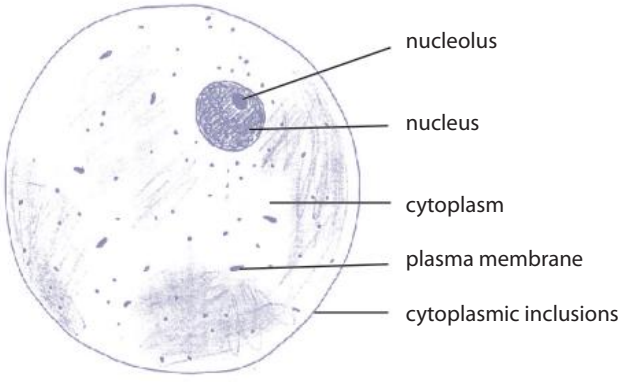
# Higher Level

**Syllabus reference: Sub-Topic 1.1 Skills**

Cells were first recognised as fundamental structures in biology by Schleiden and Schwann in 1839 when they formulated the Cell Theory. Studying cells requires the use of the microscope.

## 1A.1 Animal Cells

Animal cells compared to plant cells viewed under light microscope are characterised by what they do not possess. They usually do not possess an easily defined extracellular matrix, plastids and a sap vacuole. They tend to be smaller than plant cells and their structure is usually determined by the cytoskeleton which is not visible without special stains.




*An animal cell as seen with a light microscope*

## Preparation of a smear of liver cells (hepatocytes)

### Materials

| | | |
|---|---|---|
| microscope | forceps | filter paper |
| slides | dropping pipette | methylene blue stain |
| coverslips | mounted needle | liver tissue |
| micrometer eyepiece | cotton bud | |

Liver cells are gland cells. There are involved in a large number of functions including storing glucose as glycogen, storing vitamins and detoxifying chemicals such as alcohol. These cells divide relatively slowly sometimes at intervals of over a year.

### Method

1. Gently scrape the piece of freshly cut liver with a cotton bud.
2. Smear the cells removed onto the middle of a microscope slide. Add one drop of methylene blue and two drops of water, then place the coverslip using the mounted needle or forceps.

*Warning: Sharp instruments, handle with care*

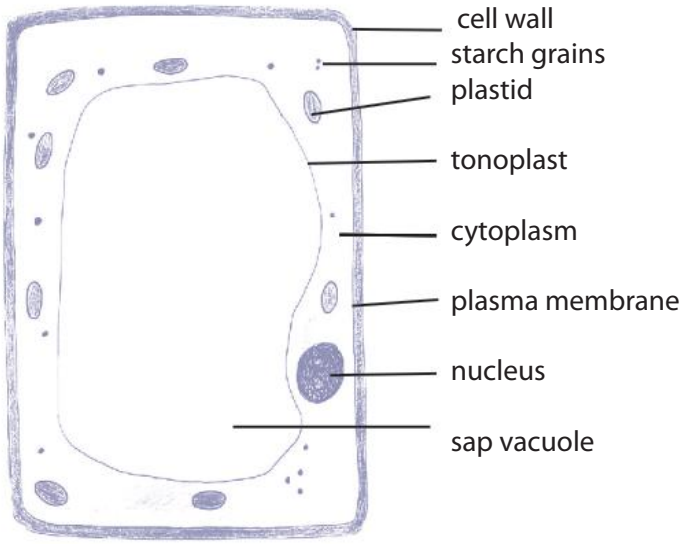

3. Observe the preparations at low power, then medium power. Scan over the slide to get a general idea of the appearance of the liver cells. Select one that appears typical, centre it and move to high power. Draw one of the liver cells from the stained preparation and label the structures that are visible. Note: Sometimes liver cells are binuclear. How does this contradict the Cell Theory?

4. Find the average size of the cells seen in your slide preparation, in micrometres (μm) by measuring a sample of the cells. Add these data on your cell drawing, showing all the data you collected clearly. Add a scale bar next to your drawing indicating the largest diameter of the cell that you have drawn.

# 1A.2 Plant Cells

Plant cells characteristically are surrounded by an extracellular matrix of carbohydrate, in particular cellulose. This cell wall supports the plant cells and determines their shape. In general plant cells are bigger than animal cells mainly due to this cell wall.

Internally plant cells have two characteristic organelles that can be observed under the light microscope; plastids and the sap vacuole. Plastids are complex organelles that include green chloroplasts. The sap vacuole, a fluid filled sack, is usually not visible without staining but some store pigments.




*A plant cell as seen with the light microscope*

| Structure | Function | Structure | Function |
|---|---|---|---|
| Cell wall | a cellulose framework. | Chloroplast | a plastid pigmented green. |
| Sap vacuole | an inflated sac containing a solution which may be pigmented. | Starch grain | a food reserve inclusion coloured blue-black in the presence of iodine solution.<br>Starch grains are laid down in amyloplasts. |

## Materials for the following Parts A, B, C and D activities

microscope
micrometer eye piece
stop watch
slides
coverslips
small beaker
forceps
lancet
scalpel
dropping pipette
filter paper
marker pen
distilled water bottle
Canadian pond weed (*Elodea*) or another suitable transparent leaf
20% sucrose solution
iodine solution
red onion
banana

# Part A: Canadian pond weed (*Elodea*)

## Method

1. Using forceps, pick a healthy green leaf of Elodea from a terminal bud and mount it in a drop of water.
2. Observe the leaf under medium and high power. The chloroplasts should be visible as small green spheres. Draw and label a few of these cells.
3. Look for cyclosis (cytoplasmic streaming). The chloroplasts will move slowly around the cell. The best area to start looking is around the midrib (midline) of the leaf. The chloroplasts that seem to disappear are moving out of the field of focus. Use the fine-focus control to follow them and to get a three-dimensional impression of the cell.
4. Use the micrometer eyepiece to measure the size of a sample of chloroplasts and using a stopwatch estimate how fast they travel. Do the chloroplasts travel at different speeds under different conditions?


*Elodea sp.*

# Part B: Red onion (*Allium*) epithelium tissue

## Method

1. Using a scalpel, cut a square, about 1cm$^2$, from an onion leaf scale. Then peel off the outer red layer cells (epithelium) using forceps.


### *Warning: Sharp instruments, handle with care*

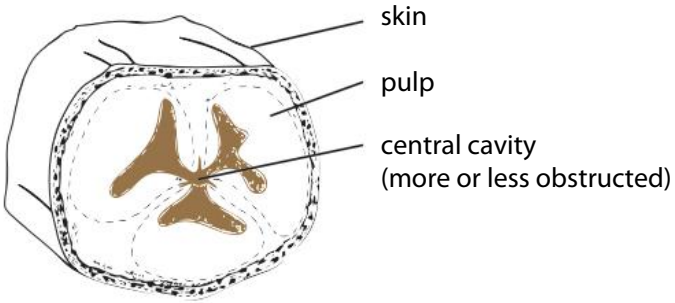
2. Mount the tissue in a few drops of distilled water. Observe the cells under medium and high power.
3. Set up another slide, this time using a few drops of 20% sucrose solution. The sucrose will make the vacuole shrink. As the vacuole shrinks, the cytoplasm will also shrink pulling the cell surface membrane away from the cell wall. In the tissue mounted in distilled water, the cells swell up with water and the cell surface membrane is pushed onto the cell wall so it is no longer visible. Mounting the cells in sucrose allows this structure to be observed.
4. Draw and measure a few of these cells, as seen in distilled water and in the sucrose solution. Label your drawing.
5. Compare the cells from inner and outer scales of the onion. Are the cells on the outer scales bigger, or are there just more of them?

## Part C: Banana (*Musa*) pulp

Some plant tissues, such as ripe fruit, are soft enough to spread the cells out into a single layer. This technique can also be used on more solid tissues that have been softened by soaking them in warm acid. This hydrolyses the cell wall material.

### Method

1. Using a scalpel, cut a slice of banana.




*Warning: Sharp instruments, handle with care*


2. Take a very small sample of pulp (the size of a pinhead) and mount it in a drop of iodine solution. Allow the fragment of banana to soak in the iodine solution for two minutes. Then place a coverslip over it and crush it lightly.
3. Observe the preparation under the microscope. Focus the image and adjust the diaphragm carefully so that the outline of the cells can be clearly seen. Draw a cell at high power and label it. Measure the cell and add a scale bar to the drawing.
4. The iodine solution will stain the starch grains blue-black. Measure the longest dimension of a sample of starch grains. Compare this to the overall size of these cells.
5. Compare bananas of different degrees of ripeness. As a banana ripens it tastes sweeter. What happens to the starch grains?

## Part D: Making a squash from tomato (*Solanum*) fruit pulp cells

### Method

1. Cut out a small piece of tomato pulp using a lancet. Mount the tissue in a few drops of water in the middle of a slide and cover with a cover slip.

*Warning: Sharp instrument, handle with care*


2. Place the slide in the middle of a piece of filter paper. Wrap the filter paper over the slide and press down firmly on the centre of the slide using your thumb.
3. Unwrap the slide and observe the cells and their contents under medium and high power. You should be able to see the coloured chromoplasts that give this tissue its red colour.
4. Using the materials available try to prepare the tissue to see if you can determine more of the cellular structure.
5. Compare normal tomatoes with cherry tomatoes. Does the cell size or shape reflect the overall shape of the fruit?
6. Compare an unripe green tomato with a ripe red one. The green colour is caused by chloroplasts and the red colour by chromoplasts. These plastids can develop from one another as do the starch grains in amyloplasts. Plastids can also be seen in the epidermal cells of red and green capsicum peppers (*Capsicum annuum*). These can be observed by pealing off the epidermal layer like the red onion cells.

# 1A.3 Drawing cells and tissues

Drawing is still a very important skill in Biology. Drawings help to record data from specimens. Drawings can highlight the important features of a specimen. Photographs can be very useful for recording data but they are not very selective - they show more detail of a specimen than you might want.

A drawing is the result of a long period of observation at different depths of focus and at different magnifications.

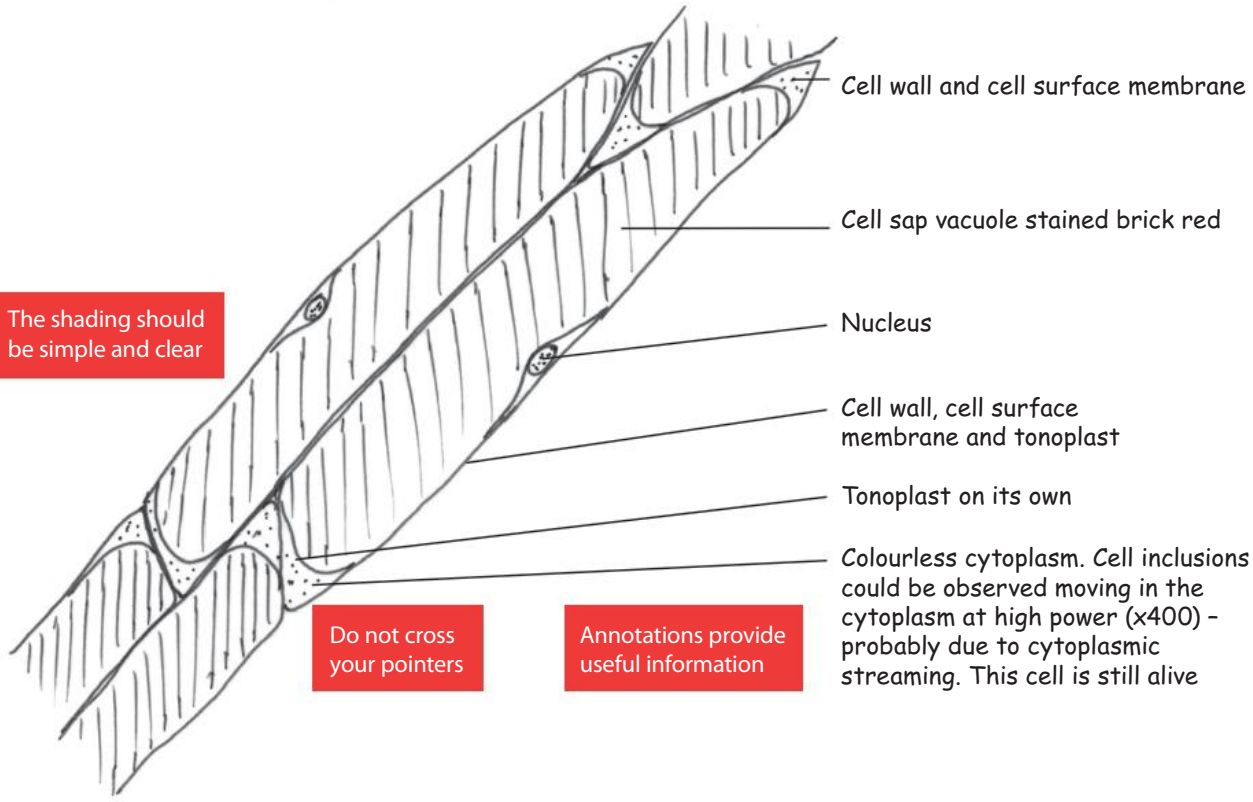
Photographs can be taken to support the drawings. It is possible to combine many photographs taken at different points of focus by stacking in programs such as Photoshop. This increases their depth of field.

## Some guidelines for drawing from specimens in Biology

- Move the specimen around, do not just concentrate on one part. Observe the general appearance first.
- Identify the most significant features (only include detail that is necessary in your drawing).
- Determine which part or parts you are going to draw.
- Use a sharp HB (medium grade) pencil.
- Use white, unlined paper for drawing.
- Make a large, clear drawing, it should occupy at least half a page.
- Keep looking back at your specimen whilst you are drawing. When drawing from a microscope it is useful to look down the eye piece with one eye and at the drawing paper with the other - it takes practice but it is possible.
- Whilst you are observing, increase the magnification to observe more details and reduce the magnification to get a more general view. Use the focusing controls on the microscope to observe at different depths of the specimen.
- A drawing is incomplete without a full title and a scale or magnification. Annotations are particularly important, they permit you to put your observations where they will have the most impact.

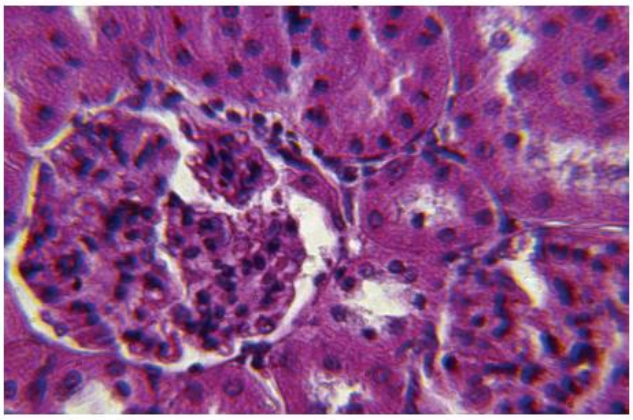
## Example from observations on onion cells

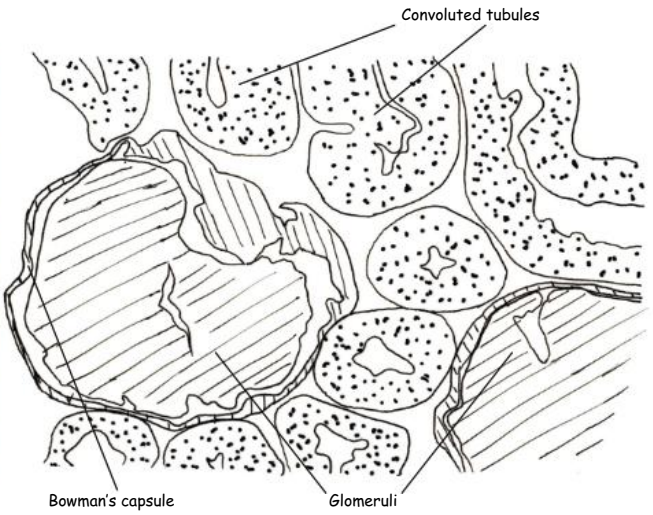
Epithelial cells from an onion bulb (*Allium cepa*) stained with neutral red at pH 7.6 maintained at 20°C.
Viewed from ×100 to ×400




## Example 2 Drawing a plan view

Identify the tissues, select your area and draw without including details of the cells.





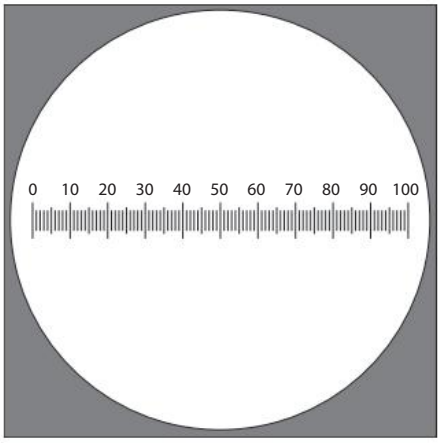
# 1A.4 Using a Micrometer Eyepiece

Measuring objects under the microscope requires a microscopic ruler. When using the light microscope, the most frequently used units are micrometres (μm). (1mm = 1000μm)

## Materials

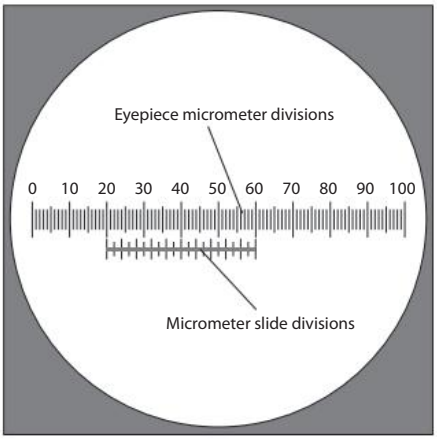
microscope lamp | eyepiece micrometer | micrometer slide | lens cleaning tissue

## Method




1. Replace the usual eyepiece lens by the micrometer eyepiece lens. Switch on the microscope lamp and look down the eyepiece. You should see a scale of 100 divisions with the numbers in the correct orientation, as shown opposite.
2. Calibrating the eyepiece micrometer: The scale in the eyepiece will depend upon the objective lens being used. You must calibrate the eyepiece for each different lens that you use.
3. The micrometer slide usually consists of 1 millimetre subdivided into microscopic divisions. (e.g. 1mm divided into 100 divisions).

   **Find out how many divisions on your micrometer slide equal 1000μm, 100μm and 10μm.**




4. Place the micrometer slide on the microscope and focus on it using the low power objective.
5. Line up the divisions on the slide with those in the eyepiece (see opposite).

   **How many divisions on the eyepiece = the divisions on the slide?**
6. Recall how many micrometres there are to each division on the slide.
7. Now calculate the number of micrometres that each division in the eyepiece represents when you are using the low power objective.

### Example for the low power objective

X eyepiece divisions = Y slide divisions

If one slide division = 10μm

Then one eyepiece division on low power = $\left[\frac{\text{Slide divisions}}{\text{Eyepiece divisions}}\right] \text{x10μm} = \left[\frac{\text{Y}}{\text{X}}\right] \text{x10μm}$

### For example

If 10 eyepiece divisions = 25 slide divisions

Then one eyepiece division on low power = $\left[\frac{25}{10}\right] \times 10\,\mu\text{m}$

Therefore, on low power of this microscope, each division in the eyepiece will measure 25μm.

Repeat this for the other Objective lenses and keep a record of your results. If you use the same microscope in the future you will not need to calibrate it again.

Now the eyepiece micrometer can be used like a simple ruler.

You might use the micrometer to measure the size of an animal cell, the size of a plant cell, the size of a nucleus or the speed of moving objects under the microscope.

# Investigation 1B - Estimating the water potential of plant tissues



**Syllabus reference: Sub-Topic 1.4 Skill**

In this experiment osmosis will be measured in a piece of tissue made of millions of cells. The water potential of the tissue can be determined by the change in size of the tissue or the change in mass of the tissue or the change in density of the liquid surrounding it.

Sucrose is a disaccharide the easily dissolves in water lowering its water potential. However, it is a molecule that is too large to pass across the cell membranes of the plant cells.

These methods can be used to compare the water potentials of plant tissues that can be cut into regular shapes.

## Materials

6 specimen tubes with caps

marker pen

potato chip cutter

scalpel

fine forceps

ruler

electronic balance

paper towel

6 labelled beakers:

one containing 50cm$^3$ of distilled water (i.e. 0 mol dm$^{-3}$ sucrose) and the other five containing 50cm$^3$ of:

- 0.2 mol dm$^{-3}$
- 0.4 mol dm$^{-3}$
- 0.6mol dm$^{-3}$
- 0.8 mol dm$^{-3}$ and
- 1.0 mol dm$^{-3}$ sucrose

12 clean, dry test tubes in a rack

methylene blue powder

spatula

Pasteur pipettes

10cm$^3$ syringe

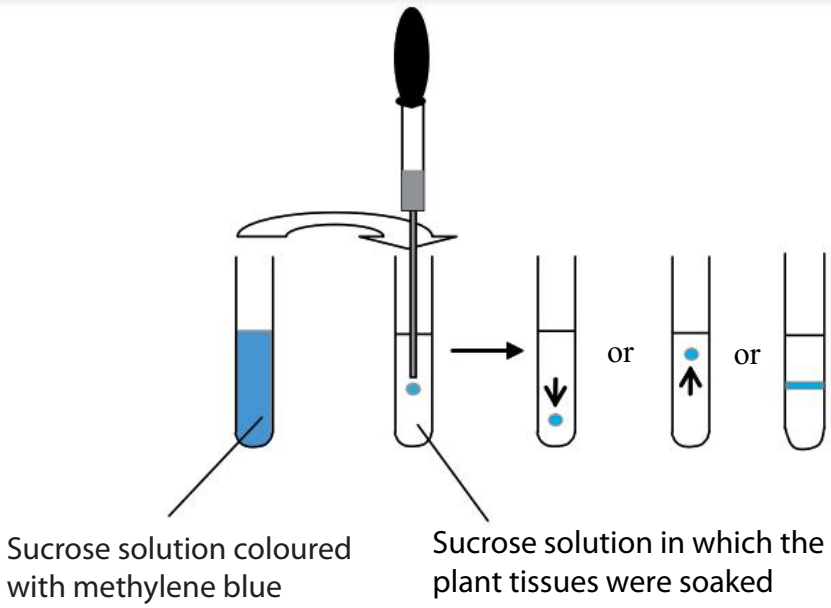
plant tissues: potato, sweet potato, carrot, swede, cassava, manioc, apple or turnip

## Method

1. Using the potato chip cutter, cut 30 chips from the plant tissue. Cut the chips lengthwise in four then trim each chip, as precisely as possible, to 50mm lengths using the scalpel. Weigh each chip and record their masses.

*Warning: Sharp instrument, handle with care*


2. Place five chips into each of six specimen tubes, one tube for each of the different sucrose solutions. Pour in enough of each respective solution to cover the tissues. Put a cap on each one of the tubes. Make your initial observations on the tissues bathing in the liquid and leave for 24 hours.
3. Prepare a set of six large test tubes, adding 25cm$^3$ of each of the solutions mixed with the smallest amount of methylene blue powder.
4. Leave the tubes overnight in a refrigerator, drain off the liquid from each of the tubes into a second set of clean test tubes. Set these tubes aside. Measure the length of each chip of tissue. Measure the mass of the chips. Make your observations and record them in an organised way.
5. Analyse your data by processing them. The initial masses of the chips should be similar but they will not be identical. Therefore, you should calculate the percentage change in mass in order to compare them.
6. Plot the percentage change in mass of the tissue against the sucrose concentration. The isotonic sucrose solution, which has the same water potential as the tissue, can be determined where the line of best fit cuts the x-axis (where there is no change in the tissue). From this and the calibration curve on the next page, you can estimate the water potential of the plant tissues.
7. Do the same thing with the percentage change in length and compare the results you get for both methods.
8. Take your series of test tubes of sucrose solutions coloured with the smallest amount of methylene blue and the series of solutions drained from the tubes containing the plant tissue.
9. Using a clean, dry Pasteur pipette and a steady hand, carefully transfer one drop from each of the methylene blue coloured solutions to its equivalent tube in the series of solutions which soaked the tissues.




10. Either the blue drop sinks, the solution has become less dense. The tissue has lost water.

    Or it rises; the solution has become denser. The tissue has absorbed water.

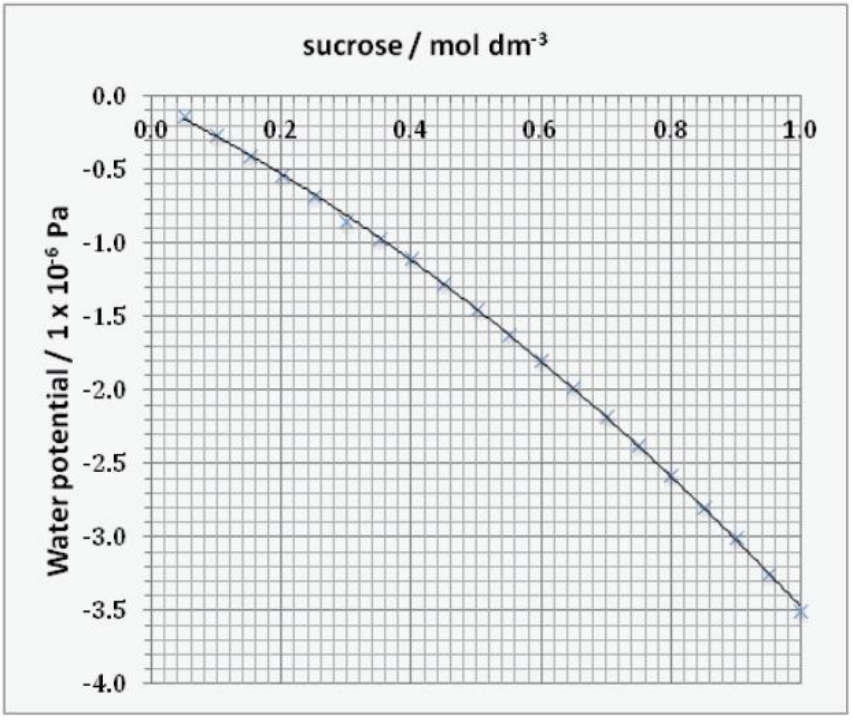
    Or it spreads out at the same level; the density has not changed.

## Discussion

Discuss your results, make your conclusions and evaluate the three methods of determining the water potential of these tissues.

### Additional information

The water potential of different sucrose solutions can be calculated. It is measured in units of pressure (Pascal). The conversion graph below shows this relationship.



**Syllabus reference: Sub-Topic 1.4 Aim 6**

Red cabbage leaf epidermal cells are pigmented purple with anthocyanins. The pigments are held in their sap vacuoles. If the membranes of the cell are damaged these pigments will leak from the cell.

What will be the effect of heat or ethanol on the membranes of red cabbage tissue?

## Materials

- data logger
- colorimeter sensor
- 10 cuvettes and caps
- thermometer or temperature probe
- electronic water bath
- marker pen
- dropping pipette
- 10 test tubes in a rack
- test tube holder
- 10cm$^3$ syringe
- distilled water
- series of solutions of ethanol: (15%, 30%, 45%, 60%, 75%, 80%, 90%, 100%)
- washed red cabbage disks

## Method

### The effect of temperature

1. Using a cork borer discs have been cut from the red cabbage and washed for several hours. Select 30 that are of uniform thickness. Place three discs in each test tube and add 10cm$^3$ of distilled water solution to each tube.
2. Put the tubes of water in the water bath and set the temperature to 40°C. Monitor the temperature of the water using a thermometer or a temperature probe linked to the computer with the data logging program installed. When the temperature reaches 40°C remove a tube and label it. Continue raising the temperature and remove a tube at 5°C intervals.
3. Withdraw liquid from a test tube and fill a colorimeter cuvette until it is 1 cm from the top (about ¾ full). Wash the pipette, take a sample from the next tube and fill a fresh cuvette. When labelling the cuvettes mark one side only near the top. Handle the cuvettes near the top too.

### The effect of ethanol

1. Select another 30 discs that are of uniform thickness. Place three discs in each test tube and add 10cm$^3$ of ethanol solution to each tube. This includes one with pure distilled water which is 0% ethanol.
2. Leave the discs to soak in the ethanol solutions for 20 min.
3. Withdraw liquid from a test tube and fill a colorimeter cuvette until it is 1 cm from the top (about ⅔ full). Wash the pipette, take a sample from the next tube and fill a fresh cuvette. When labelling the cuvettes mark one side only near the top. Handle the cuvettes near the top too.

### Calibrating the sensor

1. Plug the colorimeter probe into the first channel on the data logger.
2. The data logger should detect the colorimeter sensor, set the data collection parameters, and display the current sensor reading. If you have a choice between transmission and absorption select absorption.
3. To calibrate the colorimeter set up a blank cuvette containing distilled water. Put a cap on it and place it in the colorimeter.
4. Select the appropriate light source. Remember red cabbage is purple in neutral conditions. This is the light that is transmitted. You want to choose a wavelength of light that is absorbed by the liquid. Try a yellow or green wavelength. Calibrate the colorimeter for the selected wavelength.

## Collecting data

1. Put a cap on the cuvette with the first sample. Set the cuvette in the colorimeter, take care not to touch the sides where the light will pass.
2. When everything is ready begin data collection using an event entry mode.
3. Remove the cuvette and replace it with the next. Collect the data for every 5°C from 40 to 80°C or every ethanol solution from 0 to 100%. The data will appear in the spreadsheet of the data set. Click on 'Stop' to end the data collection. DO NOT LEAVE THE LAST CUVETTE IN THE COLORIMETER
4. Copy the data set into your laboratory logbook or record it on your USB key. The data can then be processed directly in data logging program or it can be cut and pasted into a spreadsheet like MS Excel.
5. Present your data, both quantitative and qualitative, in an appropriate way, process the data to determine the effect of temperature on the membranes of this tissue. Discuss and evaluate your results.

### Trouble shooting

As usual when evaluating investigations it is not sufficient to simply quote equipment malfunction.

However, if you get readings that appear strange or impossible try the following:

- Check the connections.
- Reboot the data logger.
- Check the liquid that you have sampled. Is it homogeneous and transparent?
- Check the cuvette that you are using is not dirty or wet on the outside.
- Check the cuvette is in the holder the right way round
- Check the cuvette holder in the colorimeter. Is it clean and dry?
- Try a different wavelength of light.

**Note:** You should try to keep your results between 0.050 – 0.550 absorbance. Outside this range the calibration curve is not linear. If your results are outside this range try a different wavelength (light source) or the liquid may need diluting by a known factor.

## Syllabus reference: Sub-Topic 1.6 Skills

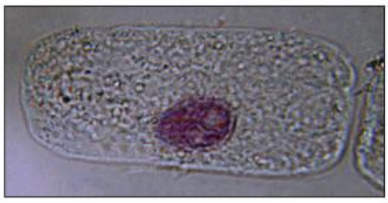
The organisation of a plant is essentially on a vertical axis (stem and roots) with branches. The plant develops by extending from the tips. Development requires cell division, then cell growth (elongation), followed by cell specialisation (differentiation). In plants, cell division occurs in zones called **meristems**. There is a meristem at the tip of the root.

Root tips are fixed to stop and preserve the cell activities. They are then sectioned longitudinally (sliced lengthwise) and stained. Each section, therefore, shows the state of the cells at one point in time.

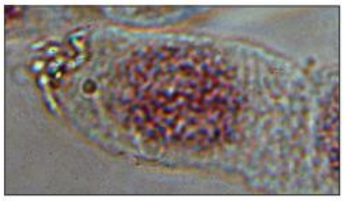
If the length of the cell cycle of the tissue is known, then by identifying and counting the stages of the cell cycle, it should be possible to make an estimate of the time the cells spend in each stage. This assumes that the cells are all randomly going through the different stages of the cell cycle.

## Method

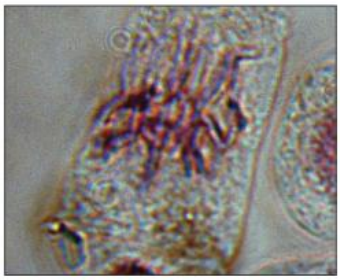
1. Prepare a slide of a root tip (or select a prepared slide) and place it on the microscope. Focus on low power then medium power, finally high power.
2. Observe the cells just behind the root tip and look for the different stages in the cell cycle. The photomicrographs of garlic root tip cells below will be helpful.



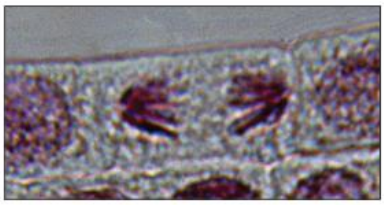
*Interphase*



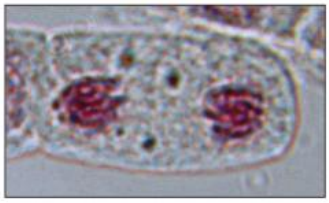
*Prophase*



*Metaphase*



*Anaphase*



*Telophase*

*(All photos are copyright property of the author Paul Billiet.)*

3. Systematically, observe at least 100 cells and record how many are in each stage of the cell cycle.
4. Present your results on the class bulletin board.
5. Search online for information on the duration of the cell cycle of the plant tissue you are observing. Estimate the time spent by the cells in each stage of the cell cycle.
6. Pool the data from the class and present your data in a suitable manner.
7. Estimate the mitotic index of this tissue = $\frac{\text{number of cells in mitosis}}{\text{total number of cells}}$

**Syllabus reference: Sub-Topic 2.1 Skill**

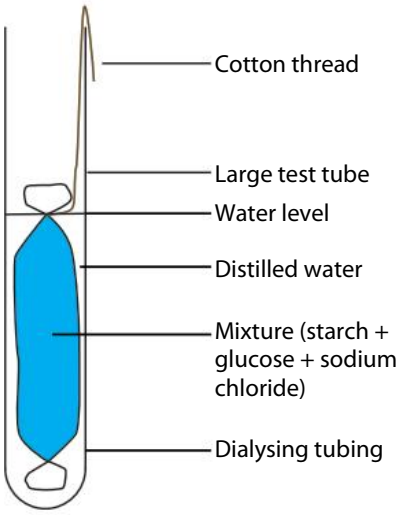
Dialysis allows the selective diffusion of molecules or ions of different sizes across a selectively permeable membrane that acts as a molecular sieve.

## Materials

A solution containing a mixture of starch, glucose and sodium chloride
small beaker
15 cm of dialysing tubing
glass rod
cotton thread
large test tube
electronic water bath
dropping pipette
pasteur pipette
3 test tubes in a rack
test tube holder
stop clock or watch
wash bottle of distilled water
iodine solution
silver nitrate solution
Fehlings or Benedicts solution
safety glasses

## Method

1. Wet the piece of dialysing tubing and open it using a round-ended glass rod. Tie a secure knot in one end of the tubing. Inject the mixture of starch, glucose and sodium chloride into the open end of the dialysing tubing using the dropping pipette.
2. When the tubing is nearly full, tie a thread of cotton tightly around the open end. Wash the outside of the tubing thoroughly, under the tap, and then wash it again using distilled water.
3. Suspend the dialysing tubing in the large test tube and fill it with distilled water up to the level of the knot (as shown opposite). Immediately start your stop clock and, using the Pasteur pipette, take three small samples from the distilled water in the large test tube. Pipette the samples into the three test tubes.
4. Test the samples for the presence of:




- STARCH by adding a few drops of iodine solution to a sample in a test tube. Positive result = blue/black colour.
- GLUCOSE by adding an equal volume of Fehlings or Benedicts solution to the sample in a test tube. Heat this mixture gently in a water bath at 80°C for a few minutes. Positive result = green, yellow or red, depending upon the amount of sugar present.
- CHLORIDES by adding a few drops of silver nitrate solution ($AgNO_3$) to the sample in a test tube. Positive result = a white precipitate.

*Warnings: Very hot water can scald, use a test tube holder. Fehling's solution is caustic. Wear safety glasses. Iodine solution is an irritant and silver nitrate is toxic and an irritant. Wash off spills with water.*


5. Leave the mixture for five minutes, take a second set of three samples and place them in clean test tubes. Repeat the test at 5-minute intervals for 25 minutes. Record your results in the form of a table. At the end of 25 minutes add several dropper-fulls of iodine solution to the large test tube and observe what happens. Record your results for this last part of the experiment and record your observations and conclusions on the whole experiment. What are the sources of error that you may have encountered?

### Additional information

| Particle | Diameter/nm |
|---|---|
| Chloride ion | 0.362 |
| Iodide ion | 0.452 |
| Glucose molecule | 70.0 |
| Starch molecule | 17500.0 |

**Syllabus reference: Sub-Topic 2.2 Application**

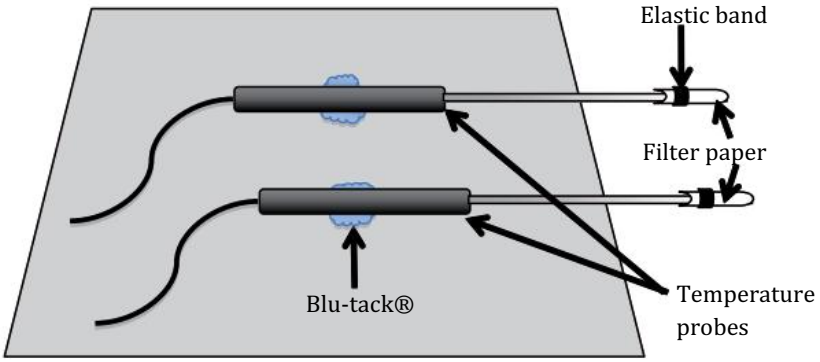
It is well known that sweat is used to cool the body. The heat of the body excites the water molecules and their evaporation helps the body to cool down. How effective is water at cooling a body?

## Materials

data logger
2 temperature probes
2 elastic bands
Blu-tack® or adhesive tape
filter paper
scissors
ruler
2cm of solvents in test tubes:
water
ethanol
propanone

## Method

1. Wrap an elastic band around each temperature probe. Cut several pieces of filter paper into to squares 2cm × 2cm.
2. Roll a piece of paper around the tip of each of the temperature probes so they fit tightly and hold them in place using the elastic bands.
3. Plug them into two channels of a data logger and set to record the temperature every 0.5s for 3 min.




4. Stick a piece of Blu-tack® on the bench where a probe will be attached. Dip one of the probes into the liquid being tested and leave the other one dry. Stick the two temperature probes to the bench, as shown above. Start recording the temperature.

*Warning: Ethanol and propanone are volatile and inflammable. Use in a well ventilated area. Do not expose to a naked flame.*


5. What variables should be controlled during the course of the experiment?
6. When the run is complete, save the data and remove the filter paper. Clean and dry the probe that was dipped in liquid and repeat the experiment using a different liquid.

## Some points for consideration

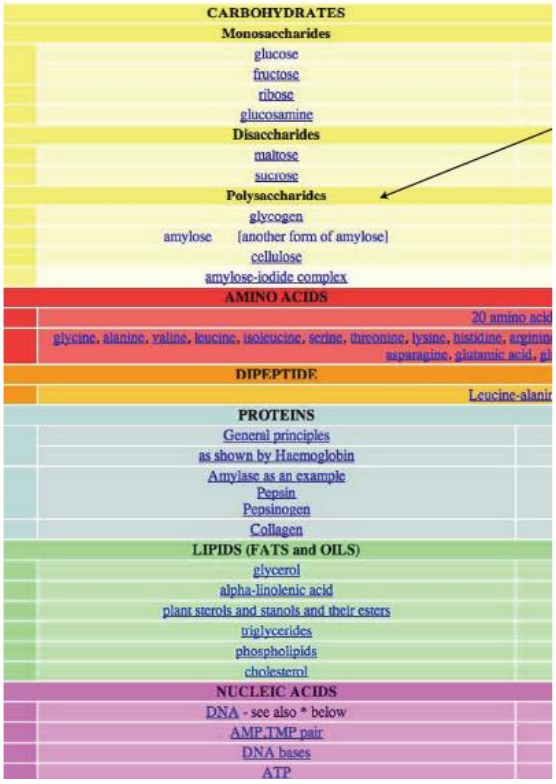
- How much did the temperature drop by for each liquid?
- Compare the size of each of these molecules. The bigger the molecule, the more energy it takes to make it evaporate.
- Compare the polarity of these molecules. Can they easily form hydrogen bonds? The stronger the hydrogen bonds between the molecules, the more energy it takes to make them evaporate.
- What are the advantages of animals and plants using water as coolant?
- How have animals and plants adapted to hot arid conditions where water is limiting?

## Syllabus reference: Sub-Topic 2.3 Skills

Carbohydrates are one of the four fundamental groups of molecules that living systems use. The basic building block, the monosaccharides, can be used to build large macromolecules, the polysaccharides.

The structures of these molecules can be visualised on the Biotopics site using:

<http://www.biotopics.co.uk/JmolApplet/jcontentstable.html>




### Select the following molecules on viewed in Jmol

Under Carbohydrates / Polysaccharides

- Cellulose
- Starches: amylose and amylopectin
- Glycogen

Note that these are only parts of the complete molecules. For example,a complete molecule of cellulose may contain 2 000 to 14 000 glucose units.

## Observations

Compare these molecules: their size and shape.

## Some points for consideration

### Compare their functions in living organisms

Branched molecules are more compact than non-branched molecules.

Large molecules are less soluble than small molecules.

Plants store starches in plastids (amyloplasts and chloroplasts). These large insoluble molecules are built up from simple sugars and broken down by hydrolysis to simple sugars when they are needed.

Unlike plants, animals store glycogen, not starches. These are stored in relatively small amounts and their glycogen stores are not long term.

### Compare the structures of glycogen and the starches from the point of view of energy storage

Why are animals inclined to store fats rather than polysaccharides?

Simple sugars would be more rapidly available for use than polysaccharides.

What is the advantage of storing carbohydrates as polysaccharides?

Cellulose is used in the cell walls of plants.

How is the structure of cellulose appropriate to its function in plant cell walls?

# Investigation 2D - Measuring the rate of reaction of an enzyme controlled reaction



**Syllabus reference: Sub-Topic 2.5 Skill**

Hydrogen peroxide ($H_2O_2$) is a toxic substance produced by the metabolism of cells. To stop this substance building up, cells break it down into water and oxygen.

$H_2O_2 \longrightarrow H_2O + ½O_2 \uparrow$

This reaction is catalysed by a group of enzymes called peroxidases. One well known peroxidase is catalase. The reaction is exothermic which means it releases heat energy.

As the reaction proceeds, the substrate will be used up, so the reaction will slow down. Therefore, where possible, when measuring the reaction rates, we take the initial reaction rates where the enzyme is performing at its maximum rate under the conditions it is exposed to.

Catalase is found in many tissues. In this case a suspension of yeast cells is used.

## Method A: Using flotation to measure enzyme activity

A product of the reaction is oxygen gas. When the enzyme extract is soaked into a piece of filter paper and dropped into the hydrogen peroxide it will sink and then float back to the surface.

### Materials

| | | |
|---|---|---|
| large test tubes in a stand | 100cm$^3$ measuring cylinder | filter paper |
| yeast suspensions (1%) | 10cm$^3$ syringes | safety glasses |
| 10 volume $H_2O_2$ | forceps | paper towel |
| 50cm$^3$ beaker | hole punch | stop watch |

1. Make up 100 cm$^3$ of a 1 Vol solution of $H_2O_2$ in a 100 cm$^3$ beaker.

*Warning: Hydrogen peroxide is corrosive, avoid contact with the skin and the eyes and wear safety glasses. Wash off any spills with plenty of water.*


2. Cut discs of filter paper using a hole punch. Holding a disk using forceps, dip it into the yeast suspension and drain off the excess liquid.
3. Put the filter paper into the beaker of 1 Vol $H_2O_2$. Observe what happens. Repeat it a few times with fresh discs soaked in yeast suspension.

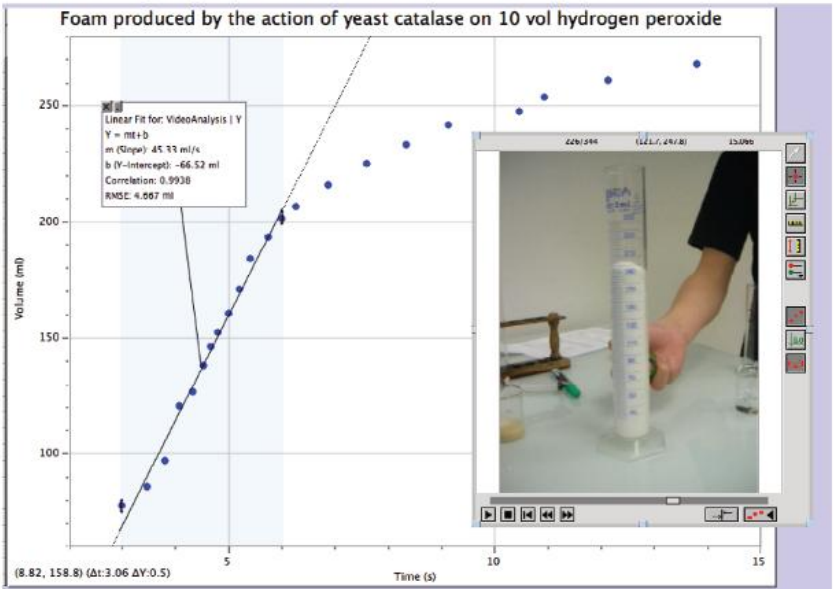
## Method B: Using foam production to measure the enzyme activity

As seen in method A the production of the oxygen gas can be measured. A foam is produced when the reaction mixture is mixed with a detergent.

### Materials

| | | |
|---|---|---|
| Yeast suspension 8% | 10cm$^3$ syringes | Detergent in dropper bottle |
| 20 volume and 10 volume $H_2O_2$ | Safety glasses | Video camera |
| 250cm$^3$ measuring cylinders | Paper towel | Computer with video capture |

1. Add 10cm$^3$ of hydrogen peroxide and two drops of detergent to a large measuring cylinder. Mix a little then add 10cm$^3$ of 8% yeast suspension.
2. Record your observations.
3. The reaction can be filmed using a video camera and processed in a video capture program. The graduations on the side of the cylinder can be used a scale to calibrate the video analysis.




## Method C: Using the heat released as an indicator of the reaction

The reaction is an exothermic reaction that releases heat. The rise in temperature of the reaction medium can be used as an indicator of the rate of reaction.

### Materials

| | | |
|---|---|---|
| thermometer | 10 volume $H_2O_2$ | safety glasses |
| large test tubes in a stand | 10cm$^3$ syringes | paper towel |
| yeast suspensions (8%) | | |

1. Measure 20cm$^3$ 10 Vol $H_2O_2$ into a large test tube. Place the thermometer into the $H_2O_2$ and let it come to the temperature of the liquid.
2. Add 10cm$^3$ of 8% yeast suspension and stir it in and observe what happens.

# Method D: Using data logging and a temperature probe

## Materials

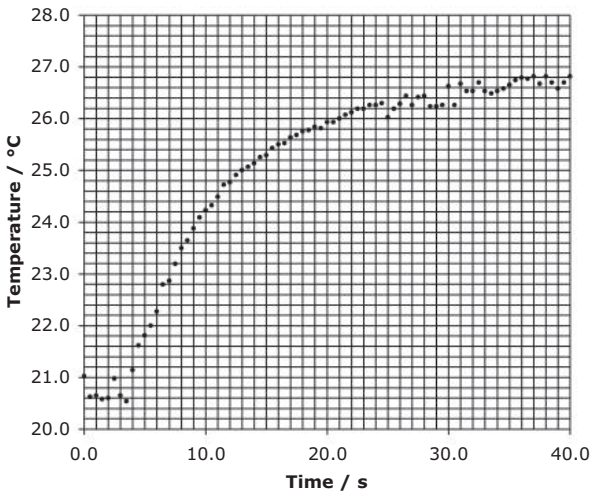
- Data logger
- Temperature probe
- Large test tube in a stand
- Marker pen
- Yeast suspension (8%)
- 20 volume $H_2O_2$
- $10cm^3$ syringe or graduated pipette with pump
- Measuring cylinder
- Safety glasses
- Paper towel

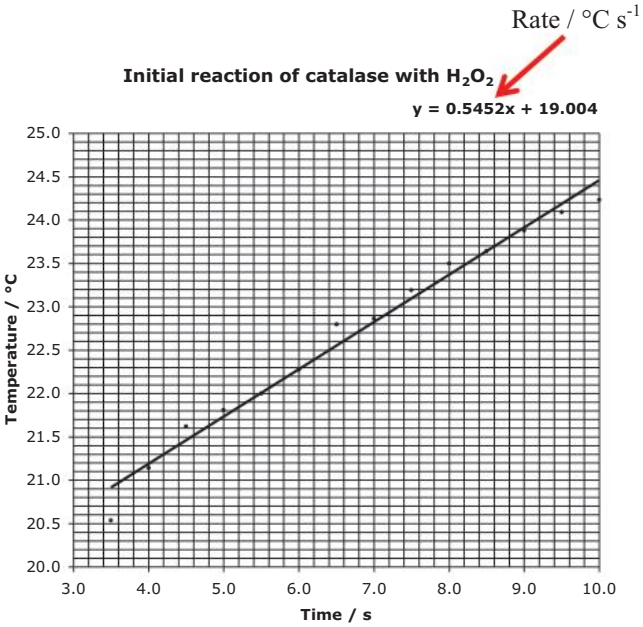
## Method

1. Plug the temperature probe into the first channel of the data logger. The data logger should automatically recognise the temperature probe and it should not need calibrating.
2. Measure $20cm^3$ $H_2O_2$ into a large test tube. Slide the temperature probe into to the $H_2O_2$ and let it come to the temperature of the liquid (let the probe equilibrate).

## Recording and Analysing the data

1. Select a time-based mode in the data collection menu. The experimental length should be about 3 minutes for a trial run and the frequency of measurement should be 60 per minute.
2. Collect $10cm^3$ of yeast suspension in a syringe. Stir the yeast suspension before taking the sample.
3. Begin data collection. A live graph should appear on the screen. Wait 15 seconds and add $10cm^3$ yeast suspension and stir it in.
4. To save the data; transfer it to a USB key or send it home as an e-mail attachment.
5. Wash the probe and wipe it dry.
6. Analyse your data using a linear fit trend line of the initial reaction. The equation of this line will give the initial reaction rate. An example is given below.



## Method E: Using data logging and a pressure sensor

A product of the reaction is oxygen gas. As this builds up in a closed vessel (e.g. a flask) the pressure can be used as an indicator of the rate of the reaction.

### Materials

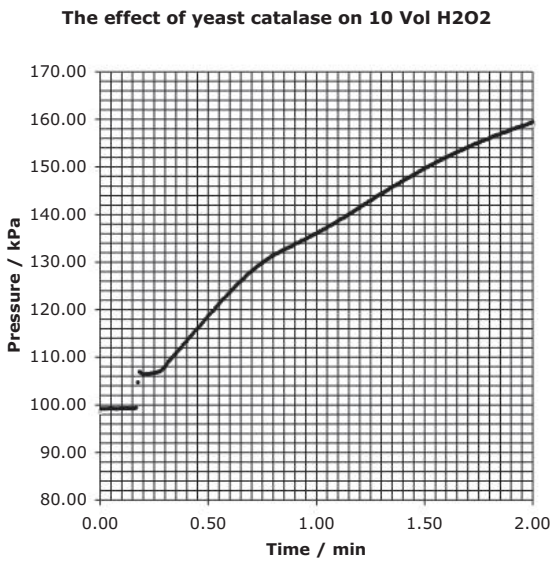
| | | |
|---|---|---|
| data logger, | yeast suspension (1%) | measuring cylinder |
| pressure sensor syringe and connectors | 10 Vol $H_2O_2$ | safety glasses |
| 50cm$^3$ flask in a clamp and stand | 5cm$^3$ syringe or graduated pipette with pump | paper towel |
| marker pen | | |

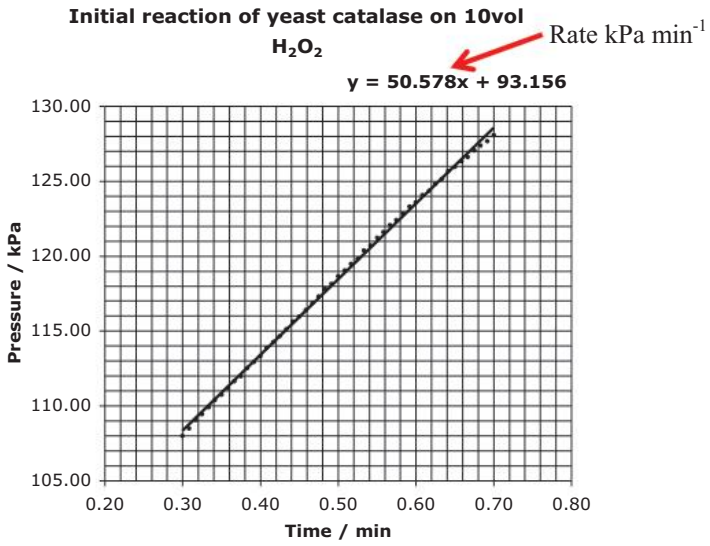
### Method

1. Plug in the sensor to the first channel of the data logger. The data logger should automatically recognise the pressure sensor.
2. Measure 5cm$^3$ yeast suspension into the small flask. Stir the yeast suspension before taking the sample.
3. Connect the pressure sensor to the rubber bung that fits in the flask, using a piece of plastic tubing.

### Recording and Analysing the data

1. Select a time-based mode in the data collection menu. The experimental length should be about 3 minutes for a trial run and the frequency of measurement should be 60 per minute.
2. Collect 5cm$^3$ of $H_2O_2$ in the syringe. Make sure the valve is turned off on the connector and screw the syringe into the connector on the rubber bung and attach the bung to the flask.
3. Begin the data collection. A live graph will appear on screen. Let it run for 10s, open the valve and inject the $H_2O_2$ into the flask, then close the valve. If the reaction is too vigorous dilute the hydrogen peroxide to 5 Vol and repeat.
4. Disconnect the syringe and wash it out.
5. Analyse your data using a linear fit trend line of the initial reaction. The equation of this line will give the initial reaction rate. An example is given below.



# ENZYME ASSIGNMENT

**Design investigations to determine the effects of temperature, pH and substrate concentration on the activity of catalase.**

You will find that some of the protocols proposed are appropriate for a factor but not others.

## Design

1. Establishing the theoretical background. This process will require some research on the properties of your enzyme and its source.
2. State a focussed research question.
3. Write a hypothesis (a justified prediction).
4. Clearly identify the variables (the 'independent' or manipulated variable, 'dependent' or measured variable and the important 'control' variables).
5. State the significance of each of the control variables and which variables cannot be controlled but need to be monitored.

## Method

1. What equipment and materials will you need? This may be best described using a labelled diagram of the experimental setup.
2. Consider any safety factors.
3. Explain exactly how the independent variable will be changed (range and intervals) and how the dependent variable will be measured and how frequently.
4. Explain how you intend to control all the other variables (how will you make your experiment a fair test) or monitor them if they cannot be controlled. Perhaps a control experiment will be needed.

   *(Note: 'Controlled variables' and a 'control experiment' are not the same thing).*
5. Using a water bath to control temperature is best but it may be possible for the enzyme to operate at room temperature. Nevertheless, the room temperature should be monitored to see if it varies significantly during the experiment.
6. To control pH you should consider using buffers. Buffers resist changes in pH, acids such as hydrochloric acid may be diluted to appropriate pH values but they will not remain stable once the reaction starts. Solutions such as lemon juice and vinegar are inappropriate when pH needs to be controlled.

# Investigation 2E - Immobilising yeast enzymes



**Syllabus reference: Sub-Topic 2.5 & 2.8 Applications**

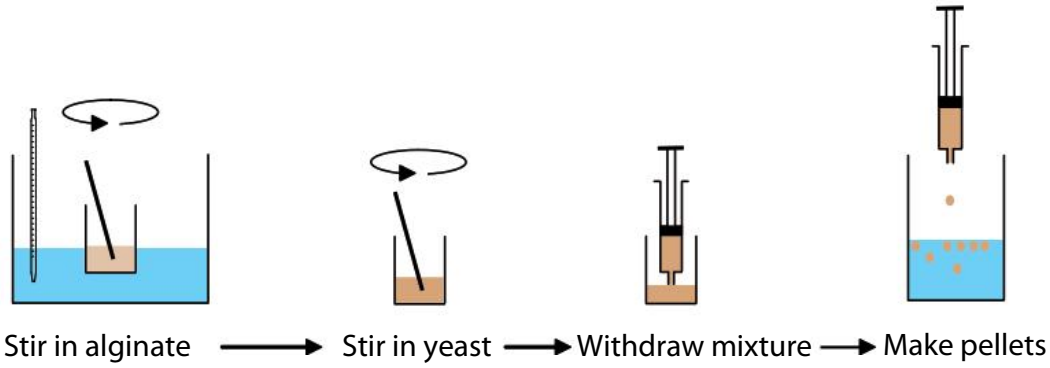
Sodium alginate is a gel that forms solid pellets when it is dripped into a solution of calcium chloride. Enzymes, or cells containing enzymes, which control reactions can be mixed in with the alginate gel so that they are immobilised. Thus, when the reaction is completed they can be recuperated and used again. In the biotechnology industries, where the strains of microbes or the types of enzymes may be very expensive, producing reusable immobilised material is an important technique.

## Materials

- balance and spatula
- 2g sodium alginate powder
- 100cm$^3$ distilled water in 250cm$^3$ beaker
- water bath at 50°C
- glass rod
- 2g baker's yeast
- 50cm$^3$ calcium chloride solution in 100cm$^3$ beaker
- filter funnel and filter paper
- 2 conical flasks 250cm$^3$
- sterile water wash bottle
- 50cm$^3$ 10% sucrose solution
- *Clinistix* glucose testing strips
- scissors
- stop watch
- 10cm$^3$ syringe

## Method

1. To prepare sodium alginate pellets, heat 100cm$^3$ distilled water in a beaker to 50°C in a water bath. Slowly add 2g of sodium alginate and then stir for 5 to 10 min.
2. Cool the beaker of alginate to 35°C and add 2g of yeast. Stir in well to mix.
3. To make the pellets, withdraw the alginate-yeast mixture into a syringe and drip it slowly into a beaker of calcium chloride solution. Stir the solution whilst the pellets are being made. Leave the pellets in the calcium chloride for 5min, then filter into a conical flask and recuperate the calcium chloride. Wash the pellets with sterile water.




### Hydrolysis of sucrose

4. Measure out 50cm$^3$ of sucrose solution into a flask. Add 30 alginate-yeast pellets. Mix and test for glucose using a *Clinistix* tester. Repeat the test every 10min for 30min.

### Fermentation of sucrose

5. Place 10 alginate-yeast pellets into the sucrose solution. They should sink. As the yeast cells begin to ferment the sucrose, carbon dioxide gas will be produced. The pellets will float to the surface of the solution. Observe and record the time taken for each pellet to rise to the surface.

### Design

6. On the basis of this experiment, plan an investigation using immobilised yeast cells, bacteria, algae or pure enzymes.

**Syllabus reference: Sub-Topic 2.8 Skill**

Soda lime is a mixture of calcium oxide granules and sodium hydroxide. It will absorb carbon dioxide. It can be used in a simple apparatus to calculate the respiration rate of a small organism, such as a meal worm, blow fly larvae, a germinating seed or even a piece of living tissue.

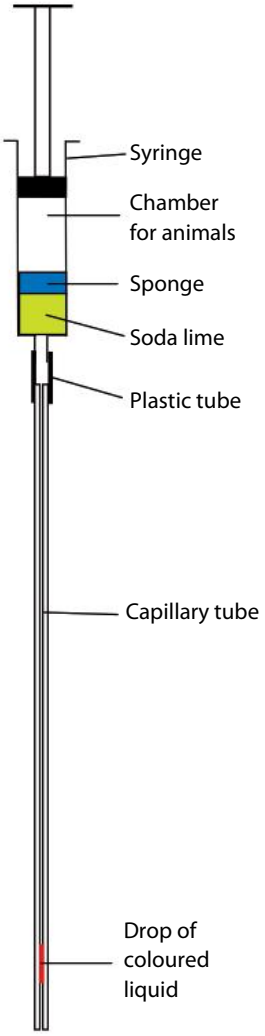
Mealworms (*Tenebrio molitor*) are insects, they are the larvae of a beetle.

## Materials

| | | |
|---|---|---|
| 2 plastic syringes (10cm$^3$) | Ink or methylene blue solution | Electronic balance |
| 2 pieces of plastic tubing 2cm long | Marker pen | 3 to 5g meal worms |
| 2 pieces of 1mm bore capillary tubing 30cm long | Ruler | Soda lime |
| | Stop watch | Clamp and stand |

## Method

The complete apparatus is set up as shown opposite:




Set up the apparatus as follows:

1. Remove the plunger from one of the syringes. Use a spatula to add the soda lime into the syringe then slide the sponge in also to cover the soda lime.
2. Measure the exact mass of the animals. Place them in the syringe. Push the plunger of this syringe up to the 10cm$^3$ mark. Leave the apparatus for 2 minutes. The syringe can be supported in a burette stand.

*Warning: Soda lime is caustic, avoid contact with the skin. Return it to the correct container for disposal after the experiment.*


3. Set up a control using the materials provided.
4. Add a drop of coloured liquid (e.g. methylene blue or ink) to the end of each capillary tube and attach them to the syringes using the short piece of plastic tubing.
5. Mark the position of the coloured liquid and start the stop clock.
6. Using the ruler, measure the distance travelled by the drop of coloured liquid at one-minute intervals for 10 minutes, in both pieces of apparatus, in the experimental apparatus and the control. Record these results in a table.

*Meal worm larvae and pupa*

*Meal worm pupa*

*Meal worm adult*

## Analysis

1. Calculate the average rate of respiration of these animals over the 10 minute period in: mm $min^{-1}$
2. What is the gas that the animals are absorbing?
3. What is the gas they are producing?
4. The diameter of the capillary tube is 1mm. Therefore, the volume of gas breathed in by these animals per minute can be calculated from the formula:

   Volume = $\pi r^2 h$

   Where: $\pi = 3.142$ $r = 0.5$mm (the radius of the capillary tube)

   $h$ = the average distance travelled by the drop of coloured liquid in mm $min^{-1}$
5. Calculate the volume of gas absorbed per minute by your animals: $mm^3$ $min^{-1}$
6. Now calculate the volume of gas inspired by the meal worms as: $mm^3$ $g^{-1}$ $min^{-1}$
7. Discuss and criticise this method for calculating the rate of respiration of an organism.

## *Ethics: Return the animals to their container*


## Some points for consideration

- If you were using a similar apparatus to measure the respiration rate of a green plant, what modifications would you need to make to the apparatus?
- What might happen if the rate of metabolism of the organism varies during the experiment?
- What might happen if the organisms start to respire anaerobically?
- Do different stages in the life cycle of an insect respire in the same way?

## Research

- What is the Respiratory Quotient (RQ)?
- Could you use the above apparatus to measure an RQ value?
- How would you modify the apparatus so that the RQ values can be calculated?
- Would the energy substrates that the organism metabolises influence the results of the RQ values?

### Additional information (equations)

Complete oxidation of sugar:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

Complete oxidation of a fat:

$C_{18}H_{34}O_2 + 25.5O_2 \rightarrow 18CO_2 + 17H_2O$

Anaerobic respiration of a sugar to lactic acid:

$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH$

**Syllabus reference: Sub-Topic 2.9 Skill**

Leaf pigments are organic compounds that absorb particular wavelengths of light. Many of them are involved in photosynthesis.

## Materials

| **Extraction**: | **Separation**: |
|---|---|
| mortar and pestle + spatula of fine sand Scissors | Pasteur pipette, 20cm$^3$ of solvent |
| funnel + filter paper | large test tube + bung |
| test tube + test tube rack | 10cm$^3$ pipette + bulb |
| 10cm$^3$ of pure ethanol | Whatman's paper no.1 (chromatography paper) |
| green leaf material | scissors, pin, hair dryer, ruler |

## Method

### Extraction with alcohol

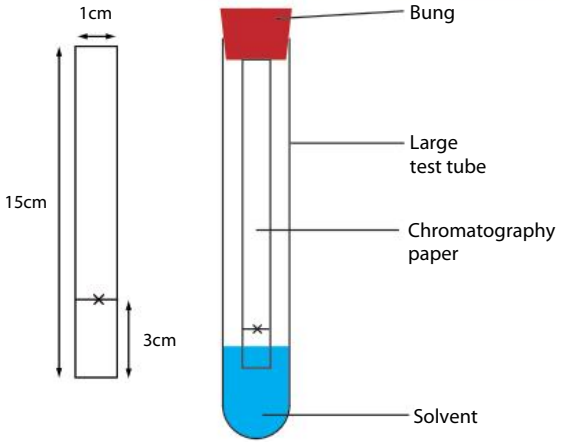
*Warning: The ethanol is volatile and inflammable. Use in a well ventilated area. Avoid contact with the eyes. Wear gloves and eye protection.*


1. Put one spatula of sand in the mortar and add 5cm$^3$ of 90% ethanol.
2. Chop a few leaves into small pieces and place them in the mortar. Grind the leaves, the pigment solution obtained should be very dark green.
3. Filter the contents of the mortar into a test tube.

### Separation by paper chromatography

*Warning: The solvent is volatile and inflammable. Use in a well ventilated area. Avoid contact with the eyes. Wear gloves and eye protection.*


4. Cut a strip of chromatography paper about 15cm long (the length of the large test tube) and 1cm wide. Hold the paper by the edges. Draw a pencil line 3cm from the bottom of the paper. In the middle of this line mark a cross. *Refer to the diagram opposite.*
5. Using the pipette, put 3cm$^3$ of solvent into the large test tube. Make sure that the solvent does not go on the sides of the cylinder. Put in the bung to allow the air inside the cylinder to become saturated with the solvent.
6. Using the Pasteur pipette put a tiny spot of the pigment solution onto the marked cross. Dry this spot with the hair dryer. Repeat this eight times to obtain a small concentrated spot.




7. Remove the bung from the test tube. Fold the chromatography paper over and stick a pin into the bung through the paper. Suspend the paper in the test tube, the solvent should be below the line marked 3cm from the bottom of the paper. Make sure that the chromatography paper does not touch the sides of the cylinder. *See diagram above.*
8. Observe what happens for 1-2 minutes, then leave this aside in the dark for 1 hour.

## Analysis of the chromatogram

As soon as the chromatogram is removed from the measuring cylinder, draw a line at the highest point the solvent has risen. This is the solvent front.

Mark the highest and widest points seen for each pigment that has been separated out by drawing a small cross at these points.

Calculate the Rf values and compare them to the values given in the table below.

$$R_f = \frac{\text{Distance moved by the pigment}}{\text{Distance moved by the solvent}}$$

| Pigment | $R_f$ value | Colour |
|---|---|---|
| Chlorophyll b | 0.45 | Green |
| Chlorophyll a | 0.65 | Green-blue |
| Xanthophyll | 0.71 | Yellow |
| Carotene | 0.95 | Orange |

Discuss and criticise this investigation.

## Some points for consideration

- Why did you grind the leaves to extract the pigment? Are you certain to extract all the pigments in this way? Explain your answer.
- How many pigments did your green leaf contain?
- Why do the different pigments separate out along the chromatogram?
- Can you be certain to have observed all the different pigments of chlorophyll?
- Is it best to identify the pigments by colour or by $R_f$ values? Justify your answer.

## Research

- Explain why the leaf is green despite the orange and yellow pigments that it contains.
- Explain why a plant has so many different pigments.

## To investigate further

- Leaf pigments in leaves during Autumn
- Leaf pigments in leaves at different heights on a tree or bush
- Leaf pigments in leaves kept in the sun and in the shade
- Leaf pigments in plants subjected to mineral deficiencies (e.g. Iron and Magnesium)
- Compare the separation of plant pigments using thin layer chromatography (TLC) and paper chromatography. For TLC, photosynthetic pigments can be separated using the solvent 2 parts of hexane : 1 part ethyl ethanoate.


*Warning: Hexane is inflammable work in a well ventilated area.*

- Other leaf pigments; some leaves have red pigments. These are usually anthocyanins or betalains. They do not move in the solvents that transport photosynthetic pigments. For these pigments use 4 parts butan-1-ol : 1 part glacial ethanoic acid : 5 parts water. Betalains are soluble in water and very insoluble in methanol and give low $R_f$ values. Anthocyanins are soluble in methanol but not water and give higher $R_f$ values.

*Warning: Butan-1-ol is toxic and inflammable. Ethanoic acid is an irritant. Use safety glasses and work in a well ventilated area.*

**Syllabus reference: Sub-Topic 2.9 Skill**

The spectroscope, due to a prism or a diffraction grating, separates out the white light into its constituent colours. The spectrum is seen when you look into the spectroscope. By putting a specimen tube containing a pigmented solution in between the light entering the apparatus and the prism, a different spectrum is obtained.

## Materials

**Extraction of pigments**
mortar and pestle + spatula of fine sand
funnel + filter paper
test tube + test tube rack
10cm$^3$ of 90% ethanol,
spinach or parsley

**Analysis of extract**
spectroscope
specimen tubes
pigment extract

## Method

### Extraction

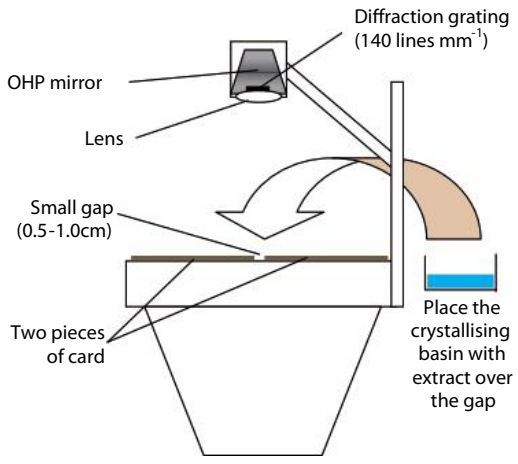
1. Put one spatula of sand in the mortar and add 5cm$^3$ of 90% ethanol.
2. Tear a few leaves into small pieces and place them in the mortar. Grind the leaves, the pigment solution obtained should be very dark green.
3. Filter the contents of the mortar into a test tube.

### Analysis

1. Fill a small specimen tube with the chlorophyll pigment solution and slot the tube into the spectroscope.
2. Hold the spectroscope towards a source of light.
3. Prepare the control that may be placed at the same time into the spectroscope.
4. Draw the two spectra that you see when you look into the eyepiece.
5. Comment on the significance of your observations.

### Using the overhead projector as a spectroscope

*Set up the overhead projector as shown in the diagram.*



### Using a spectrometer

If a spectrometer is available it can be used like a colourimeter than scans the whole width of the visible spectrum. This will give a graphical read out of the absorption spectrum of the leaf extract.

### Fluorescence

Under bright light the chlorophyll extract gives out red light. In the absence of electron acceptors, the electrons are exited out of their orbitals by the light. They fall back to their lowest energy levels giving out light energy. The effect is particularly noticeable under 'black' light (UV light source).

*Warning: UV light is harmful to skin and especially eyes.*
*Do not expose these areas directly to a UV light source.*


## Some points for consideration

From your spectroscope results, deduce what properties the leaf pigments have.

## To investigate further

- Leaf pigments in leaves during Autumn.
- Leaf pigments in leaves at different heights on a tree or bush.
- Leaf pigments in leaves kept in the sun and in the shade.
- Leaf pigments in plants subjected to mineral deficiencies (e.g. Iron and Magnesium).
- Analysing the absorption spectra from the different pigments separated by chromatography.

*New growth of pine needles clearly pigmented differently from that of the previous year*

**Syllabus reference: Sub-Topic 2.9 Skill**

The equation shows that the amount of oxygen given off equals the amount of carbon dioxide taken up by the plant.

$$nCO_2 + nH_2O \xrightleftharpoons[chlorophyll]{light} (CH_2O)n + nO_2$$

It is therefore possible to estimate the rate of photosynthesis by measuring the rate of oxygen production or the rate of carbon dioxide consumed by the plant. Several methods are outlined on the following pages.

Oxygen does not dissolve easily in water, so many water plants release oxygen as a gas. Carbon dioxide dissolves very easily in water, so a change in the level of $CO_2$ will affect the pH of the water surrounding the water plant.

# Method A: Determining oxygen production in a water plant directly

## Materials

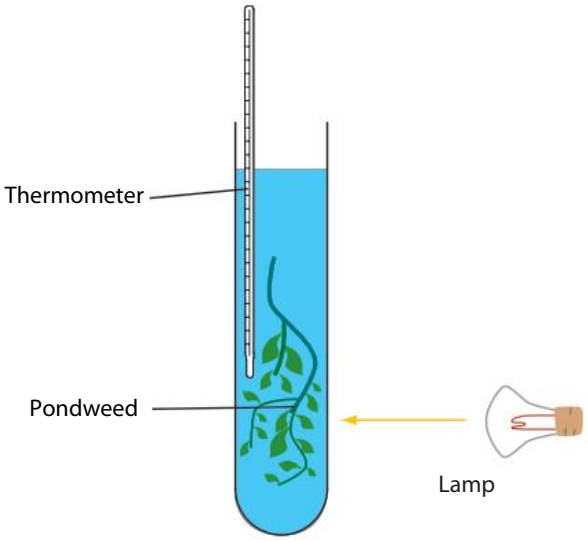
| | | |
|---|---|---|
| large test tube | lamp with 60 Watt bulb | stop watch |
| test tube rack | ruler | water enriched with carbon dioxide |
| glass rod | razor blade | 1 branch of a water plant |

## Method

1. Take a branch of pondweed that is healthy and green. Using the razor blade cut the branch 5cm from the terminal bud.

*Warning: Sharp instrument, handle with care*


2. Half fill the test tube with a solution of hydrogencarbonate.
3. Using a glass rod, push in the branch, terminal bud end first, so that the cut end is 1cm below the surface of the water (see diagram below).
4. Illuminate the tubes with the lamp and leave for five minutes. Ensure that the plant is bubbling steadily after this time. If it is not, change the plant and start again.
5. Count the bubbles over a regular period of time. Repeat the measurement. Leave the plant to adapt for 2min between each change of conditions.



# Method B: Determining oxygen production by the displacement of a liquid

## Materials

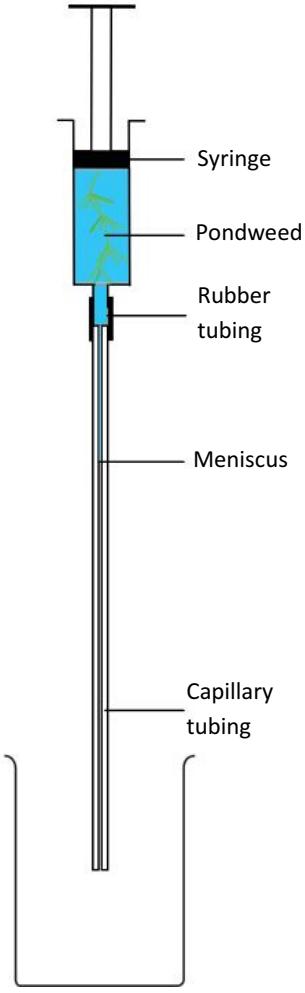
- $10cm^3$ syringe,
- 2cm tubing attached to the syringe,
- 30cm capillary tubing (1mm bore)
- $250cm^3$ beaker
- glass rod
- lamp
- stopwatch
- razor blade
- marker pen
- clamp and stand
- $20cm^3$ of 0.1 mol $dm^{-3}$ hydrogencarbonate solution,
- distilled water
- 2 branches of pond weed

## Method

1. Using a razor blade cut two branches of pondweed 5cm from the buds. Using the glass rod, push the branches, bud end first, into the syringe, with the cut ends, towards the piston of the syringe.


*Warning: Sharp instrument, handle with care.*

2. Clamp the syringe to the support stand, about 40cm above the bench. Place a small beaker on the bench just below the syringe to catch drips
3. Cover the end of the syringe with your finger. Remove the piston and fill the syringe with the hydrogencarbonate solution. Replace the piston and push it up to the $10cm^3$ graduation. Any unwanted liquid is collected in the beaker.
4. Attach the capillary tube to the end of the syringe using the 2cm of tubing. Make sure that there are no leaks. Gently push in the piston until a meniscus appears just under the bottom of the tubing. Mark the level of the meniscus with a marker pen.
5. Position the lamp 20cm from the apparatus and make observations on the level of the meniscus.



# Method C: Determining oxygen production using a pressure sensor

A product of the reaction is oxygen gas. As this builds up in a closed vessel (e.g. a large test tube) the pressure can be used as an indicator of the rate of the reaction.

## Materials

| | | |
|---|---|---|
| data logger | pond weed | glass rod |
| pressure sensor and connectors | 50cm$^3$ $NaHCO_3$ 0.1 mol dm$^{-3}$ | thermometer |
| large test tube | bench lamp (60W) | 2m tape measure |
| clamp and stand | | |

## Method

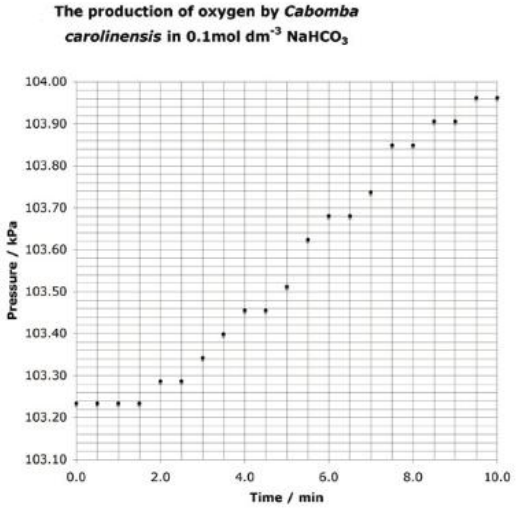
1. Plug in the pressure sensor to the first channel of the data logger. The data logger should automatically recognise the pressure sensor.
2. Using a razor blade cut a branch of pondweed 10cm from the bud. Using the glass rod, push the branch into the syringe. Fill the test tube with hydrogencarbonate solution.

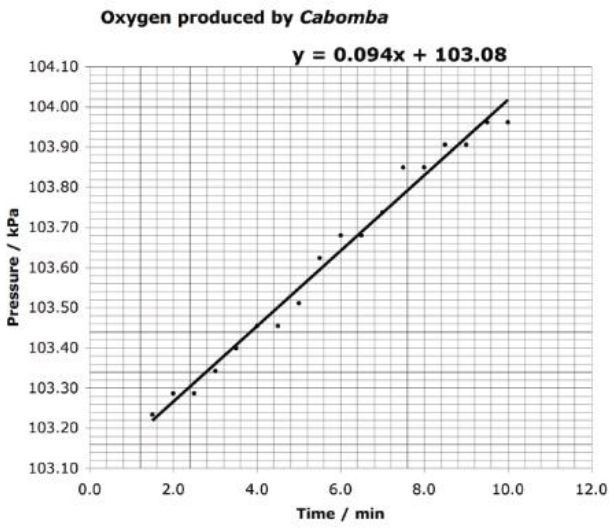

*Warning: Sharp instrument, handle with care*

3. Connect the pressure sensor to the rubber bung that fits in the test tube using a piece of plastic tubing. Mop up any spilt liquid that the bung displaces.
4. Put a bench lamp 20cm away facing the test tube. Leave the plant 2min to adapt. Verify the temperature of the liquid in the test tube after each run. You may need to consider controlling the temperature.

## Recording and Analysing the data

1. Select a time-based mode in the data collection menu. The experimental length should be about 10 minutes for a trial run and the frequency of measurement should be 60 per minute. Begin the data collection and a live graph will appear on screen.
2. Analyse your data using a linear fit trend line of the reaction where the line becomes regular. The equation of this line will give the reaction rate. An example is given below.



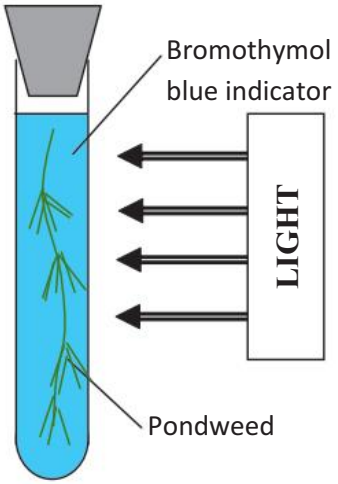
# Method D: Determining the consumption of carbon dioxide by a water plant using a pH indicator

Bromothymol blue is blue when there is no $CO_2$ (above pH 7.6), green when there is a little $CO_2$ (between pH 6 and 7.6) and yellow when there is a lot of $CO_2$ (below pH 6).

## Method

*Warning: Sharp instrument, handle with care.*


1. Take a branch of pondweed that is healthy and green. Using the razor blade, cut the branch 5cm from the terminal bud.
2. Fill the test tube with bromothymol blue indicator to 3cm from the top. Blow into the indicator using the drinking straw until it is green. Using a glass rod, push in the pondweed so that it is 1cm below the surface of the water (see diagram opposite).
3. Position the lamp at 20cm to illuminate the apparatus and observe at intervals over 30 min.




*Elodea canadensis* flowering

# Method E: Determining carbon dioxide absorption using a data logger and pH probe

## Calibrating the pH probe

### Materials

| | | |
|---|---|---|
| data logger | 3 small beakers | distilled water wash bottle |
| pH probe | buffers pH 4 and pH 7 | pond weed |

### Method

1. Plug in the probe to the first channel of the data logger. The data logger should automatically recognise the pH probe and it will need to be calibrated.
2. Prepare a beaker with pH 4 buffer and another with pH 7 buffer. Carefully remove the container of electrolyte from the end of the probe and store it in a safe place. Wash the probe with the wash bottle.
3. Follow the steps to calibrate the pH probe with both the pH 4 and the pH 7 buffers, washing the probe with distilled water between the different buffers and at the end of the calibration.
4. Replace the probe in the electrolyte until you need it for measurements.

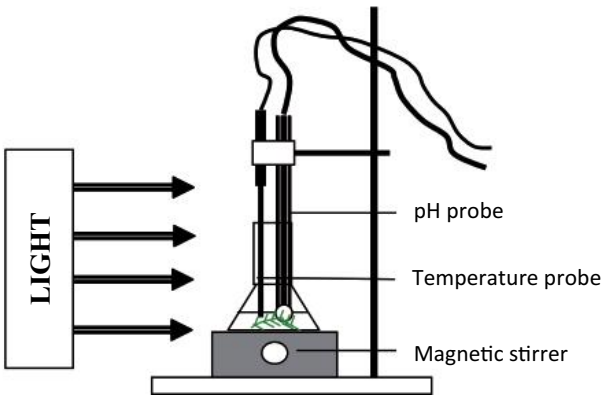
## Using the pH probe to measure photosynthesis

### Materials

| | | |
|---|---|---|
| Data logger | Magnetic stirrer | Marker pen |
| pH probe | Magnetic flea | Stop watch |
| Temperature probe | Stand and clamp | Bench lamp 60W |
| Conical flask 50cm$^3$ | 100cm$^3$ beaker | Tape measure 2m |
| | | pond weed |

### Method

1. Prepare a flask with a magnetic stirrer. Add 50cm$^3$ of tap water and a healthy piece of pondweed (approximately 10cm long).
2. Remove the probe from the electrolyte and clamp it in a stand so that it dips into the liquid in the flask. Add the temperature probe to the second channel of the data logger and clamp it so that it also dips in the liquid.
3. Select a time-based mode of data collection. The experimental length should be about 10 minutes for a trial run and the frequency of measurement should be 60 per minute.




4. Place a bench lamp 20cm from the flask. Begin recording the pH. The temperature of the liquid should be monitored at the same time to ensure that the lamp is not heating it up.
5. When the run is complete, remove the probe and wash it off. Clean out the flask, recuperate and wash off the magnetic flea. Store your latest data run.
6. Analyse the data by using a spread sheet program to draw a linear fit on the reaction rate.

# Investigation 2I - Methods to measure the rate of photosynthesis







## Assignment

Use your experience gained from the methods given below, to design an investigation into the effect of an environmental factor on the photosynthesis of a water plant.

You may use other materials that you know are available in the school laboratories.

Your method should build upon these simple protocols. You will find that some of the following protocols are appropriate for a limiting factor but not others.

## Design

- Establish the theoretical background. This will require some research on your species of plant.
- State a focussed research question.
- Write a hypothesis (a justified prediction).
- Clearly identify the variables (the independent or manipulated variable, dependent or measured variable and the important control variables).
- State the significance of each of the control variables and which variables cannot be controlled but need to be monitored.

## Method

- What equipment and materials will you need? This may be best described using a labelled diagram of the experimental setup.
- Consider any safety factors.
- Explain exactly how the independent variable will be changed (range and intervals) and how the dependent variable will be measured and how frequently.
- Explain how you intend to control all the other variables (how will you make your experiment a fair test) or monitor them if they cannot be controlled. Perhaps a control experiment will be needed.

*(Note: Controlled variables and a control experiment are not the same thing)*

### Notes

Using a water bath to control temperature is best but it may be possible for the plant to operate at room temperature. Nevertheless, the room temperature should be monitored to see if it varies significantly, especially if a bench lamp is close to the apparatus.

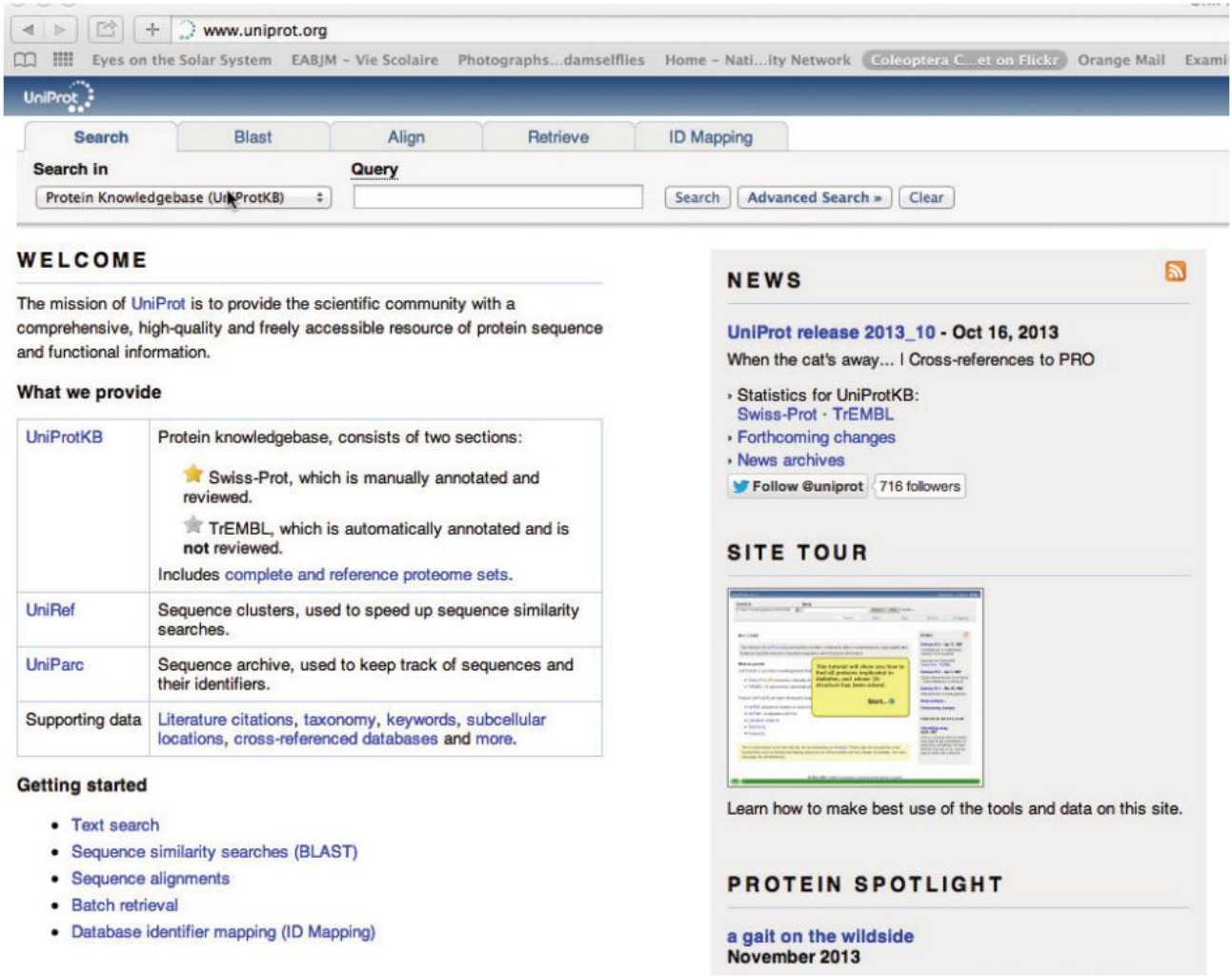
To control pH you should consider using buffers. Buffers resist changes in pH, acids such as hydrochloric acid may be diluted to appropriate pH values but they will not remain stable once the reaction starts. Solutions such as lemon juice and vinegar are inappropriate when pH needs to be controlled.

## Syllabus reference: Sub-Topic 3.1 Skill

Thanks to automated gene sequencing there are large databases that permit researchers to find out about the structure of different proteins. Here we shall investigate the structure of a well known protein; beta hemoglobin.

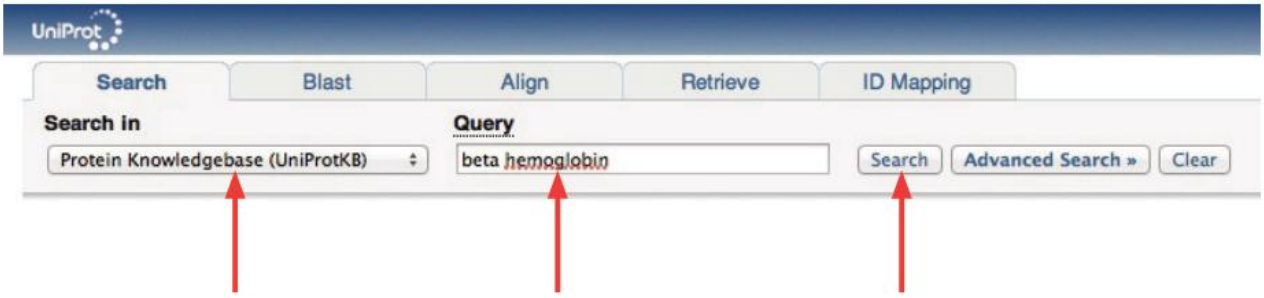
Open UniProt database: <http:/www.uniprot.org>

Select Protein Knowledgebase (UniProtKB)




Type in **Query**: (start with beta hemoglobin)

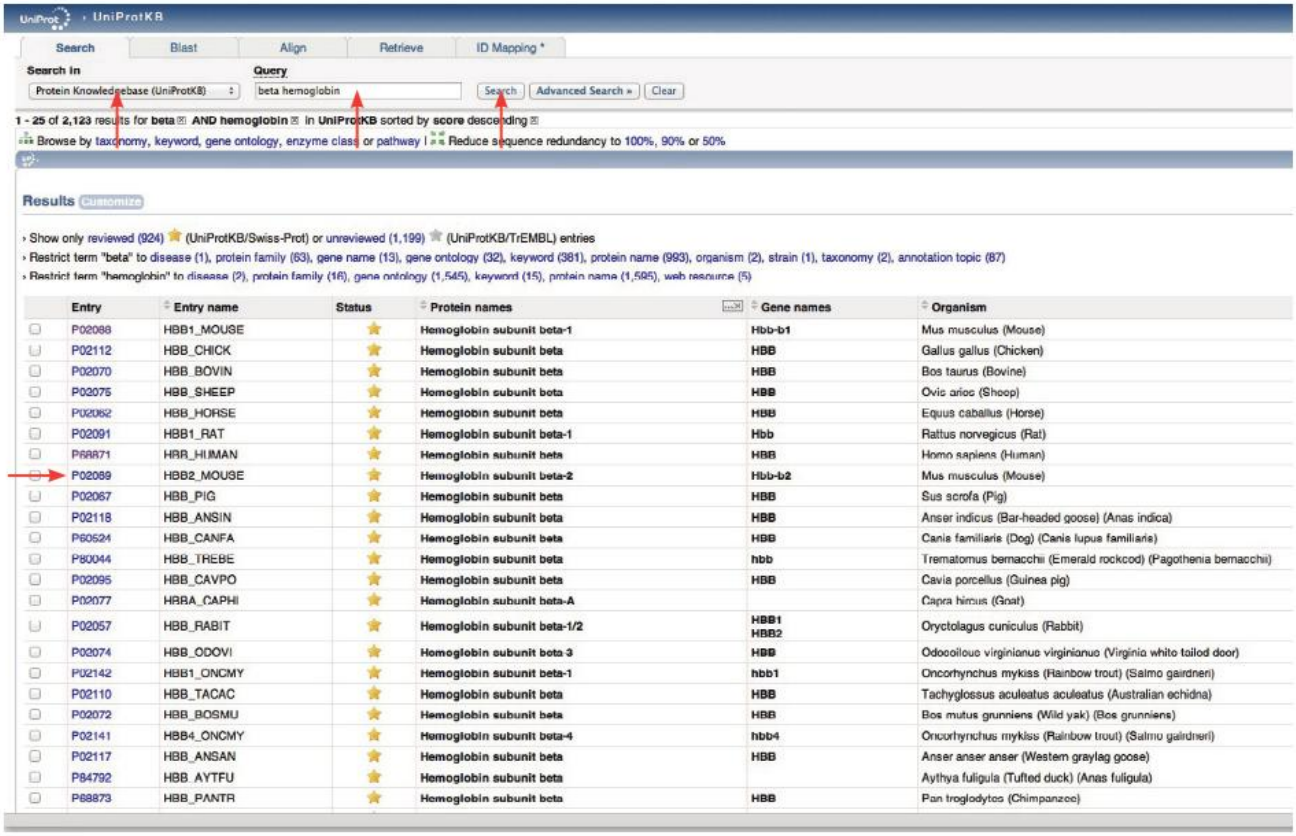
Then click on **Search**



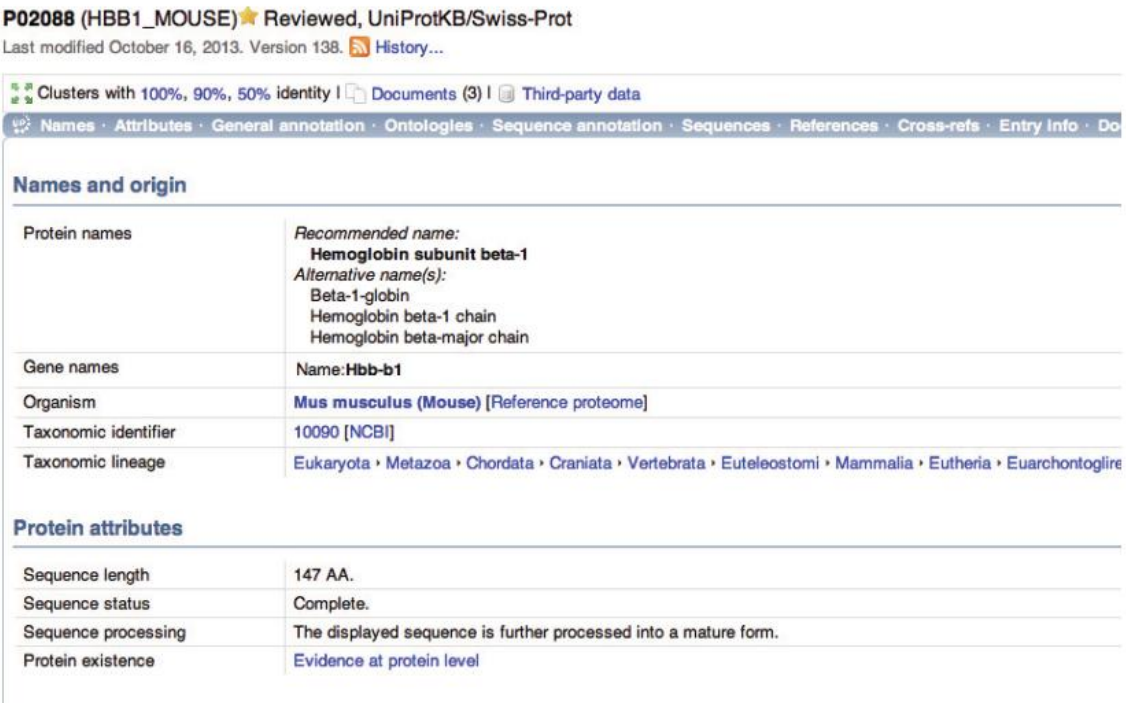
A list of species for which the sequence of amino acids is known will appear.

To see a sequence click on the **Entry**.

Try the house mouse (*Mus musculus*)




This will show the page for mouse beta hemoglobin.

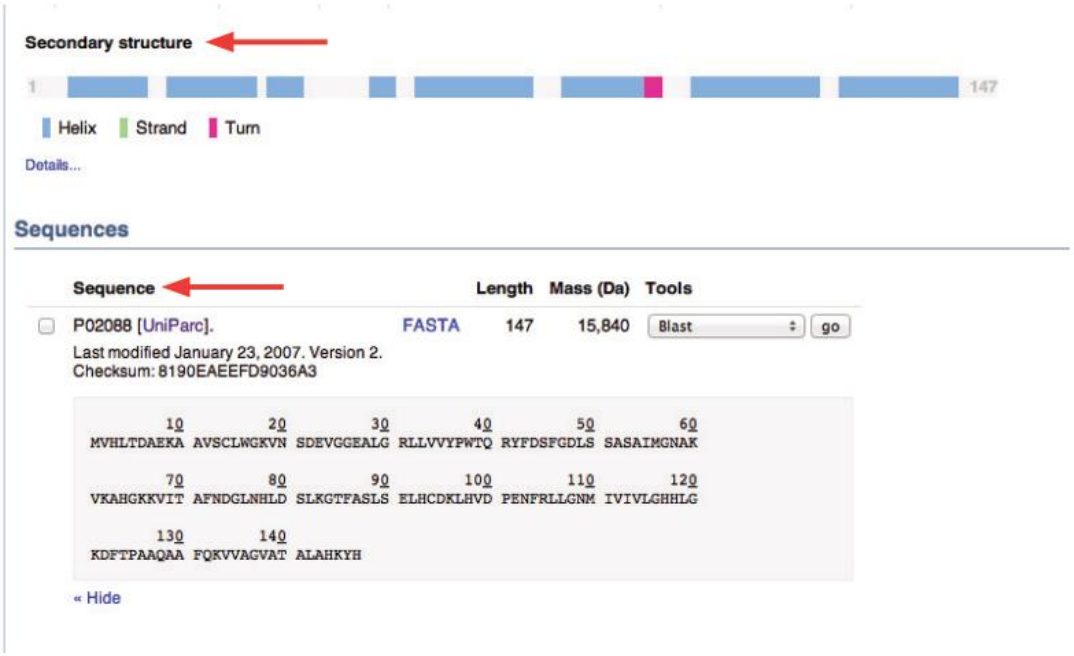


Scroll down to **Sequence**.

The amino acid sequence is given using the single letter code.

This is the primary structure of the protein. It is presented here in FASTA format (pronounced fast-a).

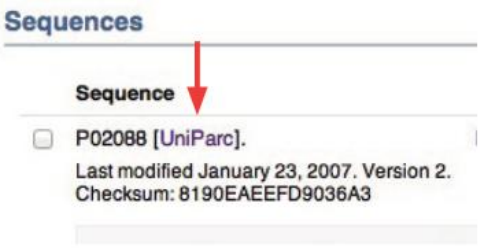
Details of the secondary structure of the protein are given above '**Sequences'**.



## The Nucleotide Sequence

To get to the nucleotide sequence for this protein click on the 'UniParc' entry next to the amino acid sequence.

This takes you to the list of all the mouse beta haemoglobin molecules in the database.




UniProt › UniParc

Search | Blast * | Align | Retrieve | ID Mapping

Search in: Sequence Archive (UniParc) — Query: Search | Advanced Search » | Clear

Sequence **UPI0000161CBA**

Entries · Sequence · Customize order

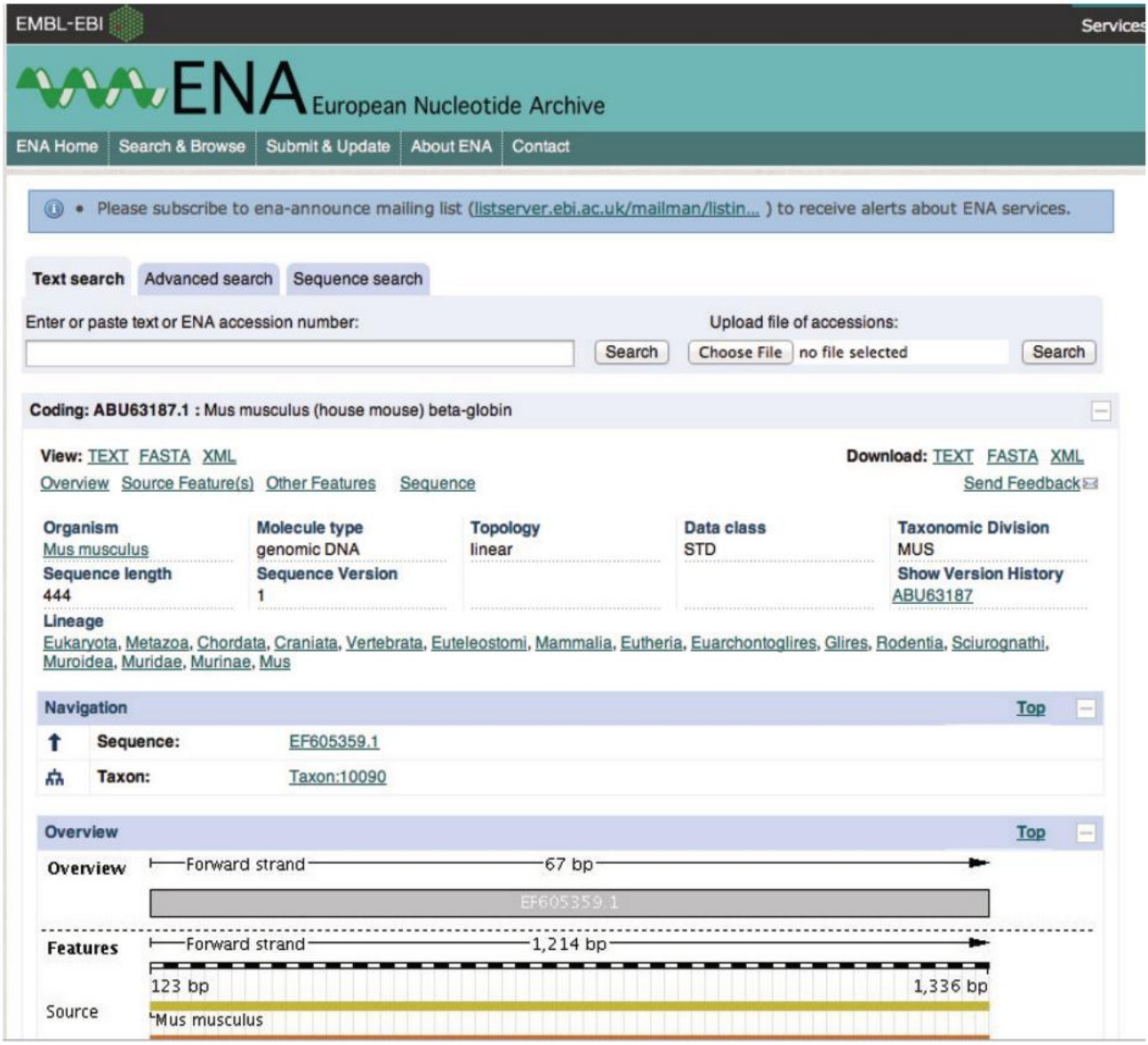
**Entries** Customize

Found in **6** databases: EMBL CDS, UniProtKB, IPI, PIR, REFSEQ and EMBL_CON

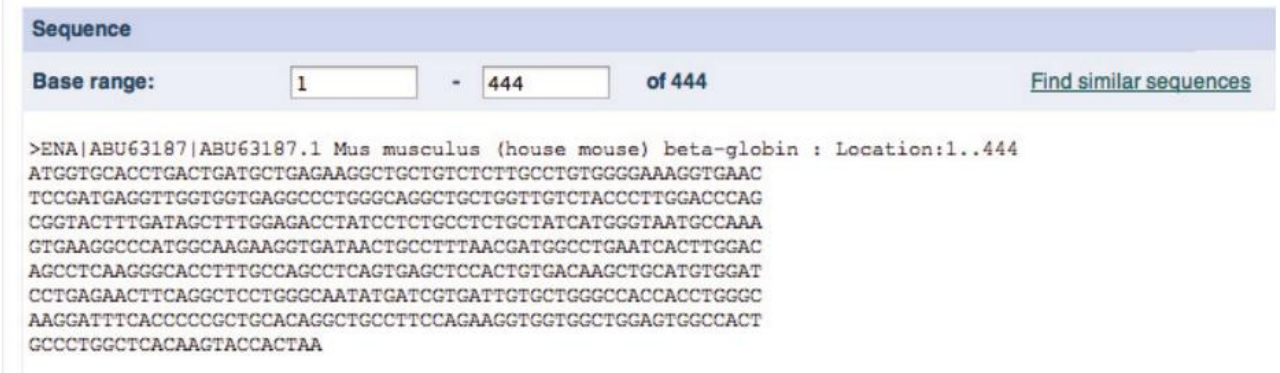
› Hide inactive entries (23)

| Database | Entry | Version | Organism |
|---|---|---|---|
| EMBL CDS | ADD52654 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | ADD52658 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | ADD52681 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | ABU63187 | 1 | Mus musculus (Mouse) |
| EMBL CDS | ADD52655 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | CAA32224 | 1 | Mus musculus (Mouse) |
| EMBL CDS | ABU63184 | 1 | Mus musculus (Mouse) |
| EMBL CDS | ADD52682 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | ADD52656 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | ABU63176 | 1 | Mus musculus (Mouse) |
| EMBL CDS | ACY03368 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | EDL16687 | 1 | Mus musculus (Mouse) |
| EMBL CDS | ADD52662 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | ADD52660 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | ADD52668 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |
| EMBL CDS | ADD52657 | 1 | Mus musculus castaneus (Southeastern Asian house mouse) |

Select one for *Mus musculus* the common house mouse.




This will take you to another database; the European Nucleotide Archive (ENA).




Scroll down to the nucleotide sequence.

## Exercise

Try the same thing for beta hemoglobin in another species of animal.

**Syllabus reference: Sub-Topic 3.5 Skill**

Many species of flowering plants can easily regenerate their tissues if they are damaged. Gardeners take advantage of this to clone plants by taking cuttings. Stimulating plants to grow from cuttings can be carried out in liquid media or solid media.

## Materials

cutting tool

alcohol

cuttings from a plant e.g. *Tradescantia, Impatiens*, geranium, bamboo, African violet, begonia

## General Method

1. Make sure that the plant to be cloned is well watered; it should not be wilting. Cuttings taken early in the day will be more successful as the plant will be fully turgid.
2. Clean the knife with alcohol and select healthy tissue from the plant to be cloned.


*Warning: Sharp instruments, handle with care*

3. Take the cuttings from the upper parts of the plant showing recent growth and remove any flower heads or flower buds. Lateral branches are better than the terminal branch.
4. Keep the cuttings cool and moist before they are used. Store the cuttings in damp paper in a fridge if they are not to be used immediately.
5. Remove the leaves from the lower third of the cuttings.

# Method A: Using a liquid medium

## Materials

wide-necked conical flasks 50cm$^3$

glass tubing 20cm

plastic tubing

aquarium pump

non-absorbant cotton wool

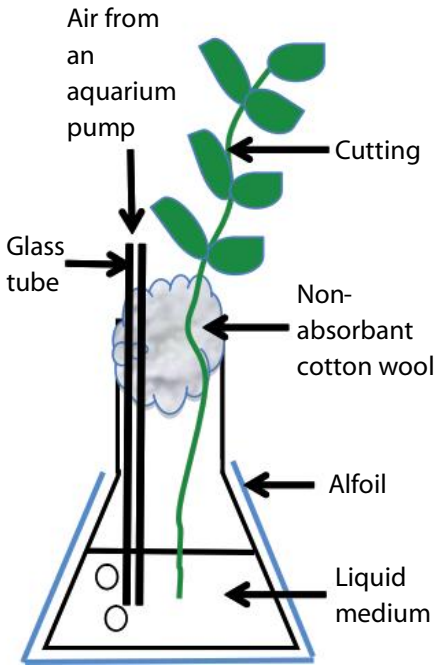
liquid media

distilled water

aluminium foil

## Method

1. Place the cutting in the flask so that the cut end is immersed in the culture medium.
2. Insert the glass tube in to the medium and hold the cutting and tube with non-absorbent cotton wool.
3. Attach the glass tube to an aquarium pump using glass tubing. Adjust the height of tube in the flask, it should be under the surface of the liquid but not touching the bottom. Adjust the aquarium pump so there is a steady flow of air to aerate the liquid.
4. Cover the flask in aluminium foil. This stops light entering the flask so algae will not grow in it.
5. Leave the plants in a cool, humid environment out of direct sunlight.



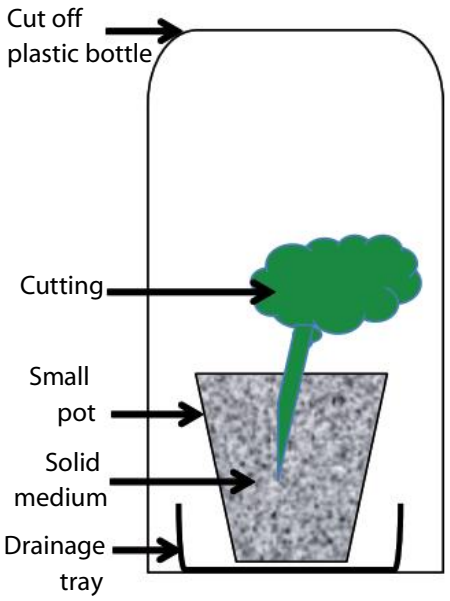
# Method B: Using a solid medium

## Materials

small pots
tray
cut off plastic bottle
medium: 40% medium grade vermiculite & 60% perlite or 50% vermiculite & 50% sand

## Method

1. Make a hole in the cutting medium big enough to put the stem of the cutting in. Gently push the cutting into the hole and firm the medium around it so it is supported.
2. Water the pot until the liquid starts to flow out into the draining tray. The medium is then at field capacity.
3. Place the plastic bottle over the pot and the cutting. Leave the plant in a cool place out of direct sunlight.




## To investigate further

- Does a particular species of plant root better in solid or liquid media?
- Hormone rooting powder or gel can be bought in garden centres these contain synthetic hormones such as indole acetic acid (IAA) or 1-naphthylactetic acid (NAA). Do they have the same effect on different species of plants? The cutting will need to be treated before they are planted.

*Warning: The rooting powders or gels are biologically active compounds. Use gloves. Do not agitate the powder form. Use in a well ventilated area.*



- Honey is said to stimulate rooting in plants. How could you test this?
- Do certain mineral nutrients affect rooting and root growth?

  Standard plant mineral nutrient solutions can be prepared (e.g. Sachs solution or Knop's solution) and equivalent solutions lacking one of the minerals can also be prepared.

- Mycorrhizal fungi are said to improve the rooting of their host species. Soil supplements containing mycorrhizal fungi can be obtained from organic garden suppliers.

*Warning: The mycorrhiza may cause allergies. Use gloves. Do not agitate the powder form. Use in a well ventilated area.*


# Investigation 4A - Ecological succession in an microbial ecosystem



**Syllabus reference: Sub-Topic 4.1 Skill**

Sealed microbial ecosystems can be set up and studied in a laboratory where simple experiments can be carried out under controlled conditions.

Such a sealed microbial ecosystem can be used to study energy flow and nutrient recycling in ecosystems and may be referred to as a mesocosm.

## Materials

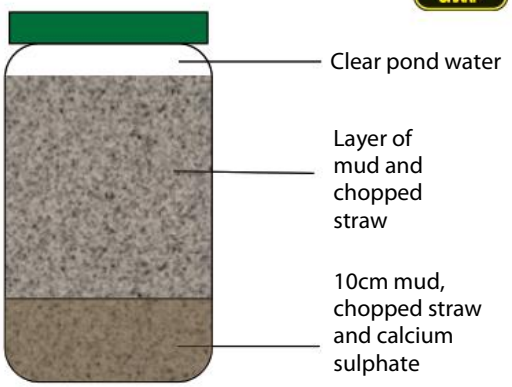
- straw
- scissors
- 2 tall glass jars with air tight lids
- pond water
- mud taken from a pond or a ditch
- calcium sulphate
- marker pen
- ruler
- glass rod
- spatula
- 2 beakers 250cm$^3$

## Method

1. Cut up a handful of straw into small pieces about 1cm long. Place the pieces of straw into the bottom of the glass jar.

*Warning: Sharp instrument, handle with care*

2. Collect 200cm$^3$ of mud in a beaker and add a heaped spatula of calcium sulphate, mix these together. Pour the mixture into the jar up to a depth of at least 10cm. Mix the components thoroughly to eliminate as much air as possible.
3. Complete the preparation by adding more of the mixture of mud and chopped straw, that has not had calcium sulphate added to it, until the jar is completely full. Screw on the lid tightly. Label your jar with your name(s) and leave it near a window where it will receive sunlight.
4. Over the following weeks, observe the ecosystem regularly and observe any changes.




5. Determine what you think are the most important variables for the growth of the microbes. Set up a second jar to investigate the influence of one of these variables on the development of the microbial community.

Two zones have been created by the addition of calcium sulphate to the bottom layer mud and not to the top layer. The lower zone will rapidly produce anaerobic conditions. The sulphate provides a substrate for chemolithotrophic bacteria.

Microbes will appear as stains growing on the sides of the glass. Their colour and their position can be used to identify them.

| Coloured stain | Microbe | Conditions in the column |
|---|---|---|
| Green | Alga or cyanophyta (photolithotrophs) | Aerobic clear water zone |
| Purple, red or orange | Purple non-sulphur bacteria (photo-organotrophs) | Upper mud zone: anaerobic and poor in sulphur |
| Purple | Purple sulphur bacteria (photolithotrophs) | Middle mud zone: anaerobic and rich in $H_2S$ |
| Green | Green sulphur bacteria (photolithotrophs) | Lower mud zone: anaerobic and rich in $H_2S$ and insoluble sulphur |

Anaerobic heterotrophic bacteria, such as *Clostridium*, decompose the straw. This produces complex organic compounds, such as fatty acids, which can be used by chemolithotrophic sulphur bacteria. These bacteria produce hydrogen sulphide by the reduction of sulphates. The hydrogen sulphide, in turn, acts as a source of hydrogen for the green or purple photosynthetic sulphur bacteria.

## To investigate further

- Different environments could be tried, e.g. light, dark, daylight, artificial light, filtered light.
- Different mixtures, e.g. with/without straw, more or less mud, different amounts of calcium sulphate.
- Top up the jar with pond water instead of mud and chopped straw.
- Mud or pond water from different locations could be compared.
- Such a mesocosm can also be setup in a tall cylinder to show various coloured microbial colonies and stages of succession. You could search the internet for 'Sergei Winogradsky' or the 'Winogradsky column'.

*A Winogradsky column*

*Source: http://upload.wikimedia.org/wikipedia/commons/0/0b/Winogradsky.jpg?uselang=en-gb*

**Syllabus reference: Sub-Topic 4.1 Skill**

Species living in the same habitat will have similar requirements. Their niches will overlap. To see if there is evidence of a significant overlap a test of association can be carried out using the $\chi^2$ test.

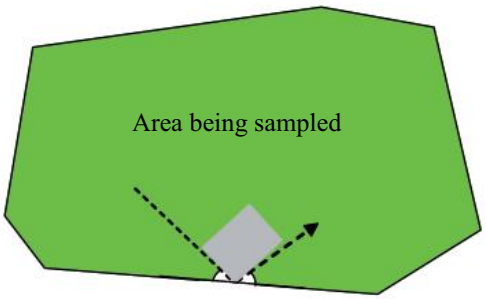
## Materials

1m$^2$ quadrat

pen or pencil

random number tables (or notebook TI calculator with Prob Sim.)

## Method




1. Identify the species to be recorded and set the limits of the sampling area. Spin your pencil and, heading in the direction that the pencil points, pace out the number of steps indicated by the first number on the random number tables. At the boundaries of your sampling area bounce off (diagram)and keep pacing.
2. Once the correct number of paces has been made, place the quadrat on the ground and record the presence or absence of the species. Look carefully in between the other plants in case they are hidden.
3. Repeat the exercise enough times so that the class results will give at least 100 quadrats.
4. Collect the class results and calculate the totals for:
   - the quadrats where both species are present
   - the quadrats where both species are absent
   - the quadrats where species A is absent but species B is present
   - the quadrats where species B is absent but species A is present

### 5. Compare the species using the the $\chi^2$ test

Set up the Null Hypothesis (NH) and the Alternative Hypothesis (AH). In this case the Null Hypothesis would be that there is no association between the species.

Set up a 2 × 2 table of the observed frequencies (O).

| | | Species A | | |
|---|---|---|---|---|
| | | Present | Absent | Row total |
| Species B | Present | | | |
| | Absent | | | |
| | Column total | | | Grand total |

There is no hypothesis that can lead us to a particular expected frequency we can calculate expected values from the observed values. According to the null hypothesis this should be what we get if there is no association.

Calculate the expected frequencies (E) for each of the boxes in the table = $\frac{\text{Row total} \times \text{Column total}}{\text{Grand total}}$

Now calculate the $\chi^2$ value = $\Sigma \frac{(O - E)^2}{E}$

This is best done in a new table.

| | O | E | (O-E) | $(O-E)^2$ | $(O-E)^2/E$ |
|---|---|---|---|---|---|
| Sp A present Sp B present | | | | | |
| Sp A present Sp B absent | | | | | |
| Sp A absent Sp B present | | | | | |
| Sp A absent Sp B absent | | | | | |
| | | | | Total ($\chi^2$) | |

Calculate the degrees of freedom = (number of rows – 1) × (number of columns – 1) which will always be 1 in these cases.

Compare the calculated $\chi^2$ value with the critical levels in the probability table.

| | Probability levels for $\chi^2$ | | | | | | |
|---|---|---|---|---|---|---|---|
| Degrees of Freedom | 0.950 | 0.900 | 0.500 | 0.100 | 0.050 | 0.010 | 0.001 |
| 1 | 0.0039 | 0.016 | 0.455 | 2.71 | 3.84 | 6.63 | 10.83 |

The difference is not significant. The NULL Hypothesis is accepted.

The difference is significant. The NULL hypothesis is rejected.

The critical value ($\chi^2_{crit}$) is taken to be p = 0.05 (or the 5% level).

Do your results reject or accept the Null Hypothesis?

State the highest value of p for which the results are not significant.

Discuss and criticise the investigation.

## Some points for consideration

- Do the species show any particular adaptations that may help them to compete easily with other species?
- Criticise this method of estimating the association between two species of plant in a habitat.

  (i) Would it make any difference if the species were clumped, uniform or random in their distribution?

  (ii) Would it make any difference if the species chosen were large or small?

  (iii) How could you improve the method to make the quadrats more randomly distributed?

**Syllabus reference: Sub-Topic 5.3 Application and Skill**

Keys are a systematic way of identifying an organism. Dichotomous keys work by asking two mutually exclusive statements. The organism being observed should fit in one category or the other.

## Materials

hand lens

type specimens of moss, fern, conifer and flowering plant

unidentified specimens

## Method

1. Observe the specimens and divide them into two groups (they do not have to be equal sized groups).

### Example

| | |
|---|---|
| (1) (a) Possesses true leaves | go to (2) |
| or (b) Only possesses leaf scales | Bryophyta (Moss plant) |

2. Repeat the process until each type specimen has been identified.

3. Test your key using the unidentified specimens.

### Points to remember

- Do not use size as it is variable. However, the presence of strengthening tissue (e.g. wood) permitting tall growth forms (e.g. tree habit) to develop is permitted.
- Do not use colour, it can be very variable.
- Avoid using the absence of a feature as a principal characteristic. However, it can be used to separate into two groups: So the following is acceptable: (a) possesses seed bearing structures OR

(b) does not produce seed bearing structures

*Euphorbia*
*Angiospermophyta*

*Moss*
*Bryophyta*

*Pine tree - Coniferophyta*

*Fern - Filicinophyta*

## Syllabus reference: Sub-Topic 6.1 Application

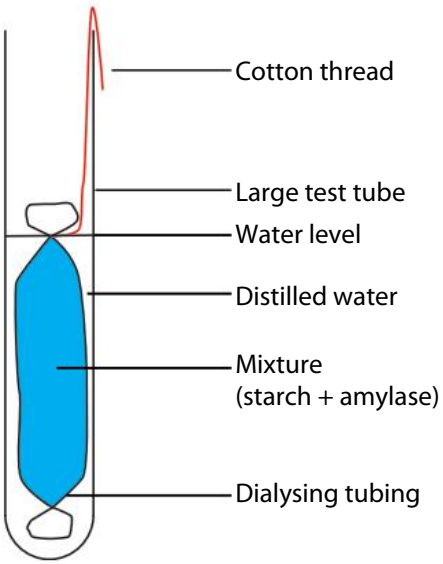
A model of the digestive system can be made from a piece of dialysing tubing. Digestion is the breakdown of large molecules into small molecules so that they can be absorbed by the wall of the intestine.

## Materials

- starch solution (2%)
- amylase solution (2%)
- 2 small beakers
- 15cm of dialysing tubing
- glass rod
- cotton thread
- syringe 2cm$^3$
- large test tube
- electronic water bath
- dropping pipette
- Pasteur pipette
- 6 test tubes in a rack
- test tube holder
- stop watch/clock
- wash bottle of distilled water
- Fehlings or Benedicts solution
- iodine solution
- safety glasses

## Method

1. Wet the piece of dialysing tubing and open it using a round-ended glass rod. Tie a secure knot in one end of the tubing. Inject the mixture of starch into the open end of the dialysing tubing using the dropping pipette.
2. Using a syringe, add 2cm$^3$ of amylase to the tube and tie a thread of cotton tightly around the open end. Wash the outside of the tubing thoroughly under the tap.
3. Suspend the dialysing tubing in the large test tube and fill it with distilled water up to the level of the knot (as shown opposite). Immediately start your stop clock and, using the Pasteur pipette, take one small sample from the distilled water in the large test tube. Pipette the samples into the three test tubes.
4. Test the samples for the presence of starch using iodine solution and for glucose by adding an equal volume of Fehlings or Benedicts solution to the sample in a test tube. Heat this mixture gently in a water bath at 80°C for 2 minutes. Positive result = green, yellow or red, depending upon the amount of sugar present.





*Warnings: Hot water can scald, use a test tube holder. Fehlings solution is caustic. Wear safety glasses. Wash off spills with water.*

5. Leave the mixture for two minutes, take a second sample and place it in a clean test tube. Repeat the test at 2-minute intervals for 24 minutes.
6. Record your results for the experiment and give your observations and conclusions.

### Some points for consideration

- What are the sources of error that you may have encountered?
- How does the model resemble the process of digestion?
- How could the model digestive system be improved upon?

# Investigation 6B - The anatomy of the heart



**Syllabus reference: Sub-Topic 6.2 Skill**

The heart of mammals is found in the thoracic cavity between the two lungs. It is the pump of the blood circulatory system. The heart is connected to the other organs of the body by blood vessels. The structure of the heart of a lamb (or a pig) is comparable to the structure of a human heart.

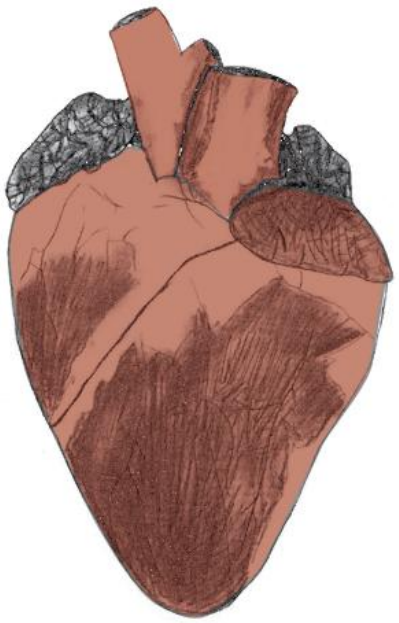
## Materials

- An intact heart of a sheep (or pig)
- Dissecting dish
- Large scissors
- Probe
- Paper towel
- Rubber tube attached to a tap
- Gloves

## Method

### PART A: Observations on the heart and the vessels attached to it

1. Record the external appearance of the heart, its shape and colour.
2. On the top of the heart are two atria. Note the colour of the atria. Locate the blood vessels on top of the heart and identify the arteries and the veins. The cross section of the arteries remains open and their walls are thick, yellowish and elastic. The thin, pinkish walls of the veins flatten and collapse where they are cut.
3. An oblique shallow groove runs across the lower part of the heart. This identifies the ventral side of the heart (See diagram opposite). What do you observe in this groove?
4. Make an annotated drawing of the ventral surface of the heart. Add any other observations that you have made (e.g. colour, distribution of fat).



Ventral view of the heart

### PART B: The examination of the interior of the heart

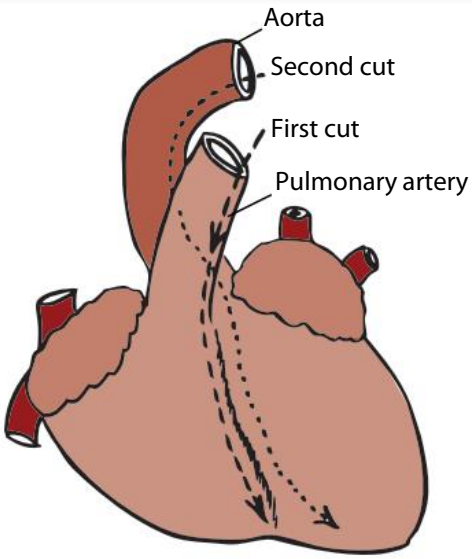
1. Reach into the interior of the heart by inserting your index finger (or a probe) successively into each blood vessel. Push against the heart wall in different places. How many cavities can you count? Do these cavities communicate with one another? Explain your answer.
2. The pulmonary artery is connected to the right ventricle and the aorta to the left ventricle. The superior vena cava and the inferior vena cava irrigate the right atrium, the pulmonary veins run into the left atrium. The right atrium receives blood from the two vena cava, which bring all the blood from the organs to the heart. The pulmonary veins bring blood from the lungs to the left atrium.

### PART C: Using water to simulate the flow of blood in the heart

1. Slide the rubber tube from the tap into the right atrium. Flush water through the right atrium. Note your observations. Now do the same for the left atrium.
2. Next, slide the tube into the pulmonary artery. Let water flow gently into this vessel whilst you observe from above the vessel. What do you observe? Now do the same for the aorta.

## PART D: Looking for structures which direct the blood flow through the heart

### The dissection of the heart



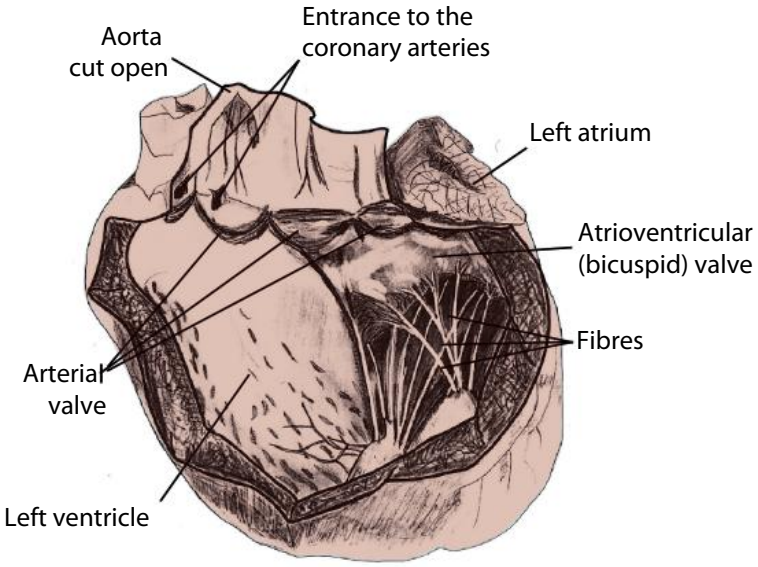

1. Put the heart in the dissecting dish with the ventral surface facing upwards and the base of the heart pointing towards you.
2. **First cut:** using a pair of scissors, cut the wall of the pulmonary artery along its entire length. Then cut the wall of the right ventricle a few millimetres from the groove that lies between the two ventricles, as indicated by the broken line (- - -) on the diagram opposite. Open the flap of the ventricle wall to observe the inside of the cavity.
3. **Second cut:** Cut the wall of the aorta and then the left ventricle. Follow the dotted line (.....) on the diagram opposite as far as the apex of the heart, staying very close and parallel to the groove between the ventricles.

*Warning: Sharp instrument, handle with care*


4. Three pockets are found at the beginning of each artery. These are the arterial (semilunar) valves.
5. Each of the openings between the atria and the ventricles is guarded by a valve made of flaps. These flaps are attached by fibres to the ventricle walls. These valves are called the atrioventricular valves, (the valve on the left side of the heart is called the bicuspid and the valve on the right side of the heart is called the tricuspid).

## Some points for consideration

- Compare the structure of the two sides of the heart.
- From your observations using water, deduce the pathway of the blood through the each side of the heart.
- Explain the role of the valves in the circulation of the blood.
- The entrance to the coronary arteries can be found just after the arterial (semilunar) valve in the aorta. Use a probe to explore this artery.




A ventral view of the heart with the left side opened

## Some points for consideration

- How can you explain the difference in thickness between the walls of the right and left ventricles?
- How do the arterial valves (semilunar valves) differ from the atrioventricular valves? At what points will they function in the cardiac cycle?
- Describe the difference in the appearance of the arteries (e.g. aorta) and the veins (e.g. pulmonary vein).

## Research

- What is the difference in the composition of the blood leaving the right ventricle and leaving the left ventricle?
- What are the respective functions of the veins and the arteries.
- The beating of the heart is a dynamic process.
- This is best observed in animations. A number of useful URLs exist:

**A clear explanation with some useful animation of the heart beat.**

<http://www.sumanasinc.com/webcontent/animations/content/human_heart.html>

**Some interesting animation and interaction with this site from Nova. You can alter the heart rate.**

<http://www.pbs.org/wgbh/nova/body/map-human-heart.html>

**Syllabus reference: Sub-Topic 6.4 Skill**

Spirometers measure the volume of air breathed in and out.

## Materials

- data logger
- spirometer sensor
- disposable breathing tubes or antiseptic wash
- bacterial filter
- exercise bike
- nose clip
- consent form (see Appendix)
- Optional: gaseous oxygen sensor
- Optional: heart rate sensor

## Method

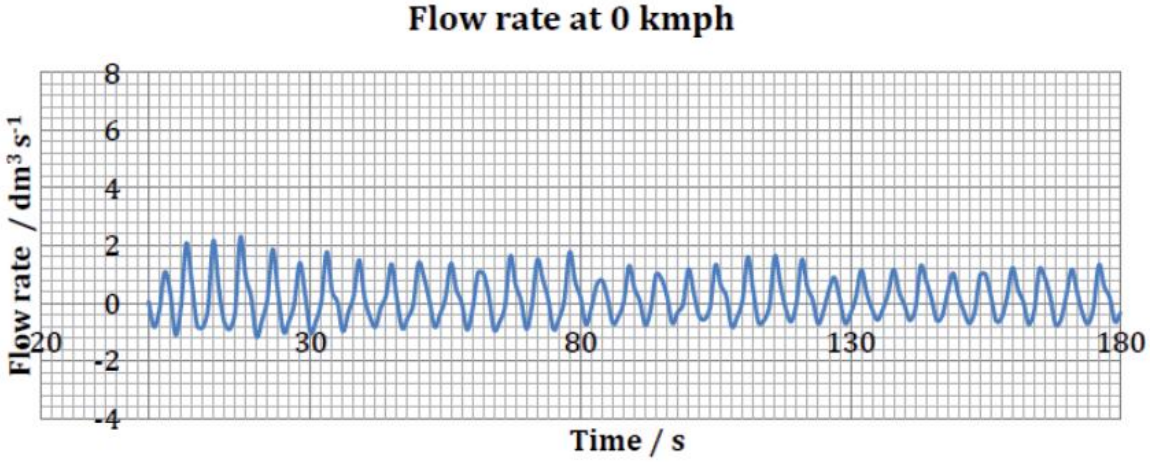
### Collecting the data

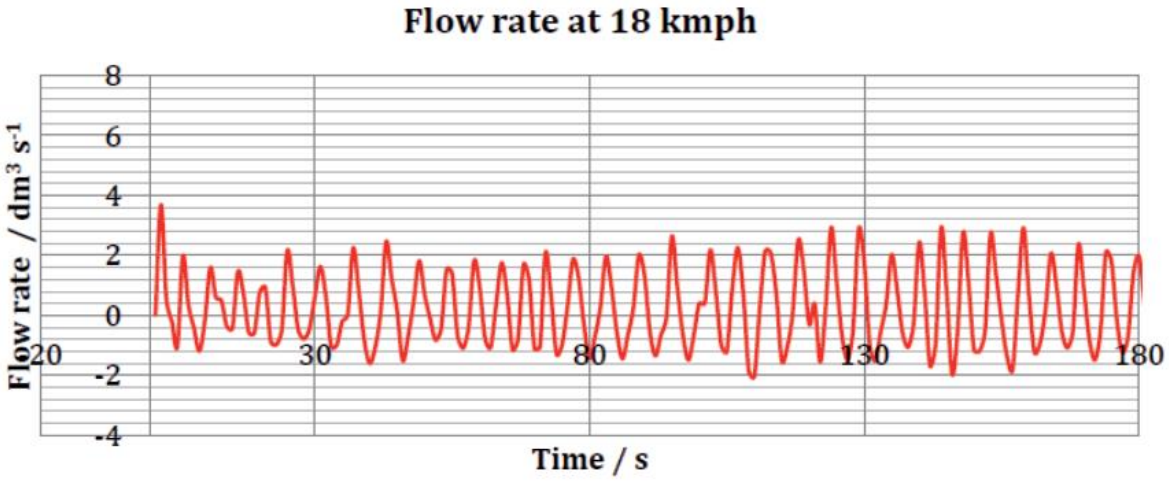
*Warning: Ensure that the subject is healthy and able to participate in the exercise. Explain the nature of the experiment and use a consent form.*


1. Set the subject comfortably on the exercise bike. Attach a nose clip.
2. Connect the spirometer sensor to the data logger and set the data logger to collect data at one measurement per second over 180s (3min).
3. Attach a disposable mouth piece to the spirometer and if available, a bacterial trap.
4. Take the subject's pulse rate at rest.
5. Ask the subject to breath in and out regularly through the disposable mouthpiece. Once a regular ventilation rhythm is obtained, start recording the ventilation using the data logger. Continue until the 180s are completed. Save the trial.
6. Ask the subject to pedal the bike at a regular speed (e.g. about 10km per hour), repeat the recording and save the trial data.
7. Check the subject's pulse rate until it returns to the resting rate.
8. Perform the experiment again pedalling at a faster speed (e.g. about 20km/h) and save the trial.

### Analysing the data

The flow rate recorded by the spirometer will show an oscillation. The frequency of the oscillation is the ventilation rate and the range of the oscillation gives the volume breathed in and out per breath. Two examples are shown below.






Sample the oscillations over the 3 minute period at 1 sample per second to obtain an average for the volumes and frequencies.

## To investigate further

- Comparing ventilation rates at different speeds.
- Comparing ventilation rates at the same speed but different loads.
- Comparing males and females of the same age.
- Comparing subjects of different ages.
- Comparing breathing rates and pulse rates.
- Calculating recovery time after a given period and intensity of exercise.
- Measuring the oxygen content using a gaseous oxygen probe.
- Calculation of the $VO_2$ max

**Syllabus reference: Sub-Topic A.2 Application**

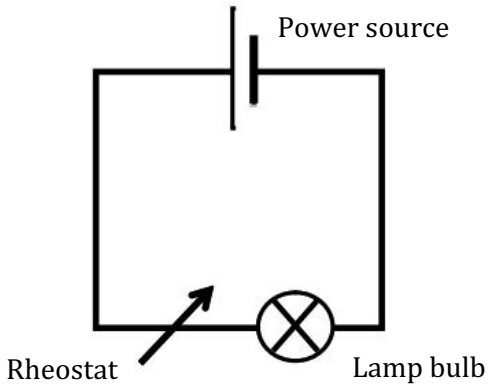
The pupil reflex is a standard test used in medicine to verify the state of the brain.

## Materials

- 4V battery or power supply
- 3 cables and connectors
- 3.5V bulb in holder
- rheostat (potentiometer)
- small mirror
- room with blackout
- mobile phone or video camera
- computer

## Method

1. Build a simple electrical circuit (shown adjacent)
2. Position the circuit so that the lamp is out of line of sight (below your eyes.)
3. Turn out the lights in the room and turn the rheostat so that the lamp glows bright. Using the mirror, observe what happens to the pupils of each eye. Do they both behave in the same way?
4. Slowly turn the light down and pause at different intensities. Note how the iris reacts. When the light intensity is constant does the pupil stay the same diameter?
5. Move the lamp so it is in front of the right eye. Shade the left eye by placing your hand in front of your nose. Now increase the light and observe what happens to the irises of the two eyes.




6. Repeat this the other way round. Put the lamp in front of the left eye and shade the right eye from the light.
7. Try this on your partner. You may find it useful to film the reaction of the pupil using a mobile phone and analyse the response more carefully on screen.

*Warning: Ensure that the subject has no eye problems and is able to participate in the exercise. Explain the nature of the experiment and use a consent form. Do not use LEDs or lasers as a light source for this investigation.*


## Some points for consideration

- How can the response of the iris be explained in terms of negative feedback?
- How can you explain the response of the shaded eye?
- Brain structure and detecting anomalies

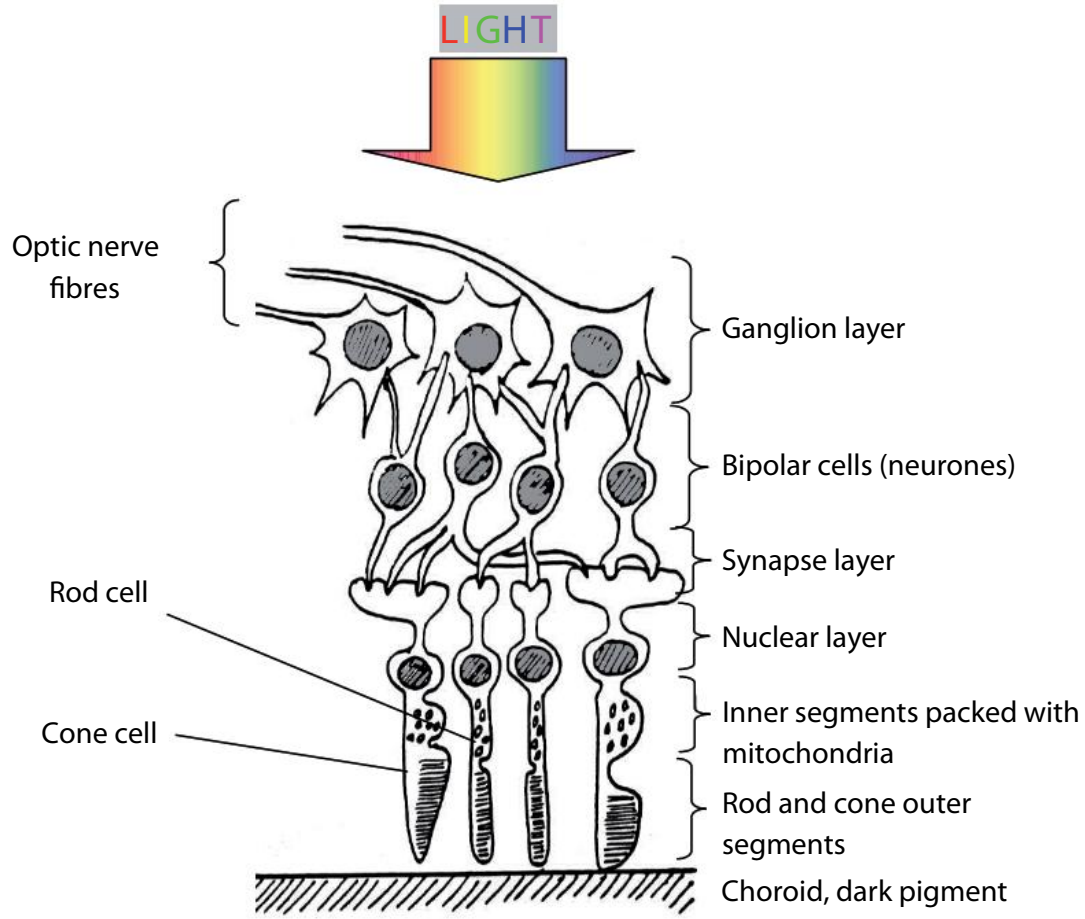
## Research

- Find out which sensory and motor nerves are involved in the pupil reflex.
- Draw a diagram of the reflex arc involved in this reflex.

**Syllabus reference: Sub-Topic A.3 Application**

The retina is made of several layers of cells. Light passes through the neurones and stimulates the rod and cone cells next to the pigmented choroid layer.

### The structure of the retina




The photoreceptors in the retina are the rod and cone cells. These are excitable cells that are stimulated by particular wavelengths of light.

| Cell | Wavelength / nm | Peak Colour Sensitivity |
|---|---|---|
| Rod cells | 500 | Yellow-green |
| L cone cells | 564-580 | Orange |
| M cone cells | 534-555 | Green |
| S cone cells | 420-440 | Blue-violet |

The photoreceptors contain pigments that absorb these different wavelengths. When the pigments absorb light they are bleached and need to be reactivated.

The rod cell pigment (rhodopsin) is very easily bleached but the pigments in the cone cells are less easily bleached.

What kind of cells are used in daylight vision and low light night vision?

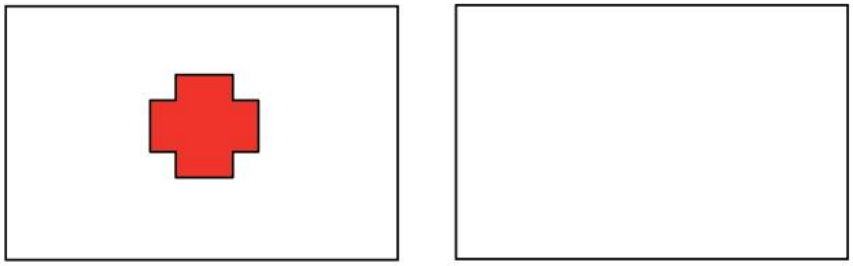
The colours of light that can be sensed are said to lie in the visible spectrum. Pure red, blue and green wavelengths are sensed by the different cone cells.

## Afterimages and complementary colours

bench lamp
stop watch
sheets of thick, white paper
coloured marker pens: bright red, green and blue

## Method

1. On one of the sheets of paper draw a large clear cross using the marker pen.



2. Shine the bench lamp onto the paper with the cross and concentrate on it for a full minute.
   - After one minute, your partner should place the other sheet of white paper covering the cross.
   - Concentrate on the new piece of paper.
   - Describe what you see.
   - You should see an afterimage of a cross. The colour of the cross is important.

3. Try the other colours.

   Which cones are stimulated when you look at a red cross?

   Which cone cells are being stimulated when you are looking at a white sheet?

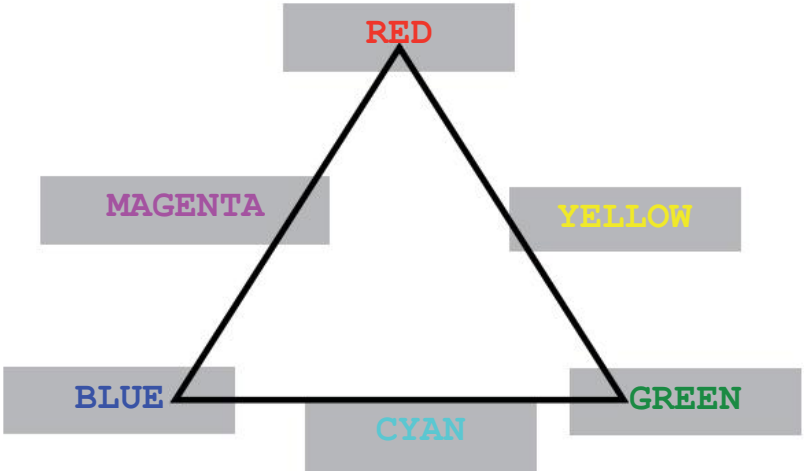
   Which cones would be stimulated if you were looking at a black object?

   How can we explain the presence of an afterimage? What is happening in the cone cells?

   Remember the pigments in the cone cells are bleached when light of the right wavelength falls on them.

   How can we explain the colour of the afterimages?

*The following diagram will help.*




The primary colours are at the corners of the triangle and the complementary colours are along the sides.

White is the presence of all colours and black is the absence of all colours.

### Afterimages from complementary colours

Fluorescent highlight markers are especially good at giving afterimages.

Predict what you will get if you stare at a yellow, cyan and magenta crosses for a minute.

You might not get quite what you expect.

Though cyan is clearly a mixture green and blue and magenta is a mixture of red and blue, what about yellow?

We do not perceive reddish-green nor bluish-yellow as colours. How can we explain this?

### Theories on colour vision

Two complementary theories try to explain colour vision are the **Trichromatic Theory** of *Young*, *Maxwell* and *Helmholtz* and the **Opponent-Process Theory** of *Ewald Hering*.

The trichromatic theory explains colour vision by the presence of three types of cone each sensitive to a different part of the visible spectrum. Colour vision is a simple mixture of the sensitivity of these three wavelengths. This works for most colour combinations.

However, some of the afterimage results can also be explained by the interaction between the signals coming from the three cells in the brain. These signals (red v green and blue v yellow) are stimulatory or inhibitory. In other words signals from different cones may oppose one another as well as add to one another. So when both L and M cones are stimulated but not S cones, we can see yellow as a distinct hue and not a greeny-red.

## To investigate further

Assessing the state of an image to a colour blind person is important for safety, education and advertising. A number of sites dedicated to colour blindness awareness exist where colour blindness is simulated. Some of these sites even permit you to upload and try out an image as it would appear to people with different types of colour blindness.

### Colour blindness simulations

<http://www.color-blindness.com/coblis-color-blindness-simulator/>

<http://www.vischeck.com/daltonize/>

<http://www.etre.com/tools/colourblindsimulator/>

<http://www.colourblindawareness.org/colour-blindness/colour-blindness-experience-it/>

# Investigation 13A – Do disinfectants kill bacteria?



**Syllabus reference: Sub-Topic B.1 Skill**

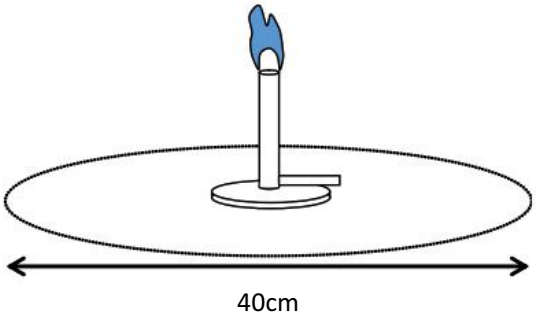
**Disinfectants** are chemicals that can kill a population of bacteria, although total sterility may not be achieved, **antiseptics** are chemicals that simply inhibit bacterial growth. The purpose of the following exercise is to demonstrate the effect of disinfectants on a population of bacteria.

## Materials

- bleach solution
- cotton wool
- soap
- disinfectants in a beaker
- discs of filter paper
- glass spreader
- beaker of alcohol
- test tube and stand
- bacterial culture (e.g. Lactobacillus)
- 2cm$^3$ syringe
- nutrient agar plate
- wire inoculating loop
- Bunsen burner
- matches
- forceps
- marker pen
- self-adhesive tape

### Working in a sterile zone

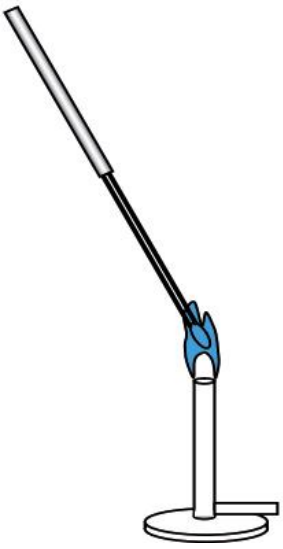
- Clean the bench with bleach using a cotton wool swab.
- Set up a Bunsen burner in the centre of the bench and leave it on a yellow flame. As soon as any sterile material is placed on the bench the Bunsen must be turned to a blue flame. The blue flame will ensure a sterile zone of about 40cm in diameter around the Bunsen burner.
- Material can now be installed in the sterile zone. Any manipulation must be carried out within this sterile zone.
- Whilst manipulating, avoid talking or excess movement as this will lead to air movement.
- Wash your hands before and after any manipulation.



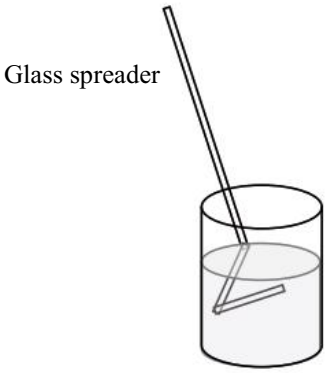

*Warning: Use goggles with Bunsen burners*


## Method

1. Measure out 0.5cm$^3$ of distilled water into a test tube using a syringe. Using a flamed loop, transfer a loopful of culture to the water and mix well.
2. Label the base of the Petri dish A, B, C and D. Place the Petri dish next to the Bunsen flame and lift the lid up a little. Pour the water and bacteria onto the surface of the agar. Using the alcohol, sterilise the glass spreader or use a disposable spreader. Spread out the bacteria as evenly as possible over the agar surface. Place the spreader in the alcohol and flame it to sterilise it and replace the lid of the Petri dish.

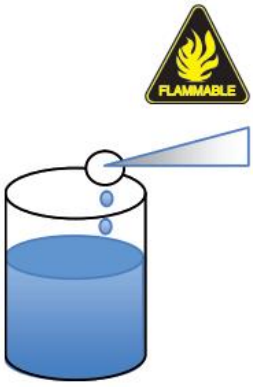


*Sterilising a wire loop in a Bunsen burner flame*




Flaming the spreader

*Warning: Do not put the beaker of alcohol near the Bunsen burner*

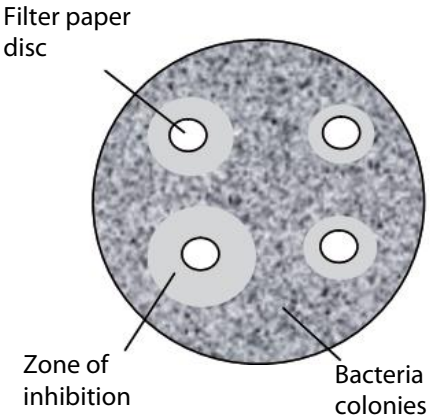



3. Dip the end of the forceps into the alcohol and pass it through the Bunsen flame. Use the forceps to pick out a filter paper disc from the beaker of disinfectant. Drain off the disk on the side of the beaker. Lift the lid of the Petri dish up a little and place the disk on one side of the agar plate over the label A.
4. Repeat the exercise with discs soaked in two other disinfectants and one disc soaked in sterile water only. Place them over labels B, C and D.
5. Label the dish (disinfectants, your name, class, date), tape down the lid, turn over the dish (so the agar is in top half) and incubate at 30°C.
6. Examine and measure any zone of inhibition through the base of the dish.

*Warning: Never open a Petri dish that has colonies growing in it.*


### Inhibition of bacterial growth by disinfectant

Zones of inhibition (i.e. regions where bacterial growth has been prevented) may be seen. Different disinfectants will give different results, as will different strengths of the same disinfectant. It is therefore possible to determine the effect of the same disinfectant on several different species of bacteria.




## To investigate further

- The bacteriocidal action of different soaps
- The effectiveness of cleaning agents at different dilutions
- The bacteriocidal action of spices and herbs
- Comparing hand sanitizers to standard hand soaps.

**Syllabus reference: Sub-Topic B.1 Skill**

The Gram stain is probably one of the most widely used stains for identifying bacteria. It was invented by the Danish microbiologist *Christian Gram* in 1884.

The technique requires four solutions:

- A basic stain (e.g. crystal violet)
- A mordant (e.g. Lugol's iodine solution)
- A decoloriser (e.g. acetone-alcohol)
- A counterstain (e.g. safranin or basic fuchsin)

**Gram positive** bacteria stain with crystal violet.

**Gram negative** bacteria stain red with safranin stain.

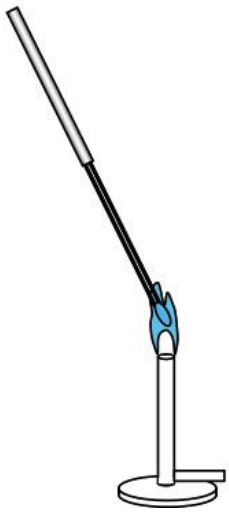
At the time it was not understood how it worked. We now know that the Gram staining properties of bacteria depend upon the nature of their cell walls.

## Materials

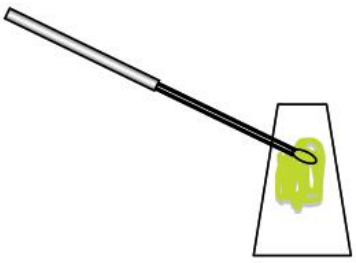
| | | |
|---|---|---|
| microscope (with ×100 objective) | sterile water | Crystal violet stain |
| immersion oil | Bunsen burner | Lugol's iodine |
| glass slides | matches | acetone-alcohol |
| wire loop | forceps | Safranin |
| stop watch | staining rack and dish | natural yoghurt |
| filter paper | rubber gloves | distilled water wash bottle |

## Method

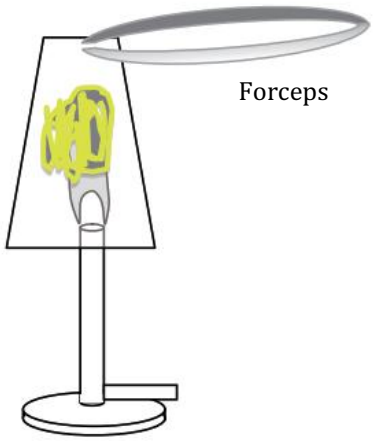
1. Flame the wire loop until red hot then let it cool. Dip the loop in sterile water (tap water will do here) and place two loopfuls on the slide.
2. Dip the loop into the yoghurt and mix with the water on the slide. Spread the mixture over 2cm of the slide. Leave the slide to air dry.
3. Hold the slide in forceps and pass it three times through a blue Bunsen flame. Do not overheat the slide. Switch off the Bunsen when complete. This heat treatment fixes the bacteria to the slide so they do not get washed off during the staining.



Sterilising a wire loop in a Bunsen flame



Spreading a bacterial culture on a slide




Heat fixing a smear in a Bunsen flame

*Warning: Take care when handling hot objects*

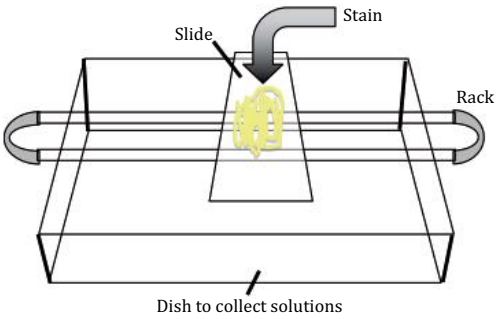

4. Place the slide on a staining rack and flood it with crystal violet stain. Leave for 30s. Lift the slide with the forceps so the stain runs off the slide and wash it in a stream of Lugol's iodine. Replace the slide on the rack and flood with Lugol's for 30s then lift up the slide and wash it off in distilled water.

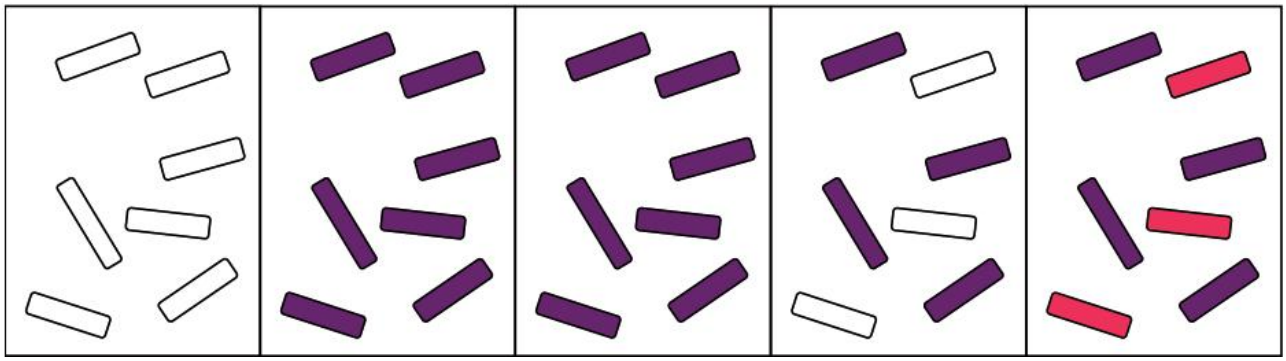
*Warning: Wear gloves when handling biological stains.*


5. Add the acetone-alcohol until no more colour is seen to come off the slide (about 3s) then immediately wash with distilled water again.

*Warning: acetone-alcohol is inflammable do not leave this near the Bunsen.*


6. Flood the slide with basic safranin and leave for 1 min. Wash off the safranin with distilled water and dry the slide gently between sheets of filter paper and let it dry in the air.
7. Add a drop of immersion oil to the centre of the slide and observe using the oil immersion objective lens on a microscope.
8. When your observations are complete, clean off the oil immersion objective using a lens tissue.
9. Dispose of the stains according to local regulations. *Do not pour them down the sink.*



| Unstained bacteria | After crystal violet all are stained violet | After mordant (Lugol) the stain is fixed | After decoloriser (acetone-alcohol) Gram negative are colourless and Gram positive are still violet | After counterstain (safranin) Gram negative become red and Gram positive are violet |
|---|---|---|---|---|

*The sequence for Gram stain*

## Research

What is the relationship between the Gram staining characteristics of a bacterium and its virulence when it has infected a patient?

**Syllabus reference: Sub-Topic C.1 Skill**

A transect is a series of observations, or measurements, taken at regular intervals along a line.

The aim is to use a transect to determine the change in a community of plant species as we progress from one habitat to another. In this exercise an interrupted belt transect will be used to study changes in vegetation with various abiotic factors.

## Materials

- tape or string marked in metre intervals
- $1m^2$ quadrat
- 2 ranging poles marked at 10cm intervals
- spirit level
- 20cm ruler
- data logger
- temperature probe
- light probe
- humidity probe
- Identification guide or species Check list

## Method

1. Lay out the tape or string across the transition zone so that it is straight and taught. Tie it down at both ends.

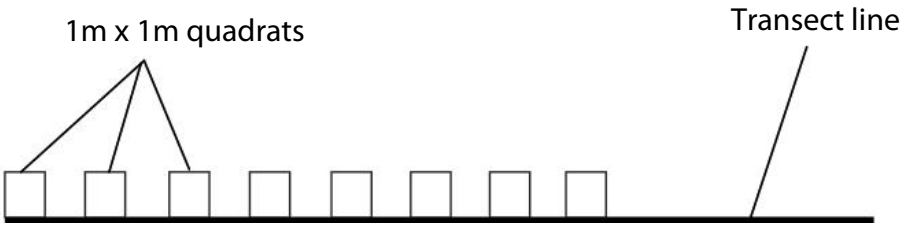
*Environmental impact: Be careful to trample the vegetation beside the line as little as possible. Leave the site as you found it.*


2. Note the orientation of the line (north-south, east-west etc.) and the major features along the transect. Use the prepared data table to record your results. Sketch the transect in your notebook. Take photographs of the site to support your observations.

3. Starting at one end, lay your quadrat by the side of the line and identify the species of plants present. Estimate their abundance using the scale given in the table below:

| Scale | Percentage Cover | Abundance Rating |
|---|---|---|
| Trace | <1% | Single individual |
| 1 | 1 - 5% | Rare |
| 2 | 6 - 25% | Occasional |
| 3 | 26 - 50% | Frequent |
| 4 | 51 - 75% | Abundant |
| 5 | 76 - 100% | Dominant |

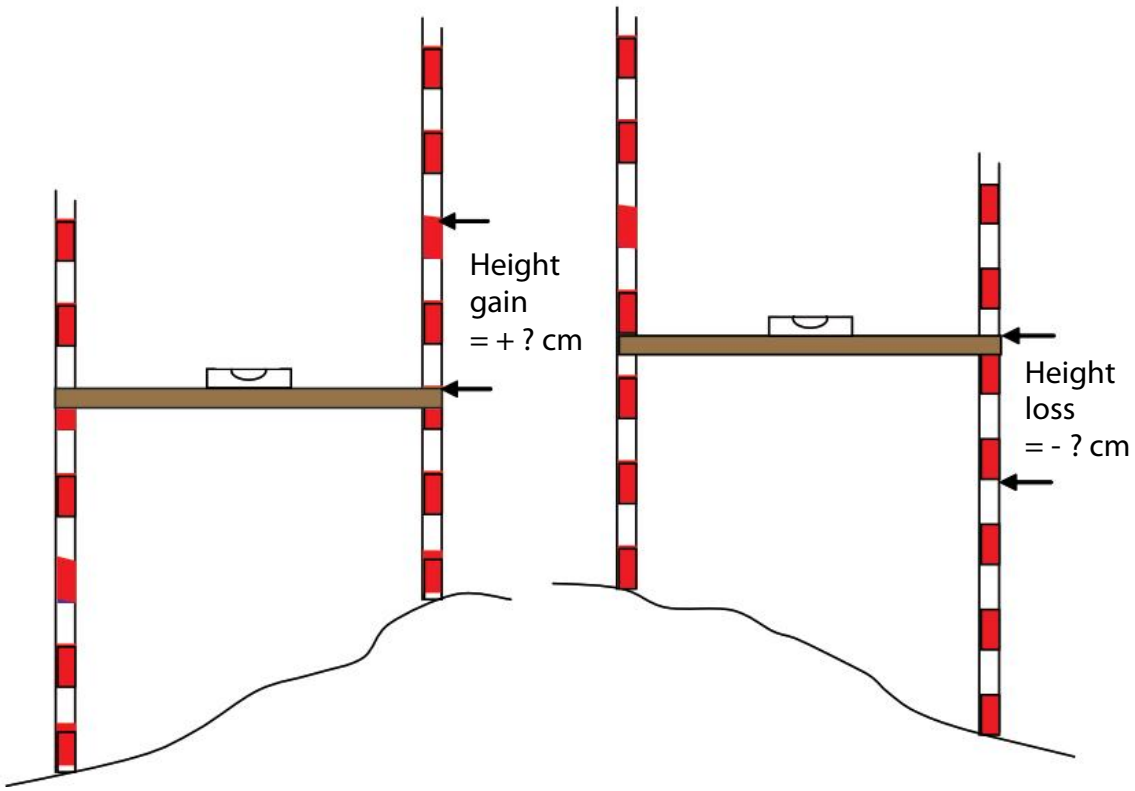
4. Record each species and their abundance in a table. Repeat this every other metre along the transect (*see diagram below*). Add new species to the bottom of the list.




5. Record the abiotic factors measured every other metre along the transect using the data logger and probes. Measure these within a relative short space of time so that variations in the weather do not influence the readings too much.

6. Record the height profile of the transect using the ranging poles and the spirit level. Place the first pole at the beginning of the transect and the second pole at 1 metre. Using the spirit level, measure the difference in height of the two poles. *See the diagram on the next page.*

7. Record an increase as a positive value and a decrease as a negative value. Where there is a sudden change in height, place the poles at shorter intervals. On soft ground be sure NOT to push the poles into the soil, they should be gently resting on the ground.
8. This data can be entered on a spreadsheet. Calculate the cumulative change in height. When this is plotted against distance along the transect it gives the height profile. Profiles of the changes in abiotic factors and the changes in the flora can be compared with this data.
9. Discuss the results and evaluate the method. Are there any correlations apparent between the distribution of the species and the abiotic factors?



# Investigation 14B - Using invertebrate pitfall traps on a line transect



**Syllabus reference: Sub-Topic C.1 Skill**

Traps can be used to sample invertebrate populations. Sampling in this way provides an idea of distribution and abundance, but it does not provide an estimate of absolute density.

## Materials

- 25m string marked at 1m intervals
- 6 traps
- trowel
- soil auger
- white sorting tray
- white plastic teaspoon
- hand lens
- data logger
- temperature probe
- humidity probe
- light probe
- Key to the orders of soil invertebrates

## Method

1. Lay out the string marked at 1m intervals along the area to study. For example covering an area that lies partly in a field, across the field to woodland boundary, then entering into the wood.
2. At every fifth meter place a pitfall trap as follows:
   - Using the soil auger, drill out a core of soil as deep as the trap. Using the trowel, clean out the hole large enough to push in the trap. Then using the soil that you have just removed, make sure that the top of the trap is level with the soil surface.
   - Set up the trap cover.
3. Using data logging every metre over the 25m measure: the soil temperature and at one metre above the ground measure:
   - the air temperature,
   - the light intensity
   - the relative humidity.

4. Leave the traps for an interval of 24 hours. Sketch the transect in your notebook. Take photographs of the site to support your observations.
5. After 24 hours, repeat the abiotic measurements and take the traps in one by one. Identify the invertebrates found using a key as far as Order. Record your data in an organised way. Use a white sampling tray to separate out the similar looking invertebrates into the different parts of the tray before identification.
6. Note anything else that may have ended up in your traps.

*Environmental impact: Release the animals in the areas where they were trapped. Remove all the traps and wooden sticks. Collect them in a rubbish sack to be disposed of in a suitable manner. Take in the string and look around to make sure that you have left nothing behind.*


7. Present the results in a suitable way.
8. Discuss any trends that are seen in the distribution and abundance of the different invertebrate groups found along the transect. Relate them to the abiotic factors that have been recorded.

## Data table

| Order | Common names, examples | Trap 1 | Trap 2 | Trap 3 | Trap 4 | Trap 5 | Trap 6 |
|---|---|---|---|---|---|---|---|
| **Class Arachnida** | | | | | | | |
| Pseudoscorpiones | | | | | | | |
| Opilones | harvestman | | | | | | |
| Acarina | mites | | | | | | |
| Araneae | spiders | | | | | | |
| **Class Crustacea** | | | | | | | |
| Isopoda | woodlice | | | | | | |
| **Class Insecta** | | | | | | | |
| Diplura | two-pronged bristletails | | | | | | |
| Protura | coneheads | | | | | | |
| Collembolla | springtails | | | | | | |
| Thysanura | silverfish | | | | | | |
| Orthoptera | grasshoppers & cockroaches | | | | | | |
| Demaptera | earwigs | | | | | | |
| Psocoptera | barklice | | | | | | |
| Thysanoptera | thrips | | | | | | |
| Hemiptera | true bugs, aphids, leaf hoppers | | | | | | |
| Lepidoptera | butterflies & moths | | | | | | |
| Diptera | true flies including mosquitos | | | | | | |
| Hymenoptera | bees, ants, wasps | | | | | | |
| Coleoptera | beetles | | | | | | |
| **Others** | | | | | | | |
| Class Oligochaeta | earthworms | | | | | | |
| Class Nematoda | roundworms | | | | | | |
| Class Diplopoda | millipedes | | | | | | |
| Class Chilopoda | centipedes | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| **Order** | **Common names, examples** | **Trap 1** | **Trap 2** | **Trap 3** | **Trap 4** | **Trap 5** | **Trap 6** |

# Investigation 14C – Measuring a Diveristy Index and a Biotic Index



**Syllabus reference: Sub-Topic C.2 & C 2.4 Skills**

Many organisms living in water are sensitive to pollution. Pollution creates stress on an environment. When an ecosystem is under stress it can result in a loss of biodiversity. Biodiversity can be measured using the *Simpson's diversity index*.

An assessment of the quality of an environment, using living organisms, is called a *Biotic index*. This is an estimate of the quality of the water by sampling organisms that show a range of sensitivity to organic pollution.

## Materials

- net
- 1m folding metre ruler
- 10m string marked at 1m intervals or tape measure
- white plastic bowl
- sorting tray
- white plastic teaspoon
- dropping pipette
- hand lens
- data logger
- temperature probe
- oxygen probe
- pH probe
- flow rate sensor
- Identification key to fresh water invertebrates

## Method

1. Place the net firmly on the bottom of the riverbed, with the opening facing up stream and measure an area in front of the net of 0.5m$^2$.
2. Remove any large stones in the sampling area causing the minimum disturbance possible. Displace the organisms from the stones by vigorous rubbing. Collect any organisms in a white plastic bowl containing a small amount of water. Leave the stones on the riverbank whilst the kick sample is carried out. Then carefully replace the stones where they were found.

### The 'Kick Sampling' technique

3. The sand and gravel in the sampling area is disturbed for at least a minute by foot. The net is held downstream and moved accordingly to catch any organisms that may be displaced. Drain most of the water out of the net before turning the net inside out into the collecting bowl.
4. Take the organisms out from the white bowl and put them into the sorting tray. Sort the organisms into groups that have similar looking characteristics. Note any particular adaptations that these organisms have to allow them to live in the habitat that you are studying.

- Only organisms of more than 0.5cm are used here.
- To calculate the Biotic Index you will need to know the number of different groups of organisms present in the sample, that is, how many different Families are present.
- If time allows, key out to Species where this is possible. To calculate *Simpson's Index* each species needs to be counted though not necessarily identified. Once they have been counted and identified, return to the sampling area. Release the animals gently into the water.

5. Measure the following abiotic factors of the river: the depth, width, velocity of the current, temperature, oxygen concentration and pH.
6. If the Biotic Index is measured at a series of sites down a river it can be used to locate points that are having an impact on the community. In your notebook sketch a map of the river with important points marked on it. Take photographs of the site to support your observations.

## Field results

| Benthic Organisms: Order* | Family | Genus/Species | Numbers found |
|---|---|---|---|
| Plecoptera | Leuctridae Nemouridae<br>Perlidae | | |
| Ephemeroptera | Baetedae Ecdyonuridae Ephemerellidae<br>Ephemeridae Leptophlebidae | | |
| Trichoptera<br>With cases | Limnephilidae Glossosomatidae Odontoceridae Sericostomatidae | | |
| Without cases | Hydropsychidae Philopotomidae Polycentropedie Rhyacophilidae | | |
| Odonatasub-orders<br>Zygoptera<br>Anisoptera | | | |
| Megaloptera | Sialidae | | |
| *Crustacea | Gammaridae<br>Asellidae | | |
| *Gastropoda | Ancylidae | | |
| Diptera | Simulidae<br>Chironomidae | | |
| Coleoptera | Dytiscidae Hydrophilidae Elminthidae | | |
| *Hirudinea | Glossiphonidae<br>Hirudinae | | |
| *Oligochaeta | Tubificidae | | |
| Other | | | |

N.B. *Class is sometimes stated instead of Order.

*The stonefly Perla is an indicator of very clean water*

**Table of abiotic results**

| Abiotic factor | Measurement |
|---|---|
| Depth / m | |
| Width / m | |
| Current /$ms^{-1}$ | |
| Volume /$m^3s^{-1}$ | |
| Temperature /°C | |
| $O_2$ /mg $dm^{-3}$ | |
| pH | |

**Description of the site(s) studied:**

## Calculating the Simpson's diversity index

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

Where:

- D is diversity
- N is the total number of all the animals sampled
- n is the numbers of each different species

A worked example for two sites on a river:

| Species | Site A | n(n-1) | Site B | n(n-1) |
|---|---|---|---|---|
| *Perla* | 10 | 90 | | 0 |
| *Baetis* | 45 | 1980 | | 0 |
| *Ecdyonurus* | 20 | 380 | | 0 |
| *Rhyacophila* | 6 | 30 | | 0 |
| *Gammarus* | 38 | 1406 | 6 | 30 |
| *Ascellus* | 15 | 210 | 498 | 247506 |
| *Ancylus* | 8 | 56 | | 0 |
| *Limnea* | 7 | 42 | | 0 |
| *Simulium* | | 0 | 256 | 65280 |
| *Oulimnius* | 10 | 90 | | 0 |
| *Hirudinea* | | 0 | 211 | 44310 |
| *Dugesia* | 18 | 306 | | 0 |
| *Hydracarina* | 30 | 870 | | 0 |
| **Totals** | **N = 207** | **Σn(n-1) = 5460** | **N = 971** | **Σn(n-1) = 357126** |
| **N(N-1)** | **42642** | | **941870** | |
| **D** | **7.8** | | **2.6** | |

## Calculating the Biotic index

In the table the invertebrates are ordered by their sensitivity to organic pollution. Those at the top are the most sensitive to pollution and those at the bottom are the most tolerant to pollution. Only those organisms that are known to be sensitive to pollution (as indicators) are present in the table.

### A worked example

Select the organism that you have found that appears highest up the biotic index table.

Suppose that you have found two different species of *Plecoptera*.

At this station suppose 13 different families were found.

On the table below proceed to the row of *Plecoptera* and follow the line 'more that one species present' along to the column 11-15 groups found.

The Biotic index found is 9.

***Biotic index:*** ***10 = clean water*** ***< 5 = polluted water***

| Organism | | Total number of families present | | | | |
|---|---|---|---|---|---|---|
| | | 0-1 | 2-5 | 6-10 | 11-15 | 16+ |
| Plecoptera present | More than one species present | - | 7 | 8 | 9 | 10 |
| | One species only | - | 6 | 7 | 8 | 9 |
| Ephemeroptera present | More than one species present | - | 6 | 7 | 8 | 9 |
| | One species only | - | 5 | 6 | 7 | 8 |
| Trichoptera present | More than one species present | - | 5 | 6 | 7 | 8 |
| | One species only | 4 | 4 | 5 | 6 | 7 |
| Gammaridae present | All above species absent | 3 | 4 | 5 | 6 | 7 |
| Asellidae present | All above species absent | 2 | 3 | 4 | 5 | - |
| Tubificidae Tubificid worm and/or Chiromomidae (red worm larvae)present | All above species absent | 1 | 2 | 3 | 4 | - |
| All above organisms absent | **Eristalis tenax* that does not require oxyen, may be present | 0 | 1 | 2 | - | - |

**Diptera; Syrphidae*

## Some points for consideration

- Biotic indices are based on the sensitivity of benthic organisms to organic pollution. How does organic pollution effect the quality of the water and the organisms living in it?
- Diversity indices are based on the variety of species present and their abundance, relative to one another. How would the diversity of an environment under stress change?
- What are the potential sources of organic pollution in your sampling area? How would you test to see if this source does influence the Biotic index?
- How would the following factors influence the biotic index and diversity index found in the river?
  - geographical region
  - season
  - current
  - distance from the source of pollution
- Comment on each of these points and suggest how the problems incurred by such variations could be overcome.
- What other factors may influence the Biotic index or the Diversity index?
- How could the evolution of pollution in the river be studied?

**Syllabus reference: Sub-Topic D.1 Skill**

Our food provides us with the energy that we need. The purpose of this investigation is to compare the amount of energy that is released when different types of dried food are burned. This gives a rough estimate of their energy content.

## Materials

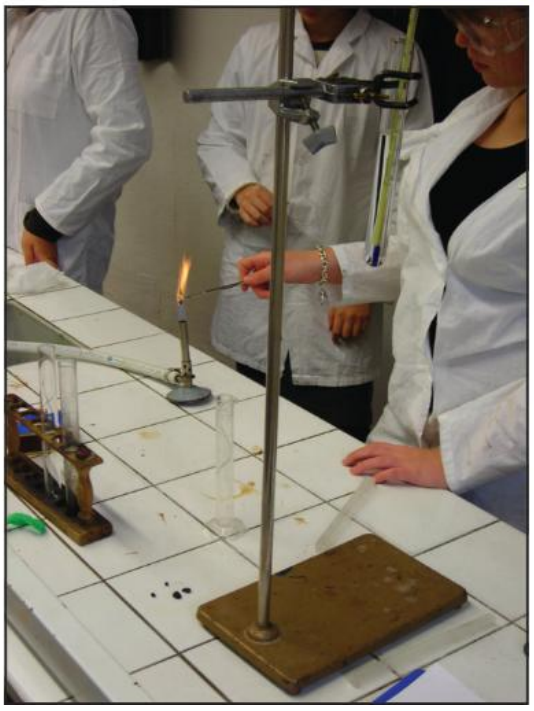
- data logger
- temperature probe
- electronic balance
- Bunsen burner
- matches
- mounted needle
- 6 large test tubes
- test tube holder
- measuring cylinder
- stand and clamp
- dry food samples

## Method

1. Plug a temperature probe to the data logger. Set the recording frequency to one reading per second for five minutes.
2. Measure out exactly 25cm$^3$ water into a large test tube. Clamp the tube upright in a retort stand. Put the temperature probe attached to a data logger in the test tube and leave it there while you go on with the next two steps.
3. Collect and weigh a piece of food. Write down its mass and the name of the food in your results table.


*Warning: As nut allergies are quite common, foods containing nuts and their products should be avoided.*

4. Light a Bunsen burner but keep it well away from the test tube.
5. Start the Data Logger.
6. Stick a mounted needle firmly into the piece of food. Set fire to the food by holding it in the flame of the burner. As soon as it is alight, hold or place the burning food under the test tube. Keep it there until it stops burning.
7. When the food stops burning, stir the water with the temperature probe until it stops recording.
8. From the temperature recorded, determine the initial temperature of the water and the highest temperature it rises to. The sample should not be so big that the water starts to boil.
9. Repeat the procedure at least once for each type of food that you use. Use fresh water and a clean test tube each time.



## Analysing the results

- 1 $cm^3$ of water weighs 1 gram. How many grams of water did you put in the test tube?
- Energy is measured in joules (J) or kilojoules (kJ). One kilojoule = 1000 joules.
- Energy produced (kJ) = $\frac{\text{(mass of water used)} \times \text{(temperature rise)} \times 4.2}{1000}$
- Some pieces of food appear to give more energy than other pieces of the same kind of food. There is a simple reason for this. What is it?
- It would be simple to compare the results if everyone had used exactly the same amount of food. Is this a practical solution?
- What must be done to each result in order to compare your results with the results of others?

Scientists have used very accurate methods to work out exactly how much energy different foods contain. Find out the literature values for your food samples. These may be available on the packets of foods.

You will probably find that they are not absolutely the same as yours.

- Consider the procedure that you used. To get an accurate value for the amount of energy, the food sample must be completely oxidised to carbon dioxide and water and all of the heat produced must go to heat the water.
- Evaluate this method and suggest improvements.

## Research

Find out how a bomb calorimeter works and compare it to your method.

### Kilojoules and calories explained

Energy values can be confusing, several systems of notation are used.

- A kilojoule (kJ) is a unit of measure of energy and it is the accepted scientific unit.
- Previously, energy was measured in calories. It takes 1 cal of energy to raise 1g of water by 1°C.
- Food energy can also be measured in terms of the 'nutritional' or 'large' Calorie. There are 1,000 (small) calories in one (large) Calorie, which is why it is also sometimes known as a 'kilocalorie'. The terms 'calorie' and 'Calorie' are often used interchangeably, which can be very confusing.
- One Calorie (Cal) or kilocalorie (kcal) has the same energy value as 4.184 kilojoules (kJ).
- Therefore: 4.184 kilojoules = 4 184 joules = 1 Calorie = 1 kilocalorie = 1 000 calories

**Syllabus reference: Sub-Topic D.4 Skills**

Heart rate and blood pressure are important physiological indicators of health and fitness.

## Materials

| | | |
|---|---|---|
| stethoscope | paper towel | Heart rate monitor |
| ECG sensor and electrodes Data logger | stop watch | Blood pressure monitor |

## Method

### Heart Rate

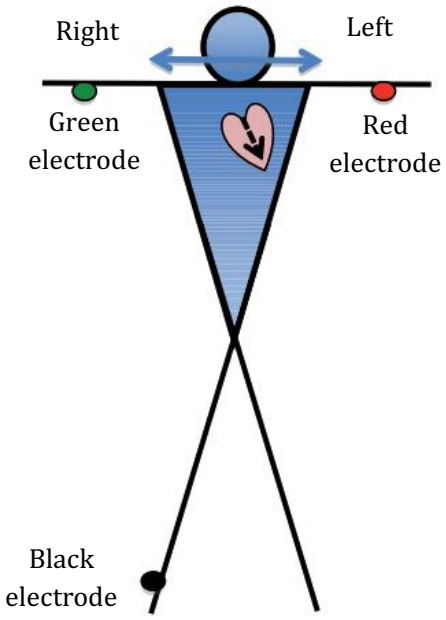
There are several ways the heart rate can be measured:

- by auscultation using a stethoscope
- electronically using an electrocardiogram or heart rate monitor
- indirectly, by palpation of the pulse in an artery

**Do different ways of measuring the heart rate give the same result?**

*Warning: Use a consent form for each one of your subjects*


1. Sit your subject, or yourself, down in a quiet room and allow the subject to settle down for a few minutes.
2. **Auscultation** requires the stethoscope to be positioned, by your subject, a little to the left of centre of the chest. The double beat of the heart can be heard and counted directly for 10s. Multiply by 6 to calculate the heart rate.
3. **Palpation** requires you to use your fingers, not the thumb, to feel for the pulse at a pressure point, either the radial artery in the wrist or the carotid artery in the neck. A light pressure will be needed to feel the pulse when the subject is at rest. Count the beat for 10s. Multiply by 6 to calculate the heart rate.
4. The **electrocardiogram** detects the electrical activity of the heart.
   - Three electrodes must be attached as follows.
     - Attach an electrode to the right wrist and clip the green lead (negative) to it.
     - Attach the red lead (positive) to an electrode stuck to the left wrist.
     - Finally attach the black lead (reference) to an electrode stuck to the inside of the right ankle.




   - Clean the skin where the electrodes are to be attached with soap and water then dry thoroughly using a paper towel.
   - Attach the ECG probe to a data logger and set to record every 0.01s for 10s. Multiply by 6 to calculate the heart rate.
5. **Electronic heart rate monitors** can be gripped by the hand. They need to be cleaned to give a good contact with the skin. Monitors signalling to data loggers will have default values for the sampling frequency and the duration of sampling. The duration for this exercise should be set to 1 min. This will provide enough data for the monitor to calculate the pulse rate.
6. Compare the results from the different methods of calculating the pulse rate at rest.

### Does posture make a difference to the heart rate?

1. Using a convenient method for recording heart rate, record the heart rate or pulse
   - Sitting
   - Lying down
   - Standing
2. The subject must adjust to the posture for a few minutes before measurement.
3. Collect data from the rest of the class. Calculate the average, maximum, minimum and range of heart rates for your population.
4. Collect the heart rate before and after a period of exercise.

## Blood pressure

Blood pressure is the force exerted by the blood on the blood vessels. This varies depending on the stage in the cardiac cycle. When the heart is fully contracted (systole), the pressure is the highest. When the heart is fully relaxed (diastole), the pressure is the lowest. The pulse pressure is the difference between these two values.

Blood pressure is measured by using a pressure cuff and listening for the sounds of the pulse at the pressure point in the elbow joint. Measuring the blood pressure using a pressure cuff requires paramedical training, however, it is possible to measure the blood pressure safely using an electronic sensor. Electronic blood pressure monitors work by sensing the pulse after compression of the arteries in the arm using a programmed pressure cuff.

### Measuring the blood pressure at rest

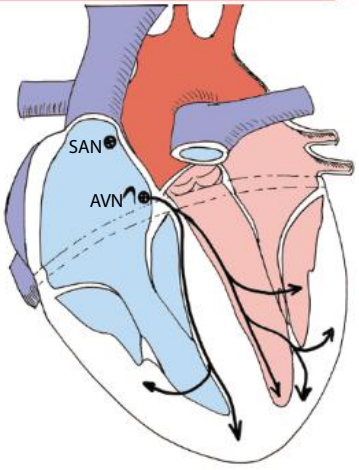
The blood pressure is measured sitting down in a quiet environment.

1. Attach the pressure cuff and allow the subject, or yourself, 2-3 minutes to rest. To neutralise the effect of gravity on the blood pressure, the pressure cuff must be at the same level as the heart. Attach it to the upper arm or if it is a wrist model attach it to the wrist and hold the wrist over the position of the heart in the chest. Make sure the pulse sensor is over the pressure point. The subject should not talk or move during the measurement.
2. Start the pulse reading. The pressure cuff will inflate until the sensor no longer detects the pulse. Then is when you will slowly relax sensing for the pulse. Usually this is indicated by a beeping sound. When the cuff is deflated the reading can be recorded.

### Exploring blood pressure under different conditions

- Repeat the measurement standing up and lying down.
- Try the left arm and the right arm.
- Try taking the blood pressure before and after some light exercise.
- If the subjects permit it, try subjects of different:
  - size
  - gender
  - ages

**Syllabus reference: Sub-Topic D.4 Skill**

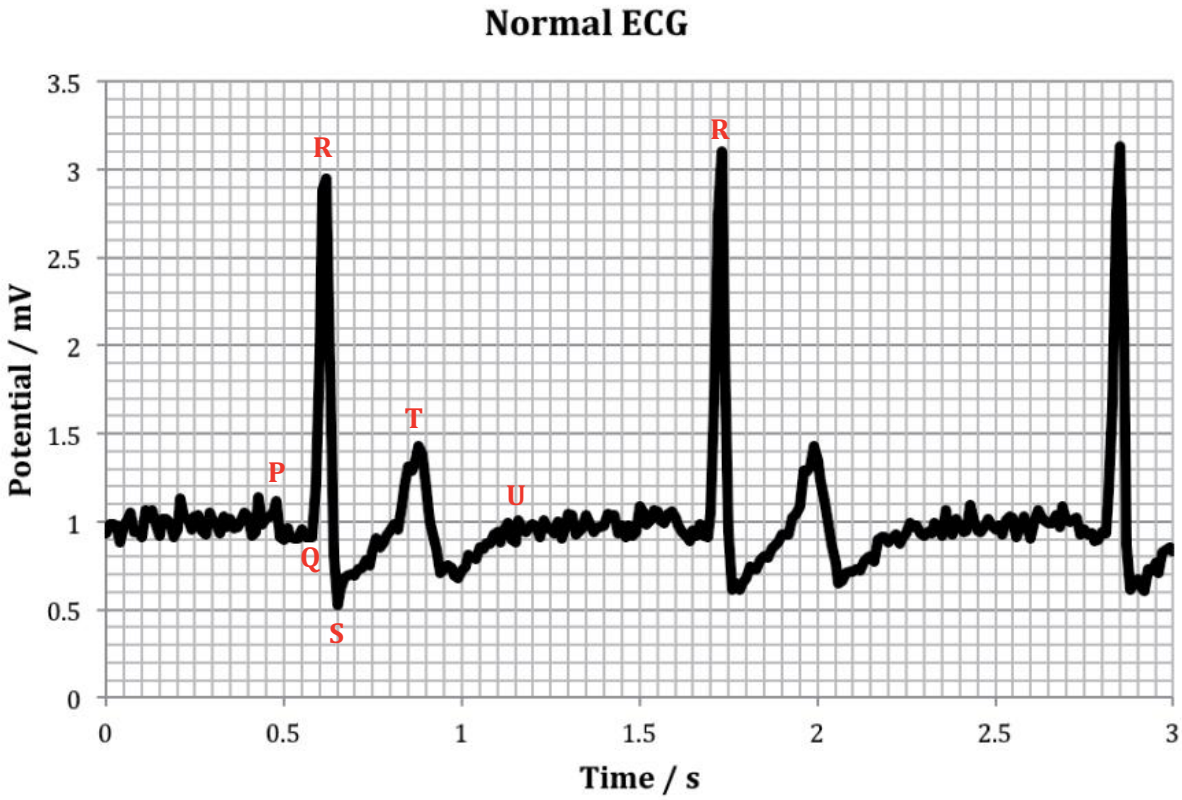



An electrocardiogram (ECG or EKG) is a recording of the electrical activity of the heart. The heart is mainly made of muscle tissue, composed of muscle cells. Muscle cells are excitable cells, that is they can become depolarised causing them to contract.

The heart has a built in rhythm. The heart starts to contract at the pacemaker or sinu-atrial node (SAN). This is found on the wall of the atrium near where the superior vena cava enters. The contraction crosses the atria to a point between the atria and the ventricle called the atrio-ventricular node (AVN). Once across the AVN, the contraction spreads rapidly across the ventricles.

The spread of the contraction across the heart can be seen in the electrocardiogram (ECG or EKG)

## Observations from the ECG




- The cardiac rhythm from R to R
- The conduction of the wave of contraction from the atria to the ventricles P to R. This should be < 0.2s at rest.
- The QRS complex represents the contraction of the ventricles, which should be about 0.1s.
- The T wave represents repolarisation of the ventricles. Q-T represents the whole of the ventricular activity. It should be less then half of R-R at rest.

*Note: The wave of atria repolarisation is hidden by the huge wave of ventricle depolarisation, so it cannot be seen on the ECG.*

## Materials

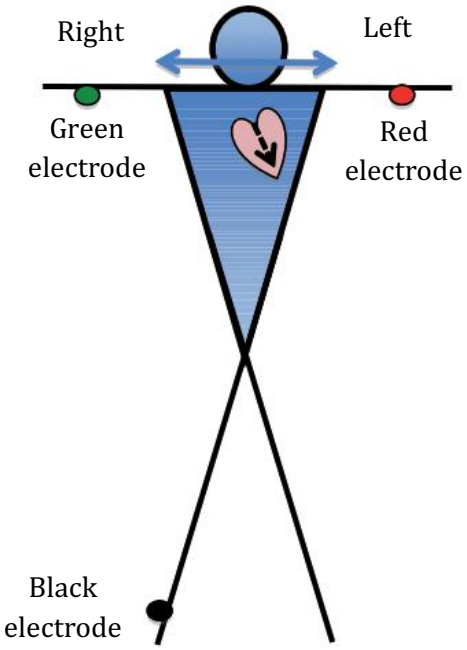
| | | |
|---|---|---|
| Data logger | adhesive electrodes | paper towel |
| ECG sensor | | soap |

## Method

### Taking an ECG


*Warning: Make sure your subject reads and signs a consent form*

1. You will need to attach three electrodes to the body to pick up the ECG. These can be attached to the limbs.
2. Clean the skin with soap and water, where the electrodes are to be attached, then dry thoroughly using a paper towel. Attach an electrode to the right wrist and clip the green lead (negative) to it. Attach the red lead (positive) to an electrode stuck to the left wrist. Finally, attach the black lead (reference) to an electrode stuck to the inside of the right ankle.
3. Attach the ECG probe to a data logger and set to record every 0.01s for 3s.
4. Try recording the ECG in different postures: sitting, standing, lying down.
5. Try a little exercise (e.g. knee bends) and record the ECG before and after.



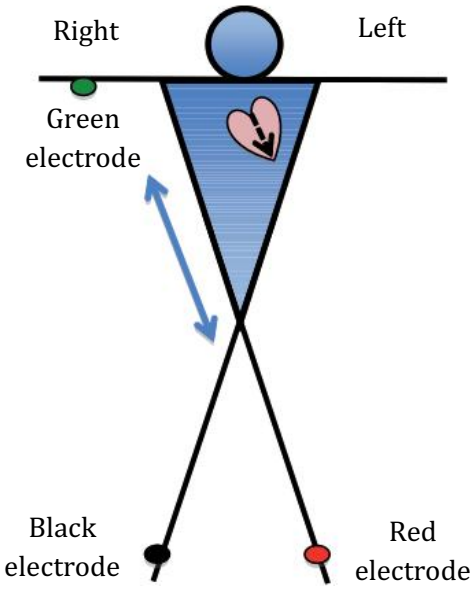

### Analysing the axis of the heart

The orientation of the heart can be determined using the ECG. The heart can rotate on three axes:

- Left-right
- Forwards and backwards
- Around its vertical axis

The ECG can detect the orientation of the heart. This can be determined by attaching the electrodes in different positions.

1. The diagram of the subject shown on the previous page is the basic position that gives the classic ECG. A line crossing between the red and green will follow the shoulders, as shown on the diagram on the previous page.
2. Try two other positions (as shown) and see what happens to the ECG.

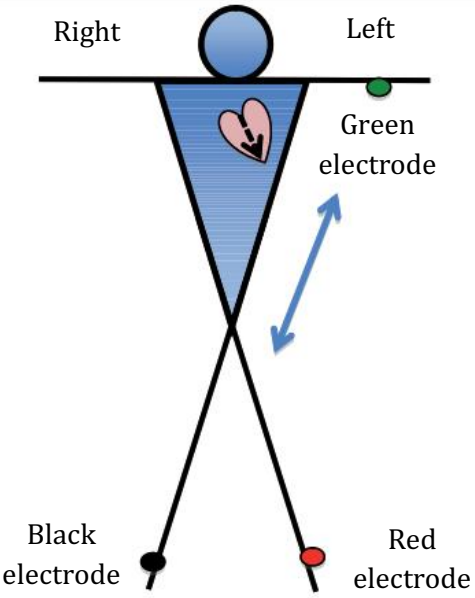


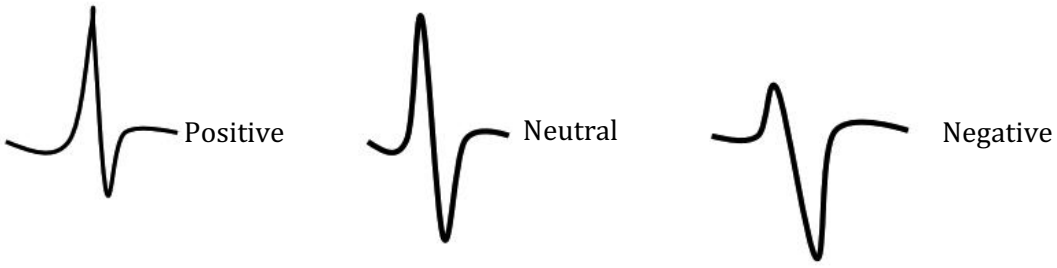

Green on the right wrist, red behind the left ankle and black behind the right ankle.

Green on the left wrist, red behind the left ankle and black behind the right ankle.

The lines crossing between the red and green electrodes are not in the same direction. These three positions form a triangle across the body called 'Einthoven's' triangle.

Which one gives the highest QRS wave amplitude? To work this out you need to consider both the rise at R and the drop at S.




The overall height of the QRS wave gives an indication of the angle of the axis of the heart.

- If the highest QRS amplitude is at position 1 the angle is about 0° to the norm
- If the highest QRS amplitude is at position 2 the angle is about 60°
- If the highest QRS amplitude is at position 3 the angle is about 120°

Note: Even though the norm is taken to be a heart apex pointing towards the left hip, the orientation of the axis of the heart varies with the individual. For example the age of your subject may make a difference to the results.

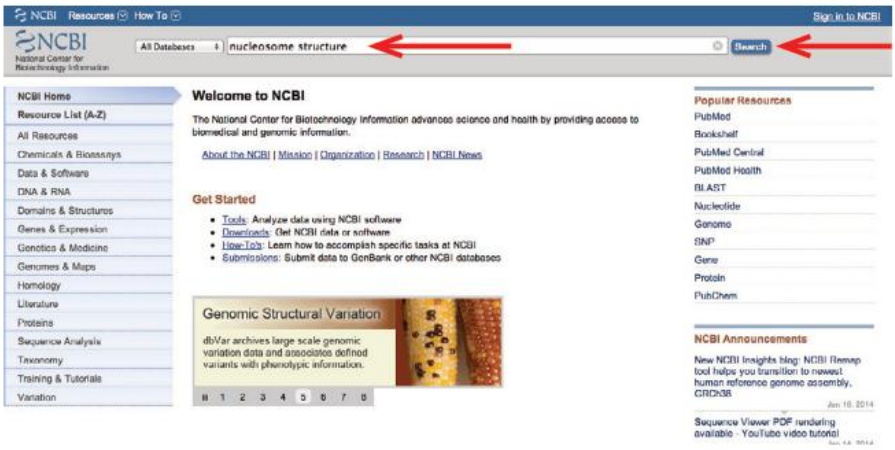
## Syllabus reference: Sub-Topic 7.1 Skill

The nucleosome is a complex of DNA and proteins that make up chromatin. It is a nucleoprotein and the proteins associated with the nucleosome are called histones.

Go to the National Center for Biotechnological Information (NCBI) site:

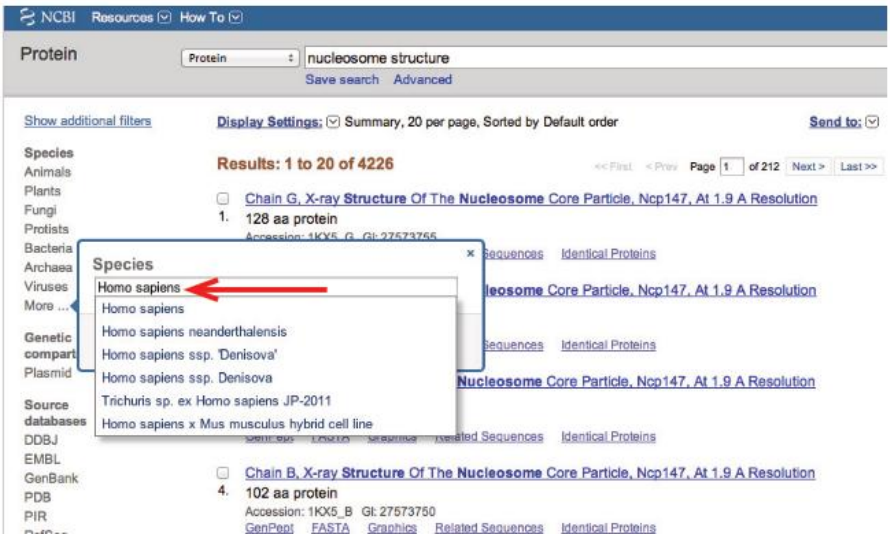
<http://www.ncbi.nlm.nih.gov/>

Use the Search tool by entering '**nucleosome structure**'.

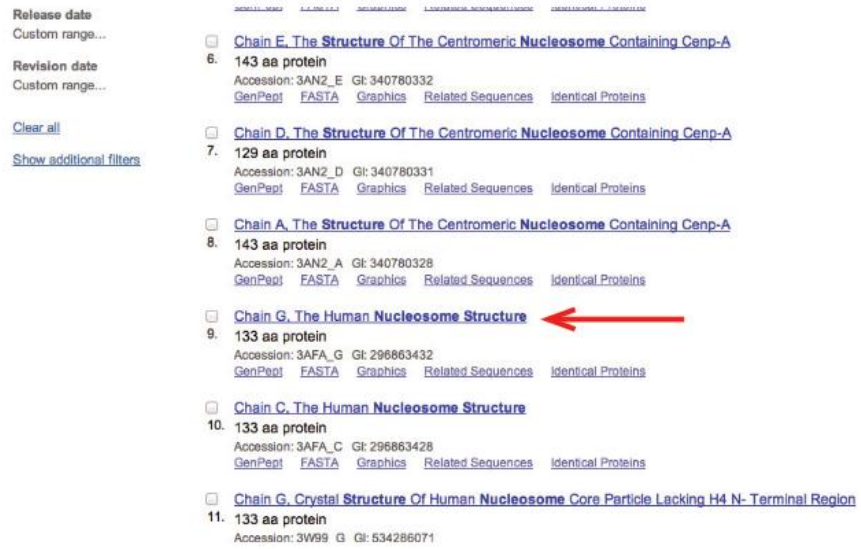



Scroll down and click on '**protein sequences**'.

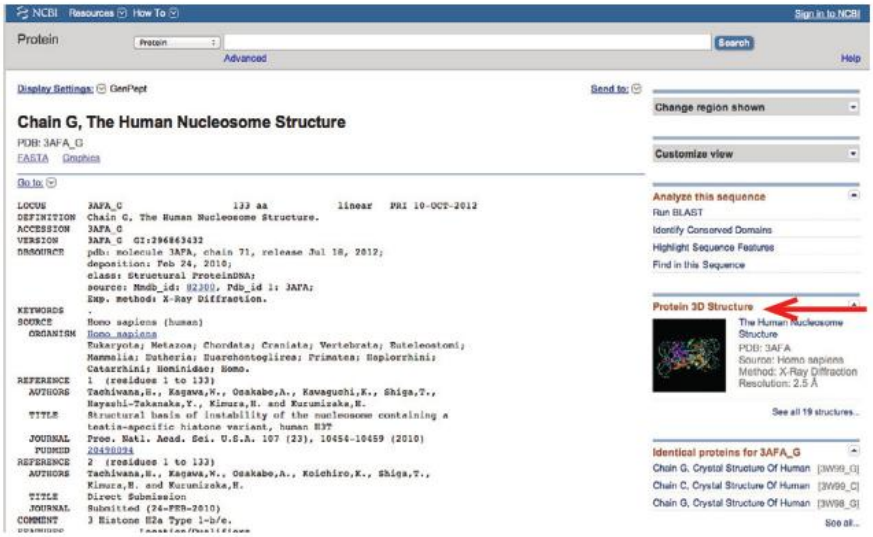

Then select '**Homo sapiens**' in the '**Species**' menu.



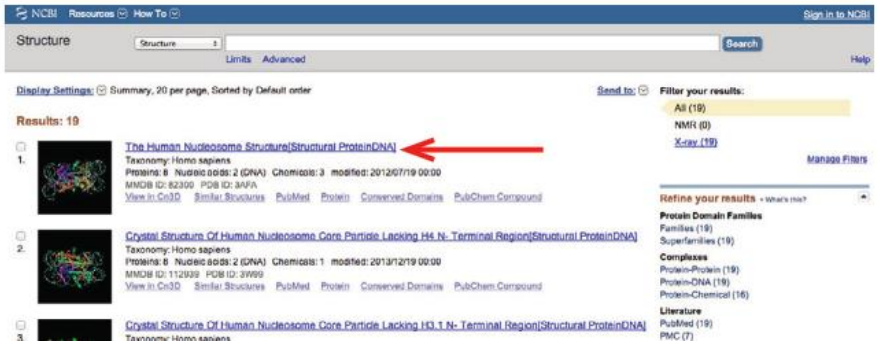
Scroll down to find a '**Human Nucleosome Core Particle'** entry and click on the link.




Click on '**Protein 3D structure'**.




Select the first in the list. It will display the '**Structure Summary'** of the nucleosome core particle.



## Nucleosome structure summary

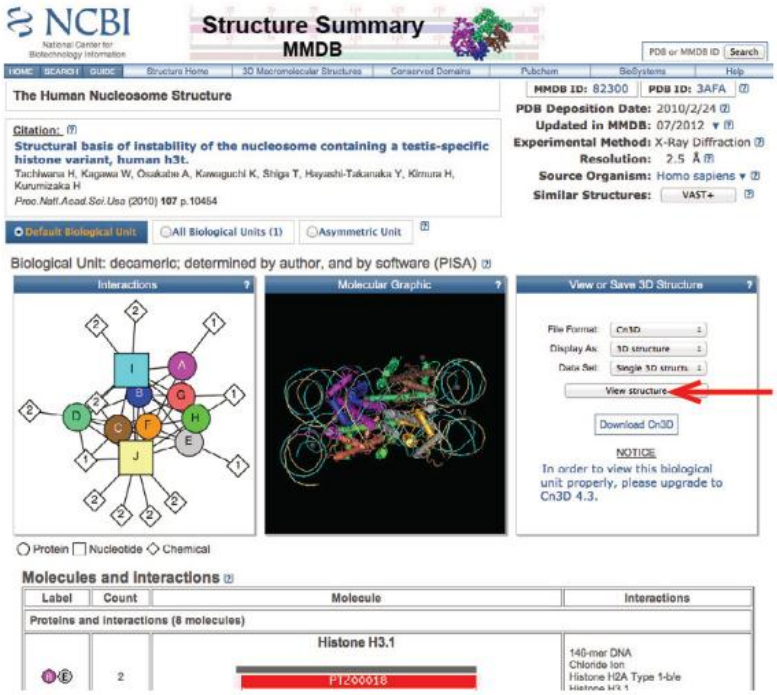
*How many protein sub units are in the core?*

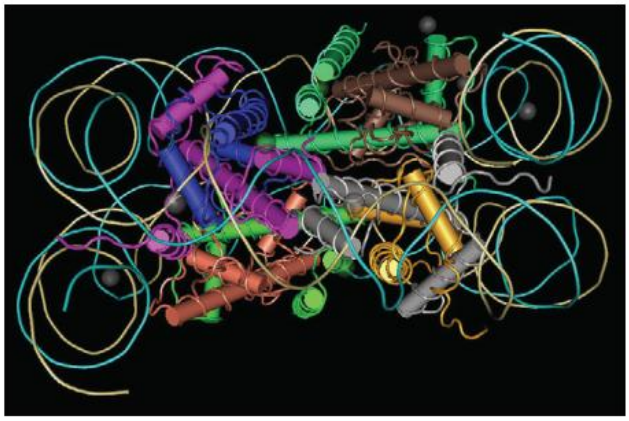
View the core particle in 3D using Cn3D viewer. (You will need to install this on the computer).

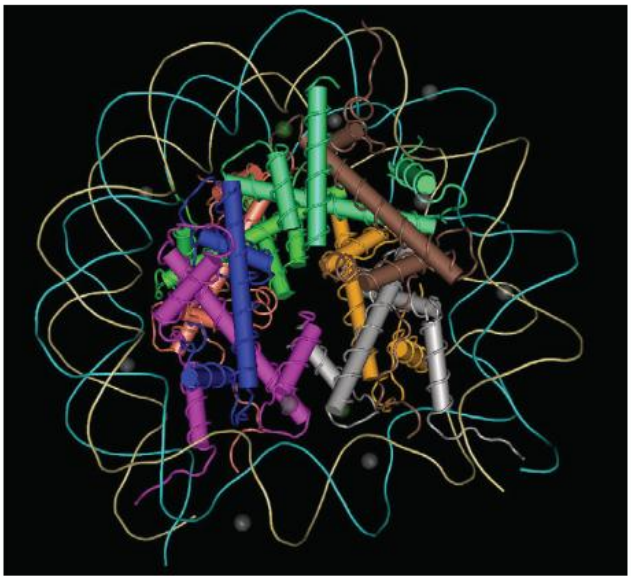
Using this viewer you can turn the molecule round to see it from various angles.

*Describe the structure of the nucleosome of humans.*

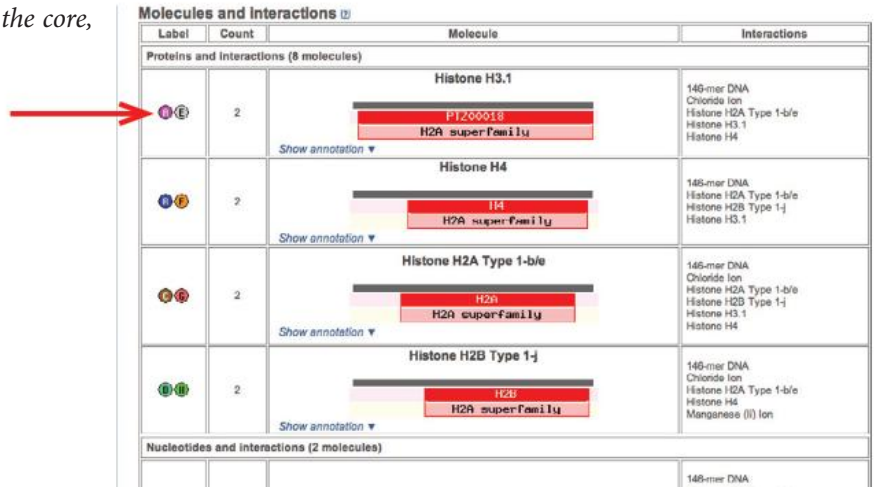
What is the relationship between the DNA molecule and the histone proteins of the core particle?





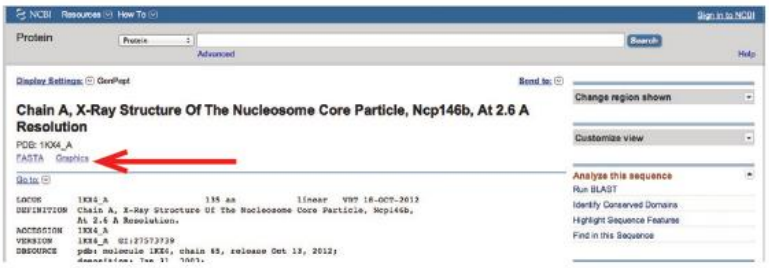
*Select one of the histone proteins of the core,* **e.g. H3.1**



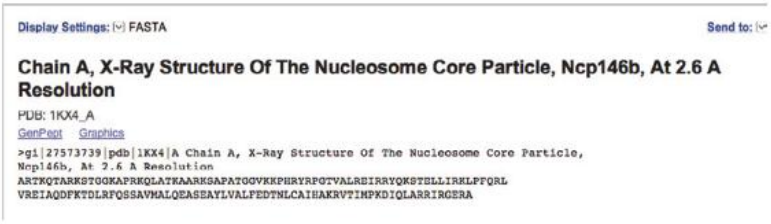
## Using FASTA format (pronounced fast-a)

*Determine the amino acid residues of using FASTA format*

These sequences will be displayed as single letters for each amino acid. It permits a comparison between proteins in programs such as BLAST (below)




*What is the total number of amino acids in human H3.1?*

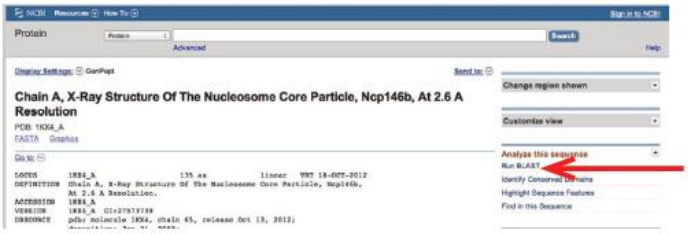



DNA is an acidic molecule because the phosphate groups are on the outside of the molecule. Acids and bases combine to make neutral compounds.

*Identify the basic amino acids: Histidine (H), Arginine (R) and Lysine (K)*

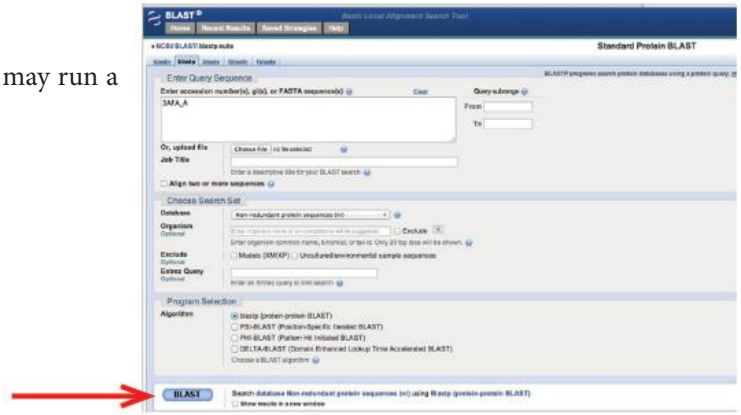
*What is the proportion of basic amino acids in H3.1?*

Try analyzing the other histones in the core particle of the nucleosome.




## Basic Local Alignment Tool (BLAST)

To compare the histones of different species you may run a BLAST analysis.




The BLAST program compares the FASTA sequences of other proteins and rates them by similarity.

*Would you say histones are variable or conservative proteins?*

**Syllabus reference: Sub-Topic 8.1 Skill**

Urease is an enzyme that can be extracted from soya bean seeds. It was the first enzyme to be purified and crystallised in 1926 by J. B. Sumner who got the Nobel Prize for Chemistry in 1946. It catalyses the hydrolysis of urea (also called carbamide):

$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$$

The ammonia produced makes the solution more basic so this reaction can be followed using a pH probe.

***Here we will investigate the effect of two other chemicals on the activity of urease:***

thiourea, $CS(NH_2)_2$ (which is also called thiocarbamide) and lead nitrate, $Pb(NO_3)_2$

# A Calibrating the pH probe

## Materials

| | | |
|---|---|---|
| Data logger | 3 small beakers | Distilled water wash bottle |
| pH probe | Buffers pH 4 and pH 7 | |

## Method

1. Plug in the probe to the first channel of the data logger. The data logger should automatically recognise the pH probe and it will need calibrating.
2. Prepare a beaker with pH 4 buffer and another with pH 7 buffer. Carefully remove the container of electrolyte from the end of the probe and store it in a safe place. Wash the probe with the wash bottle.
3. Follow the steps to calibrate the pH probe with both the pH 4 and the pH 7 buffers, washing the probe with distilled water between the different buffers and at the end of the calibration.
4. Replace the probe in the electrolyte until you need it for measurements.

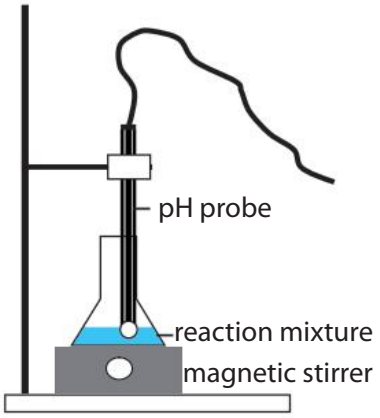
# B Using the pH probe to observe an enzyme controlled reaction

## Materials

| | | |
|---|---|---|
| 2 conical flasks 50cm$^3$ | Stop watch | **Solutions:** |
| Magnetic stirrer, flea, stand and clamp | 15cm$^3$ buffer pH 4 | 30cm$^3$ 1% urease |
| 6 small beakers to collect solutions | 30cm$^3$ distilled water | 80cm$^3$ 1 mol dm$^{-3}$ urea |
| 2 syringes 10cm$^3$ | Universal pH indicator solution + colour chart | 20cm$^3$ 1 mol dm$^{-3}$ thiourea |
| Marker pen | | 20cm$^3$ 1 mol dm$^{-3}$ lead nitrate |

## Method

1. Prepare a flask with a magnetic stirrer. Add 2cm$^3$ pH 4 buffer, 4cm$^3$ of urease and 10cm$^3$ distilled water. Put 2 to 3 drops of universal pH indicator in the flask to provide a visual check.
2. Remove the probe from the electrolyte and clamp it in a stand so that it dips into the liquid in the flask.
3. Select a time-based mode of data collection. The experimental length should be about 8 minutes for a trial run at 60 measurements per minute.
4. Start the stirrer, collect 10cm$^3$ urea solution in a syringe and start the recording the data. Record the pH for a few seconds, then add the urea.

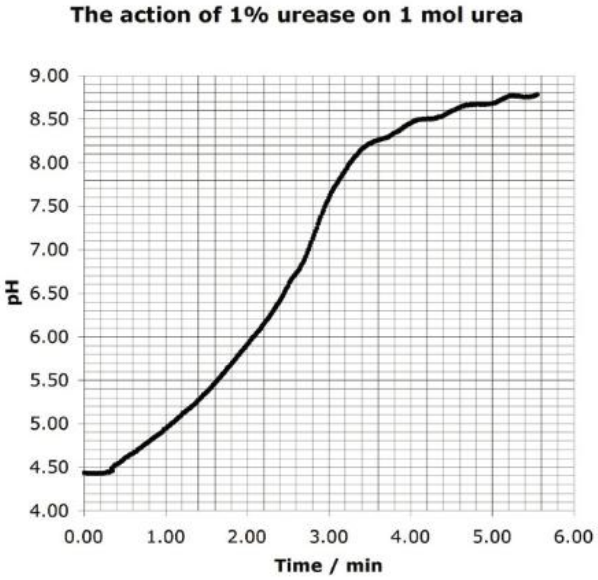


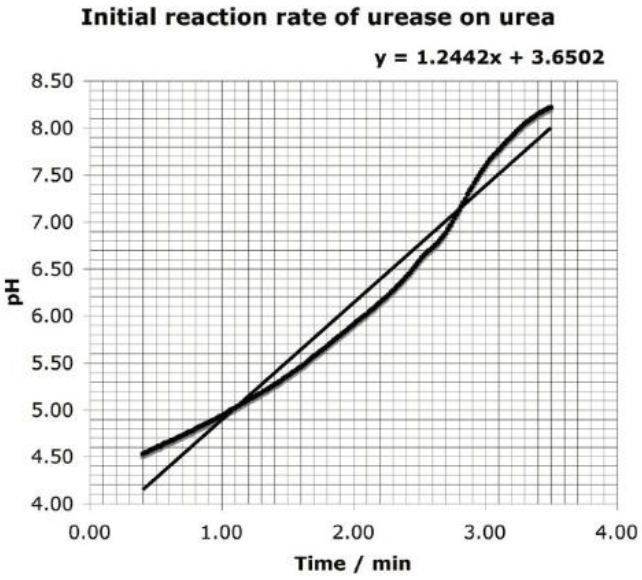
5. When the run is complete remove the probe and wash it off. Clean out the flask, recuperate and wash off the magnetic flea. Add 2cm$^3$ pH 4 buffer, 4cm$^3$ of urease, 5cm$^3$ distilled water and 5cm$^3$ thiourea. Put 2 to 3 drops of pH indicator. Start the stirrer, collect 10cm$^3$ urea solution in a syringe and start the recording. Store your latest data run. The second run will start. Let it run for a few seconds and add the urea.
6. Repeat the recordings using:
   - 10cm$^3$ thiourea
   - 5cm$^3$ lead nitrate + 5cm$^3$ distilled water
   - 10cm$^3$ lead nitrate

| Summary | Volumes / cm$^3$ | | | | | |
|---|---|---|---|---|---|---|
| Run | pH 4 buffer | Urease | Distilled water | Thiourea | Lead nitrate | Urea |
| 1 | 2 | 4 | 10 | 0 | 0 | 10 |
| 2 | 2 | 4 | 5 | 5 | 0 | 10 |
| 3 | 2 | 4 | 0 | 10 | 0 | 10 |
| 4 | 2 | 4 | 5 | 0 | 5 | 10 |
| 5 | 2 | 4 | 0 | 0 | 10 | 10 |

**Warning**: *Lead nitrate is toxic, dispose of it in the container provided, not in the sink.*


7. Analyse the data by using a spread sheet program to draw a linear fit on the initial reaction rate. An example is given below.




8. Compare the molecular structures of urea, thiourea and lead nitrate.
9. Discuss and evaluate this investigation. How might it be extended?

**Syllabus reference: Sub-Topic 9.1 Skills**

Transpiration is the loss of water through the stomatal pores of a plant. The potometer is simple tool that can be used to measure the volume of water entering a leafy shoot from a plant.

## Materials

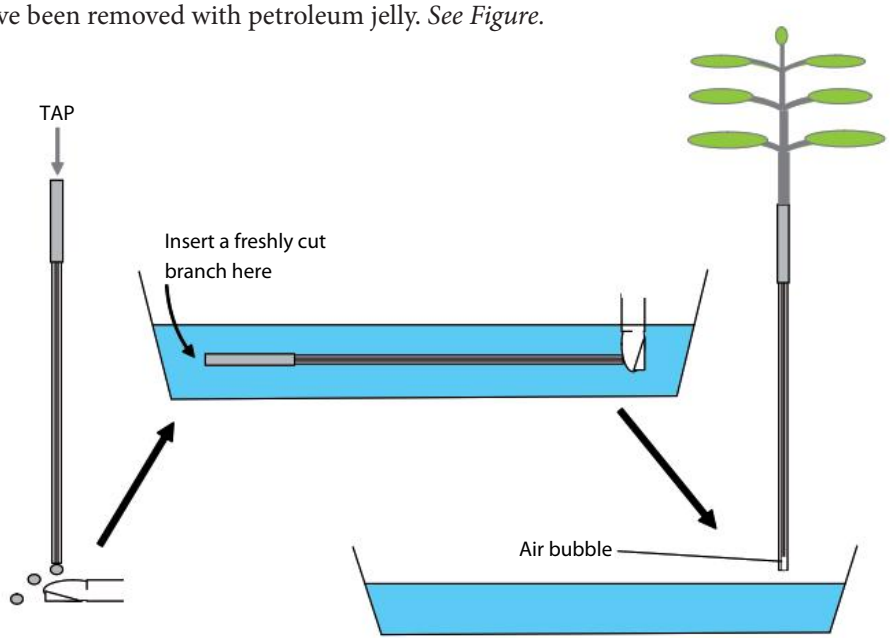
| | | |
|---|---|---|
| 30cm capillary tubes (1mm bore) | Secateurs | Ruler |
| 5cm plastic tubing | Clamp and stand | Stop watch |
| Plant trough (50cm) with drainage holes filled | Petrolium jelly (Vaseline ®) | Leafy branches |
| | Marker pen | |

## Method

1. Mark three 10cm intervals on the capillary tubing starting from the glass end.
2. Fill the potometer by running water into the plastic-tubing end. Once the water runs out from the glass tubing and you are sure that there are no air bubbles in the tubing, block the glass tubing end with your finger and remove the potometer from the stream of water. Submerge the potometer lengthways in the water basin.
3. Cut the end of the branch again and insert the end into the plastic tubing under water

*Warning: Sharp instrument, handle with care.*


4. Turn the tubing into an upright position keeping the glass end under water. Check that there are no air bubbles in the potometer. If the water level drops beneath the branch, try to push the branch in further. If this fails, set up the apparatus again using a thicker branch.
5. Lift the glass tube out of the water to allow an air bubble to enter it and then quickly plunge it back into the water basin. Mark the level of the bubble on the tube and note the time. Use a clamp and stand to support the apparatus.
6. Time how long it takes for the bubble to rise 10cm in the tubing. Repeat this for the next two 10cm intervals.
7. Repeat procedures 1 to 5 with the second leafy branch but remove the leaves. Cover the wounds where the leaves have been removed with petroleum jelly. *See Figure.*




8. Calculate the rate of transpiration through the branch in $cm^3\ h^{-1}$.
9. Record all the results in a table and discuss them fully.

## Some points for consideration

- How would the absence of leaves influence the rate of transpiration?
- What must be assumed when calculating the rate of transpiration through the branch in $cm^3\ h^{-1}$, using the potometer?
- What other measurement could be made in order to make the calculation more valuable? Explain how you would carry out this measurement.

# Assignment

Design an experiment to determine the effect of temperature or humidity on the rate of transpiration of a leafy shoot.

## Design

- Establishing the theoretical background. This will require some research on your species of plant.
- State a focussed research question.
- Write a hypothesis (a justified prediction).
- Clearly identify the variables (the independent or manipulated variable, dependent or measured variable and the important control variables).
- State the significance of each of the control variables and which variables cannot be controlled but need to be monitored.

## Method

- What equipment and materials will be needed? This may be best described using a labelled diagram of the experimental setup.
- Consider any safety factors
- Explain exactly how the independent variable will be changed (range and intervals) and how the dependent variable will be measured and how frequently.
- Explain how you intend to control all the other variables (how will you make your experiment a fair test) or monitor them if they cannot be controlled. Perhaps a control experiment will be needed.

N.B: Controlled variables and a control experiment are not the same thing. Controlled variables are those variables that need to be held as stable as possible. A control experiment is performed when you suspect that an uncontrolled variable may have an affect on the experiment.

**Syllabus reference: Sub-Topic 9.1 Aim**

Stomata are pores found on the surface of the leaves. They are surrounded by two guard cells that control the size of the pore. In this way the flow of water is regulated up the plant in the xylem tissue.

# PART A Observation of the potted plant

## Materials

- A potted plant with some of the leaves enclosed in plastic bags and left for 24 hours
- Petri dish
- Forceps
- Dry cobalt chloride paper
- Self-adhesive paper

## Method

1. Look at the plastic bags covering the leaves. Make a hypothesis to explain what you see. Using the forceps and self-adhesive paper, stick one square of cobalt chloride paper onto the upper side of the leaf. Ensure that the paper is not in contact with the air. Repeat this on the lower side of the leaf.

***Warning**: Cobalt chloride is an irritant and toxic, handle the cobalt chloride paper using goggles, gloves and forceps*


2. Observe the cobalt chloride paper from time to time over the next 30 minutes.
3. In damp conditions the cobalt chloride paper turns pink. Make a hypothesis to explain what you see.

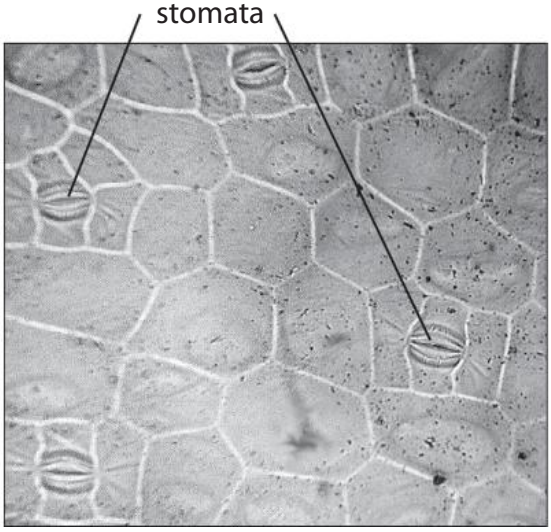
# PART B The site of transpiration: Observation of stomata

## Materials

- Microscope
- Micrometer slide
- Slides
- Coverslips
- Fine forceps
- Distilled water bottle
- Nail varnish
- $2cm^2$ of graph paper
- Marker pen
- Leaves

## Method

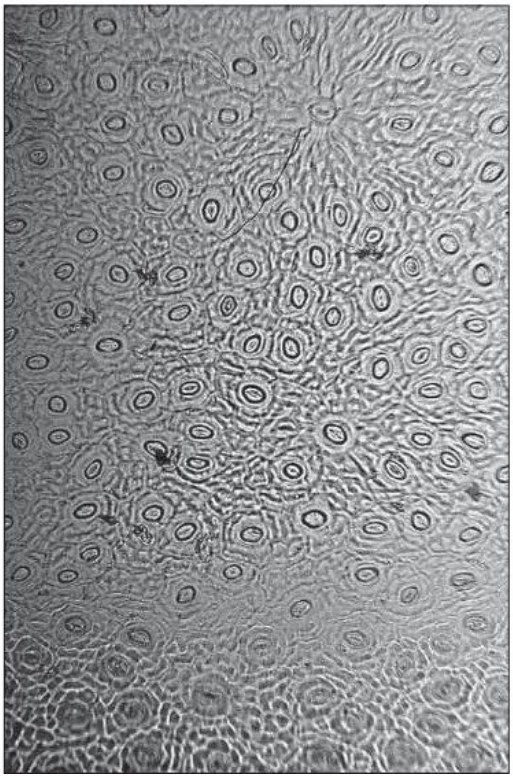
1. Paint a thin coat of nail varnish over approximately $1cm^2$ surface of the upper and lower leaf surfaces.
2. Allow the nail varnish to dry thoroughly.
3. Using the forceps peel away the nail varnish and mount on a microscope slide in a drop of water. Make sure that the face that was sticking to the leaf is facing upwards on the microscope slide. Do not forget to note the type of leaf and the surface from which you took the nail varnish. Compare the nail varnish imprints of the two surfaces.
4. Under high power, count the number of stomata in the microscope field view. Move the nail varnish and count again. Repeat this at least five times. Now take your next nail varnish imprint. Record you results in a table. Use a micrometer slide to measure the diameter of the field of view at high power.
5. Record the results in a table and analyse them.
6. Discuss your results and criticise this investigation.




*Impression of the stomata of the plane tree (Platanus hispanica) viewed at ×100*

## To investigate further

- Compare the stomatal densities on the upper and the lower surfaces of a leaf.
- Compare the stomatal densities on the surfaces of the leaves from different species of plant.
- Comparing monocotyledon with dicotyledon leaves.
- Looking at stomatal densities and transpiration rates.
- Comparing mesophytes, xerophytes and hydrophytes.



Holly (*Ilex aquifolium*) and its stomata (viewed at ×100)

# Investigation 9C - Cell types and tissues in the petiole of celery (*Apium graveolens*)



**Syllabus reference: Sub-Topics 9.1 & 9.2 Skills**

The structure of a plant cell largely depends upon the growth of its cell wall. (a) The cell wall may remain thin and flexible as in parenchyma cells, (b) it may have secondary thickening at the corners as in collenchyma cells or (c) secondary thickening may develop all around the cell wall as in sclerenchyma cells. In addition the secondary cell wall may be further strengthened by the addition of lignin to the cellulose fibres. Secondary thickening leads to extra mechanical support but it also isolates the plant cell protoplast from its neighbours. Cells with extensive secondary thickening tend to die.

## Materials

Binocular microscope
Compound microscope
4 slides and coverslips
Forceps
Razor blade
Needle
Dropping pipette
Stop watch

3 Petri dishes separated into lids and bases containing:
(a) 10cm$^3$ bleach
(b) 10cm$^3$ distilled water
(c) 10cm$^3$ dilute ethanoic acid
(d) 10cm$^3$ carmine stain
(e) 10cm$^3$ iodine green stain
(f) 10cm$^3$ distilled water

10cm$^3$ measuring cylinder

A small petiole from a celery plant (0.5 to 1.0cm in diameter)

## Method

### Cutting the sections

1. Using a sharp new razor blade, cut several transverse sections from the celery petiole. Cut them as thinly as possible and keep the razor blade perpendicular to the petiole. Complete sections of the petiole are not essential.

*Warning Sharp instrument, handle with care.*


2. With forceps, transfer the sections to a Petri dish containing bleach.
3. Repeat and carry on until you have at least 5 sections to ensure that at least one of the five will be a good section.

### Staining the sections

1. Leave the sections in the bleach for 5 minutes or until the tissue decolourises.
2. Using a needle, transfer the sections to a second Petri dish containing distilled water. Stir the sections gently in the water to rinse them. Leave them in the distilled water for 1 minute.
3. Transfer the sections in the same way to the third Petri dish containing dilute ethanoic acid for 2 minutes.
4. Transfer the sections to a fourth Petri dish containing distilled water for 1 minute.
5. Transfer the sections to a fifth Petri dish containing carmine stain for 10 minutes.
6. Using the forceps, pass the sections through the iodine green whilst counting to 3 seconds.
7. Finally, transfer the sections to the distilled water to wash off excess stain. Leave the sections for 5 seconds before mounting them on slides for observation.

The bleach destroys the inside cell structure (protoplast) leaving only the cell walls. The ethanoic acid removes any excess bleach and helps to fix the stains. The iodine green colours the lignin green (as found in xylem and schlerenchyma). The carmine colours cellulose pink (as found in parenchyma, phloem and collenchyma).

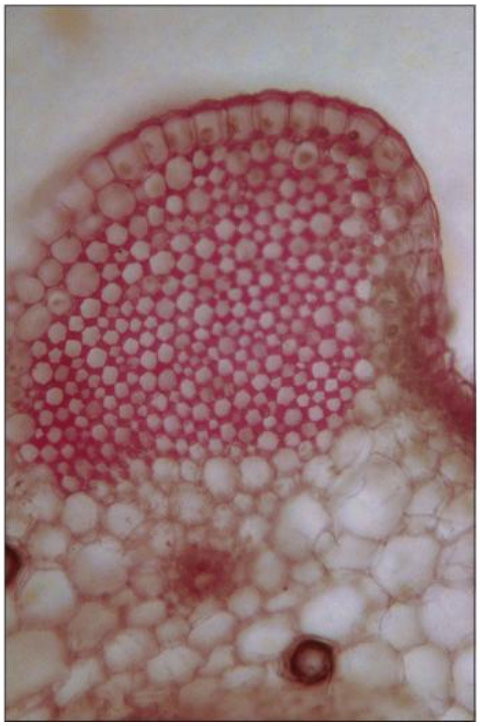
### Observing the sections

1. Place two or three sections of one petiole in a drop of water on a slide and cover with a coverslip.
2. Observe under low power and choose the best section: the thinnest one, cut transversely and not too badly torn. It is rare to get complete sections. Different sections probably contain different parts that are clear to see. A general idea of the entire section can be built up by looking at different sections.
3. Draw a plan view (no cellular detail) of a transverse section of celery petiole based upon your observations. On your plan mark the limits of the different tissues: dermal, vascular and ground tissue. Within these limits, shade the areas occupied by the following cell types: parenchyma, collenchyma and sclerenchyma in the ground tissue; xylem and phloem in the vascular tissue.
4. Now, using medium and if possible, high power, observe and draw an example of each type of cell observed in your section: parenchyma, sclerenchyma, collenchyma, xylem vessel, phloem sieve tube and epidermal cells.

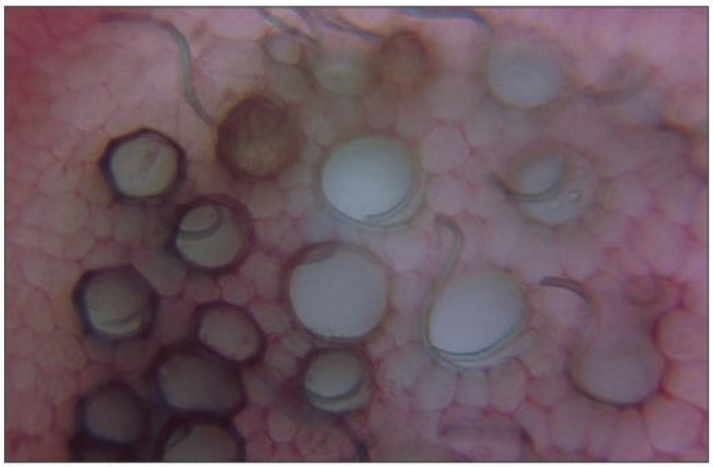
*TS Celery vascular bundle viewed at ×100*

## Research

- The petiole supports the leaf and connects it to the plant stem. How are the tissues and their cells adapted to these functions?
- How are the vascular tissues distributed in the stem and the root of flowering plants?
- Does the petiole have any other functions?



*TS collenchyma supporting ridge, celery viewed at ×100*



*TS xylem vessels celery viewed at ×400*

# Investigation 9D - The structure and adaptations of flowers



**Syllabus reference: Sub-Topic 1.4 Skill**

Flowering plants use different mechanisms to transport the pollen from the anther to the stigma, preferably of another plant of the same species. Two principal agents of dispersal are used, the wind and animals, in particular insects.

## Materials

Hand lens and/or binocular microscope

Fine forceps

Scalpel

2 flowers or inflorescences, one from an insect pollinated and one from a wind pollinated plant

## Method

1. Identify the wind pollinated plant. Using the hand lens or binocular microscope, make a large drawing of a single flower. Label and annotate this drawing.
2. Cut the insect pollinated flower carefully into two equal halves. Draw this half flower. Label and annotate the drawing.

*Warning: Sharp instrument, handle with care.*

## Some points for consideration


- What features of these two flowers permitted you to identify them as being insect pollinated or wind po Explain how these features adapt the flower to insect or wind pollination
- How may these plants avoid self pollination?
- The floral formula (explained on the next page) does not include the colour of the flower. In what way is the colour important for the flower's function? Is the colour important in identifying the flower?

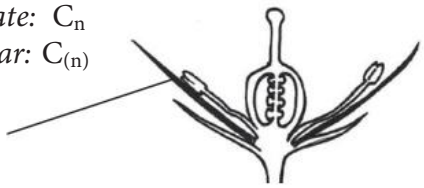
*Honey bees (Apis melifera) are important pollinators of flowering plants*

# Floral Formulae

Ecologists and plant biologists can identify a species of plant by accurately describing its flower structure. Thus we can say that the structure of a flower is species specific. The description can be abbreviated into a standard formula using letters to indicate the different parts of the flower.

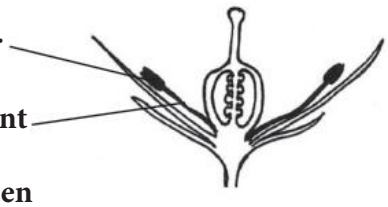
**K = CALYX** (ring of sepals)
*Separate:* $K_n$
*Tubular:* $K_{(n)}$


**C = COROLLA** (ring of petals)
*Separate:* $C_n$
*Tubular:* $C_{(n)}$

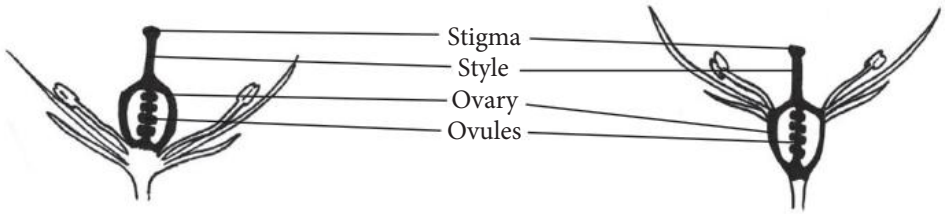



Note: P = PERIANTH (combined corolla and calyx) also $P_n$ or $P_{(n)}$

**A = ANDROECIUM** (ring of stamens)

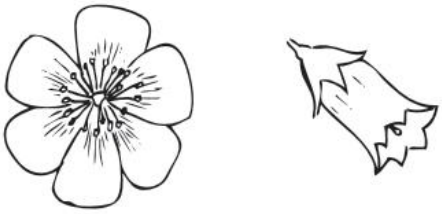



**G = GYNOECIUM** (ring of carpels)
*Superior ovaries* : $G^n$ *Inferior ovaries:* $G_n$

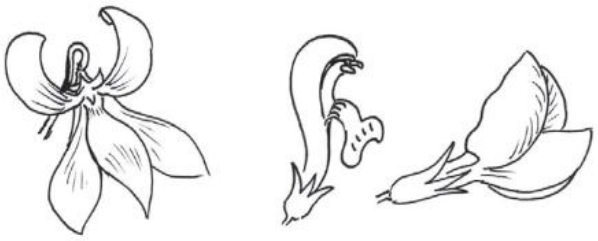



## Examples

⊕ = RADIALLY SYMETRICAL FLOWER (actinomorphic)



↑ = BILATERALLY SYMETRICAL FLOWER (zygomorphic)



e.g. ⊕ $K_{(6)}$ $C_{(6)}$ $A_6$ $G^3$ This is a flower which is radially symmetrical, it has 6 sepals fused into a tubular shape, 6 petals also fused into a tube, 6 stamens and 3 carpels sitting inside the ring of petals.

**Syllabus reference: Sub-Topic 9.4 Skills**

Each year millions of seeds are released by plants. Only a fraction of them are capable of germinating, these seeds are called viable seeds. Of these viable seeds only a fraction will actually germinate immediately, the rest will lie dormant until conditions permit them to germinate. These dormant seeds form what is called the soil seed bank.

It is estimated that up to 62 000 seeds fall onto each square metre of soil per year, and that there are up to 20 000 dormant seeds per square metre of soil.

## Materials

| | | |
|---|---|---|
| 5 petri dishes | Filter paper | *Solutions*: |
| 10cm$^3$ measuring cylinder | Hand lens | Tetrazolium indicator |
| Forceps | Gloves | Iodine solution |
| Scalpel | Safety glasses | Ponceau S |
| Scissors | Pre-soaked seeds | Sudan blue |

# PART A Using tetrazolium indicator to determine seed viability

## Method

1. Select 25 seeds at random and carefully cut each one, lengthways, in half. The embryo should be visible inside the seed.

*Warning sharp instrument handle with care.*


2. Keep one half of each seed and place it, cut surface down, in a Petri dish. Cover the seeds with 10cm$^3$ of tetrazolium solution. Keep the other half of the seeds for Part C.

*Warning: Avoid touching tetrazolium with your fingers, use forceps to handle the seeds. Wear gloves and use eye protection. Wash any splashes with plenty of water.*



3. Leave the seeds to soak in the tetrazolium for 25 minutes. Remove the seeds with your forceps and examine the cut surfaces of each one. A red or pink coloration of the embryo indicates living tissue.

4. Record your results and process them. Compare them with other groups in the class or other types of seeds.

*Wheat seeds (Triticum) stained with tetrazolium*

# PART B Germinating a sample of seeds to determine seed viability

## Method

1. Prepare a Petri dish by placing 2 pieces of filter paper, cut to size, in the bottom. Moisten the filter paper with water and arrange randomly chosen seeds over the surface of the paper.

2. Keep the seeds at a cool temperature (10 to 20°C) and observe them regularly over a period of at least two weeks. Add water to moisten the filter paper when necessary.

3. Record the number of seeds that have germinated on each occasion. Analyse your results and compare them with other groups in the class or other types of seeds.

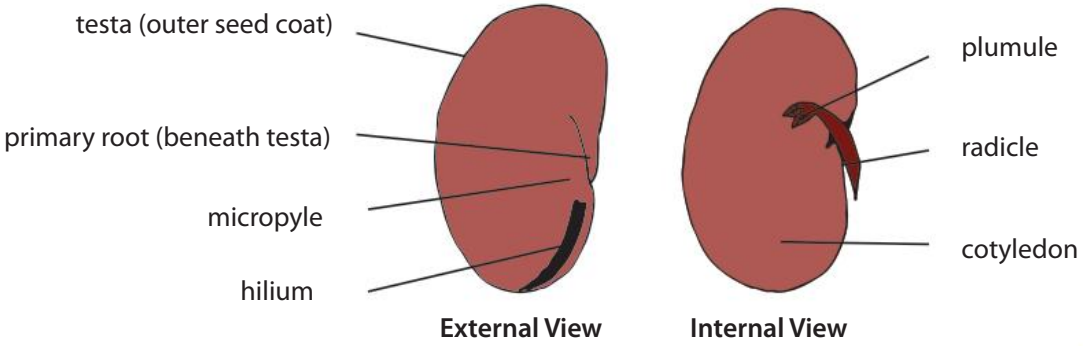
# PART C Structure of seeds and distribution of food reserves

## Method

Use both the dry and soaked seeds for observations.

### Broad beans (*Vicia faba*): a dicotyledonous seed

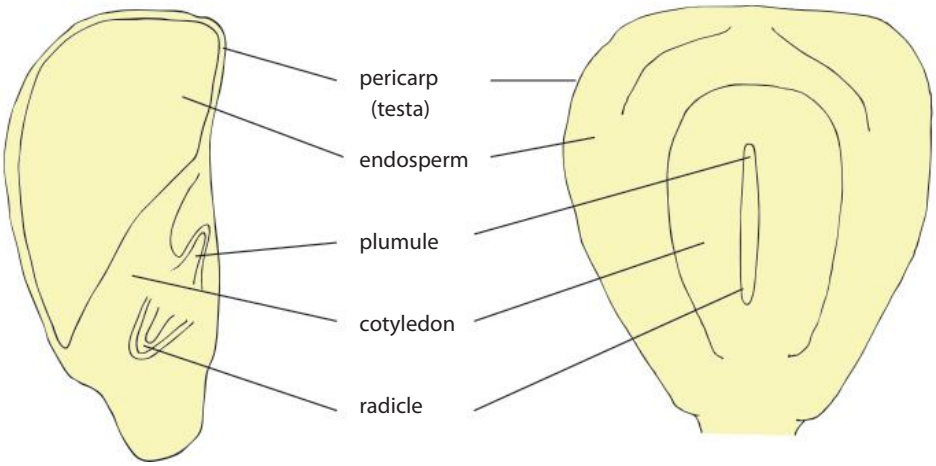
1. After soaking, the seed swells with water. The outer skin or testa covers the seed. A brown scar, the hilum is seen at one end of the seed. This shows where the ovule was attached to the ovary of the fruit. Below the hilum is a tiny pore the micropyle through which water can enter the seed.
2. Using a scalpel, remove the testa to observe the two cotyledons of the embryo. Separate the two cotyledons. On one side observe the folded shoot, the plumule, which lies above the primary root, the radicle.




*Warning: Sharp instrument handle with care.*


### Maize (*Zea mais*): a monocotyledonous seed

1. Monocotyledon plants follow the same trend of development, however, only one cotyledon develops in the embryo. Strictly speaking, the maize kernel is not a seed but a fruit.
2. Cut the maize kernel in half. The inside is divided obliquely into an upper section made up of the white endosperm tissue and a lower section in which the embryo lies. Examine the different structures using the diagram to help you. The face view may be seen by cutting off the outer pericarp-testa, this reveals the embryo. The pericarp develops from the ovary wall.
3. Carry out observations on the following seeds and classify them according to whether the are dicotyledons, monocotyledons: sunflower (*Helianthus annuus)*, kidney bean (*Phaseolus vulgaris*), wheat (*Triticum spp*), and pea (*Pisum sativum*).




*Maize kernels shown in lengthwise section (LS).*

## Identifying and localising food reserves

1. **Stain three of the cut surfaces of bisected seeds using the following stains:**
- Iodine stain: identifies starch (black)
- Ponceau S: identifies protein (red)
- Sudan blue: identifies lipids (blue)

2. Make drawings of the seeds, identifying their parts and the location of their food reserves.

*LS garden peas (Pisum sativum) stained with Ponceau S (top), Sudan blue (left) and iodine solution (bottom right)*

*LS White bean (Phaseolus vulgaris) stained with iodine solution*

# PART D Factors affecting seed germination

Design an experiment to determine the factors that influence the germination of a seed.

## Design

- Establish the theoretical background. This will require some research on your species of plant.
- State a focussed research question.
- Write a hypothesis (a justified prediction).
- Clearly identify the variables (the independent or manipulated variable, dependent or measured variable and the important control variables).
- State the significance of each of the control variables and which variables cannot be controlled but need to be monitored.

## Method

- What equipment and materials will I need? Can this be best described using a labelled diagram of the experimental setup?
- Consider any safety factors.
- Explain exactly how the independent variable will be changed (range and intervals) and how the dependent variable will be measured and how frequently.
- Explain how you intend to control all the other variables (how will you make your experiment a fair test) or monitor them if they cannot be controlled. Perhaps a control experiment will be needed.

N.B: Controlled variables and a control experiment are not the same thing. Controlled variables are those variables that need to be held as stable as possible. A control experiment is performed when you suspect that an uncontrolled variable may have an affect on the experiment.

# Investigation 10A - Using $\chi^2$ test to analyse inheritance patterns



**Syllabus reference: Sub-Topic 10.2 Skill**

Maize shows a number of hereditary characteristics for the colour and shape of the kernels on the maize cob. Black kernels are dominant to yellow.

## PART A Monohybrid cross

The photograph below shows a cross that just involves the colour of the maize kernels. Analyse this photograph of the F2 generation.

The pure bred parents, one producing black kernels the other yellow kernels, are crossed. They produce the F1 which are all black. These F1 are then selfed to produce the F2, shown in the picture above.

- Produce a genetic diagram to explain the data.

Mendel's Law of Segregation predicts that there should be 75% black kernels to 25% yellow kernels.

### Setting up the hypotheses

We need to use the $\chi^2$ test because the data is in categories (nominal). Here we are comparing our data with Mendel's prediction so we are using the $\chi^2$ as a goodness of fit test.

NH: The results agree with the prediction. Called the **Null Hypothesis** because we expect no difference.

AH: The results disagree with the prediction. This is called the **Alternative Hypothesis**.

- Which one is correct?

Set up a 1 x 2 table (one row x two columns) for your observed frequencies.

| | Type of kernel | |
|---|---|---|
| | Black | Yellow |
| Number of kernels | | |

Calculating the $\chi 2$ statistic from the results:

| Trait | (Observed) | Mendelian Prediction (Expected) | Difference (O-E) | $\frac{(O-E)^2}{E}$ |
|---|---|---|---|---|
| Black kernels | | 75% of total | | |
| Yellow kernels | | 25% of total | | |
| **Totals** | | | $\chi^2$ | |

### Comparing the calculated value for $\chi^2$ with values for $\chi^2$ found in probability tables

Calculate the number of Degrees of Freedom = the number of classes - 1.

In this case there are two classes (black kernels and yellow kernels) = 2 - 1 = 1

| Probability levels for $\chi^2$ | | | | | | | |
|---|---|---|---|---|---|---|---|
| Degrees of Freedom | 0.950 | 0.900 | 0.500 | 0.100 | 0.050 | 0.010 | 0.001 |
| 1 | 0.0039 | 0.016 | 0.455 | 2.71 | 3.84 | 6.63 | 10.83 |

The difference is not significant. The NULL hypothesis is accepted.

The difference is significant. The NULL hypothesis is rejected.

The critical value ($\chi_{crit}2$) is taken to be p = 0.05 (or the 5% level)

- Do your results reject or accept the Null Hypothesis?
- State the highest value of p for which the results are not significant.

## PART B Dihybrid cross

The photograph below and its accompanying outline drawing show the F2 results of a cross between a pure breeding smooth, black maize plant with a pure breeding wrinkled, yellow plant. Smooth kernels are dominant to wrinkled.

Analyse the data and explain the heredity of the two characters (seed colour and seed shape). Mendel's law of independent assortment predicts that there should be a 9:3:3:1 ratio for the four phenotypes. How would you determine if the genes for seed colour and seed shape were linked or not?

Set up the Null Hypothesis (NH) and the Alternative Hypothesis (AH). Once again, this is a 'goodness of fit test.

Set up a 1 × 4 table of the observed frequencies (O)

| Type of kernel | | | | |
|---|---|---|---|---|
| | Black Smooth | Black Wrinkled | Yellow Smooth | Yellow Wrinkled |
| Numbers of kernels | | | | |

Calculate the expected frequencies (E) for each of the boxes in the table using Mendel's prediction.

Black smooth = 56.25%

Black wrinkled = 18.75%

Yellow smooth = 18.75%

Yellow wrinkled = 6.25%

Now calculate the $\chi 2$ value = $\sum \frac{(O - E)^2}{E}$

| | O | E | (O-E) | $(O-E)^2$ | $(O-E)^2/E$ |
|---|---|---|---|---|---|
| Black and smooth | | | | | |
| Black and wrinkled | | | | | |
| Yellow and smooth | | | | | |
| Yellow and wrinkled | | | | | |
| | | | | Total ($\chi^2$) | |

Calculate the degrees of freedom = (number of classes – 1).

Compare the calculated $\chi^2$ value with the critical level ($\chi_{crit}^2$) in the probability table

| Probability levels for $\chi 2$ | | | | | | | |
|---|---|---|---|---|---|---|---|
| Degrees of Freedom | 0.950 | 0.900 | 0.500 | 0.100 | 0.050 | 0.010 | 0.001 |
| 1 | 0.0039 | 0.016 | 0.455 | 2.71 | 3.84 | 6.63 | 10.83 |
| 2 | 0.10 | 0.211 | 1.39 | 4.61 | 5.99 | 9.21 | 13.82 |
| 3 | 0.35 | 0.584 | 2.37 | 6.25 | 7.81 | 11.34 | 16.27 |
| 4 | 0.71 | 1.06 | 3.36 | 7.78 | 9.49 | 13.28 | 18.47 |
| 5 | 1.14 | 1.61 | 4.35 | 9.24 | 11.07 | 15.09 | 20.52 |
| 6 | 1.64 | 2.20 | 5.35 | 10.64 | 12.59 | 16.81 | 22.46 |
| 7 | 2.17 | 2.83 | 6.35 | 12.02 | 14.07 | 18.48 | 24.32 |
| 8 | 2.73 | 3.49 | 7.34 | 13.36 | 15.51 | 20.09 | 26.13 |
| 9 | 3.32 | 4.17 | 8.34 | 14.68 | 16.92 | 21.67 | 27.88 |
| 10 | 3.94 | 4.87 | 9.34 | 15.99 | 18.31 | 23.21 | 29.59 |

The critical value ($\chi_{crit}^2$) is taken to be p = 0.05 (or the 5% level)

Do you reject or accept the **Null Hypothesis**?

State the highest value of **p** for which the results are not significant.

**Syllabus reference: Sub-Topic 11.3 Application**

The maintenance of a stable environment for red blood cells is of vital importance. Physiological saline is a solution used to rehydrate patients in hospital. It is perfused by an intravenous drip directly into a blood vessel.

# Part A Exposing blood cells to different solutions of sodium chloride

## Materials

- microscope with micrometer eye piece
- 9 slides and cover slips
- 9 test tubes and test tube rack
- 9 bungs
- 100cm$^3$ flat bottomed flask
- 100cm$^3$ beakers
- Pasteur pipette
- dropping pipette
- 10cm$^3$ pipette and pump
- 1 pipette 1cm$^3$ and pump
- marker pen
- physiological saline and NaCl solutions
- distilled water bottle
- bench centrifuge
- centrifuge tubes
- fresh animal blood (pig's blood)

## Method

1. Pipette 10cm$^3$ of physiological saline into test tube number 1. Put a bung on it.
2. Set up the next 8 test tubes with bungs using 10cm$^3$ of the prepared solutions: 0%, 0.1%, 0.3%, 0.5%, 0.7%, 0.9%, 1% and 2 % NaCl.
3. Collect a few cm$^3$ of blood in the fat bottomed flask. Using the 1cm$^3$ pipette add 0.1cm$^3$ of blood to each test tubes. Replace the bungs and shake the tubes gently to mix them.
4. Set up 9 labelled microscope slides on the bench. Using a Pasteur pipette, take one drop of the each of the dilutions on the appropriate slide. Make sure that you rinse the pipette thoroughly with the new dilution before taking the drop.
5. Observe the shape of the red blood cells under high power until no further change in appearance is seen. Start with the highest concentrations. Record your results.
6. After having left the test tubes undisturbed for 1 hour, observe the appearance of the tubes and note your observations.
7. Transfer the blood dilutions to centrifuge tubes and spin for 1 minute. Record your observations on the sediment (solid at the bottom of the tube) and the supernatant (liquid layer above the sediment).
8. Clear away all the materials, apart from the centrifuge tubes of blood in the different NaCl solutions.

## Some points for consideration

- What is the shape of the cells observed in the physiological saline solution?
- Explain your observations for the shape and the numbers of the red blood cells in the different salt concentrations.
- In which salt concentrations was little or no change seen? Explain why.
- In the tubes that were left for 1 hour explain why some tubes became clear whilst others became cloudy.
- At the top of some of the tubes, there is a clear layer of liquid. What is this liquid? Explain the appearance of this liquid in these particular tubes.
- Explain your results from the observations made after centrifugation.

# Part B A colorimetric analysis of the supernatant

## Materials

| | | |
|---|---|---|
| Data logger | Colorimeter cuvettes and caps | Centrifuge tubes with blood samples |
| Colorimeter sensor | 10 cuvettes | Pasteur pipette |

## Calibrating the sensor

1. Plug the colorimeter probe into the first channel on the data logger.
2. The data logger should detect the colorimeter sensor, set the data collection parameters, and display the current sensor reading. If you have a choice between transmission and absorption select absorption.
3. To calibrate the colorimeter set up a blank cuvette containing physiological saline. Put a cap on it and place it in the colorimeter.
4. Select the appropriate light source. You want to choose a wavelength of light that is absorbed by the hemoglobin released by the red blood cells. Calibrate the colorimeter for the selected wavelength.

## Collecting data

1. Using a Pasteur pipette, withdraw the supernatant from a centrifuge tube, without disturbing the sediment, and fill a colorimeter cuvette until it is 1 cm from the top. Wash the pipette, take a sample from the next tube and fill a fresh cuvette. When labelling the cuvettes mark one side only, near the top. Handle the cuvettes near the top too.
2. Put a cap on the cuvette with the first sample. Set the cuvette with the first sample in the colorimeter, take care not to touch the sides where the light will pass.
3. When everything is ready begin data collection using an event entry mode.
4. Remove the cuvette and replace it with the next. Collect the data for each solution of sodium chloride. The data will appear in the spreadsheet of the data set. Click on 'Stop' to end the data collection. **Do not leave the last cuvette in the colorimeter.**
5. Copy the data set into your laboratory logbook or record it on your USB key. The data can then be processed directly in the data logging program or it can be cut and pasted into a spreadsheet like MSExcel.
6. Present your data, both quantitative and qualitative, in an appropriate way and process the data to determine the effect of different salt concentrations on red blood cells. Discuss and evaluate your results.

## Trouble shooting

### If you get readings that appear strange or impossible try the following:

- Check the connections.
- Reboot the data logger.
- Check the liquid that you have sampled. Is it homogeneous and transparent?
- Check the cuvette that you are using is not dirty or wet on the outside.
- Check the cuvette is in the holder the right way round.
- Check the cuvette holder in the colorimeter. Is it clean and dry?
- Try a different wavelength of light.

*Note: You should try to keep your results between 0.050 – 0.550 absorbance. Outside this range the calibration curve is not linear. If your results are outside this range try a different wavelength (light source) or the liquid may need diluting by a known factor.*

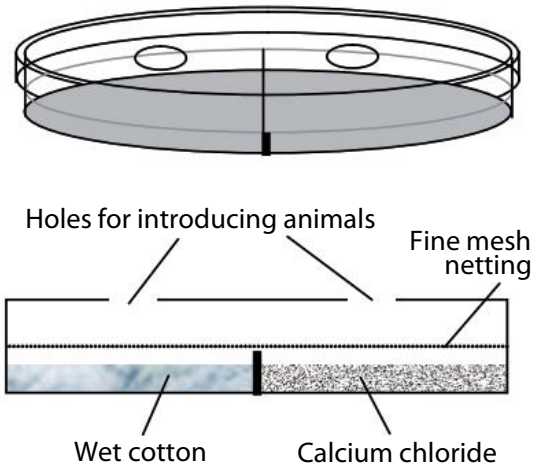
## Syllabus reference: Sub-Topic A.4 Aim

Behavioural responses to particular environments can observed in an organism when it is presented with a choice of environments. The choice presented is usually two extremes of one variable. For example, humidity, the extremes being, dry or wet, with all other variables kept constant. The organism's orientation and movement towards one of these choices is a positive tactic response.

The rate of response to the stimulus is a kinetic response. It is not related to the direction of movement, but to the intensity of the stimulus. The animal may move less in the environment it prefers so it spends more time there.

## Material

| | |
|---|---|
| choice chamber | anhydrous calcium |
| fine mesh netting | chloride granules |
| elastic band | spatula |
| cotton wool | forceps |
| self-adhesive tape | 20 woodlice (slaters) |




## Method

1. Set up the choice chamber so that one half is damp and the other half is dry. Damp cotton wool can be placed at the bottom of the chamber on one side and anhydrous calcium chloride that absorbs water on the other.

*Warning: Calcium chloride is an irritant.*
*Avoid eye contact and wash off any in contact with the skin with plenty of water.*


2. Cover the bottom half with fine mesh netting. Pull it taught, so that the organisms cannot come into contact with the conditions set up below. Use the elastic band to secure the netting. Replace the lid. Leave the apparatus five minutes before introducing the animals, why? All other variables that may influence the experiment should remain constant. If this cannot be guaranteed, a control experiment should be carried out.
3. Introduce 5 woodlice on one side of the choice chamber and 5 on the opposite side. Cover up the holes using the self-adhesive tape.
4. Observe and record the movements and the resulting distribution during five minutes. Design and carry out a suitable control for this experiment. What conclusion can you draw concerning this organism's preference for the variable humidity?
5. Select other variables to test and record the results in the same way.
6. Discuss the results and criticise the investigation. Can you make a conclusion about the habitat that this animal prefers?
7. Set up the same experiment as above, but this time record activity per variable, per unit of time. Record the results in a suitable way. Where are these organisms most active or least active?

Woodlice *Porcellio scaber*

8. Discuss the rate of movement seen. From this part of the investigation explain how you would determine whether the behaviour animal was using taxis or kinesis.
9. Set up other variables to look at the response of these animals to the intensity of a variable.

***Ethics**: Return the woodlice to the environment where they were captured.*

# Investigation 12C - Taking an electromyogram



## Syllabus reference: Sub-Topic A.4 Aim

An electromyogram is a trace of the electrical activity of a muscle. Muscles are excitable cells, when they depolarise they contract and repolarise afterwards. We can use the electrodes of the ECG probe to record this.

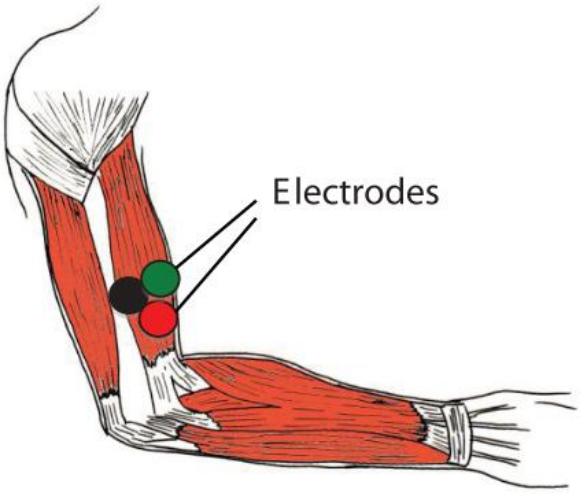
## Materials

| | | |
|---|---|---|
| data logger | soap | adhesive electrodes |
| ECG probe | paper towel | 2kg, 5kg and 10kg masses |

## Method


*Warning: Make sure your subject reads and signs a consent form.*

1. Clean the skin where the electrodes are to be attached with soap and water then dry thoroughly using a paper towel. (e.g. the ends of the biceps muscle of the arm.)
2. Stick the three electrodes to the skin covering the muscle
3. Take the red green and black leads and clip them to the electrodes. It does not matter which way round they go.
4. Set the data logger to record the electrical activity every 0.001s for 10s.
5. Do a trial run with the arm relaxed. Do a second run flexing your elbow. Do a third run lifting a mass of 2kg.
6. Record your data and compare the traces.




## To investigate further

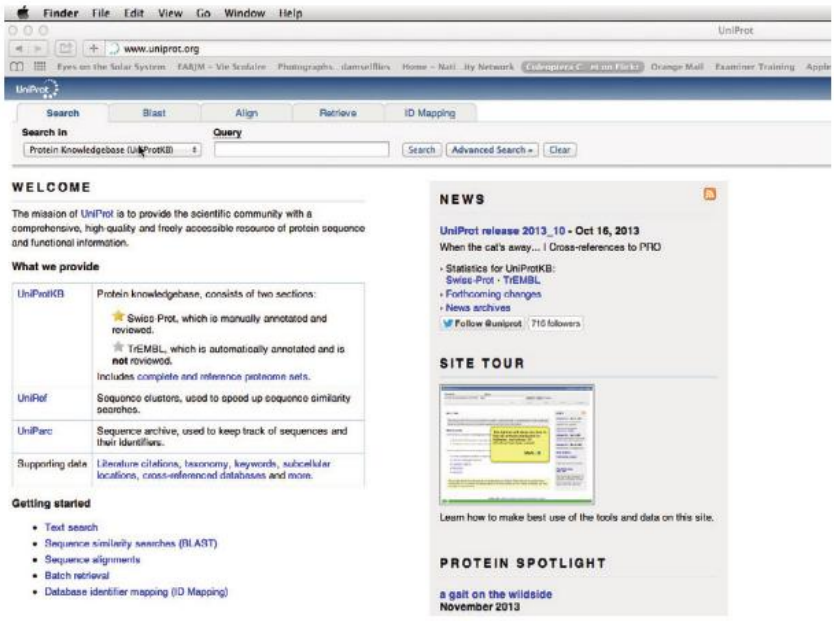
- Try a series of runs recording the same muscle but lifting different masses.
- Place electrodes on the other muscles of the arm (triceps and forearm extensor and flexor muscles) to record their effort doing the same activities.

## Syllabus reference: Sub-Topic B.5 Skill

The sequence of amino acids in the same protein from different species can be used to determine how similar they are.

Open UniProt database:

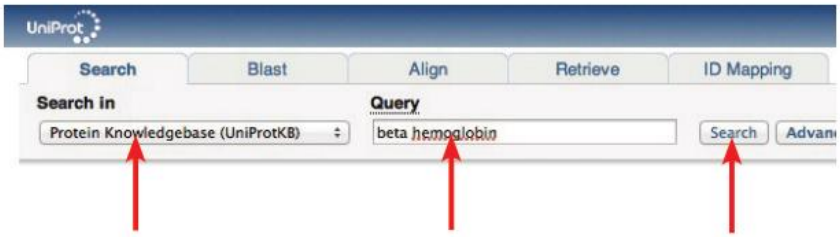
<http://www.uniprot.org>




Select '**Protein Knowledgebase**' (UniProtKB):

Type in '**Query**': (start with beta hemoglobin)

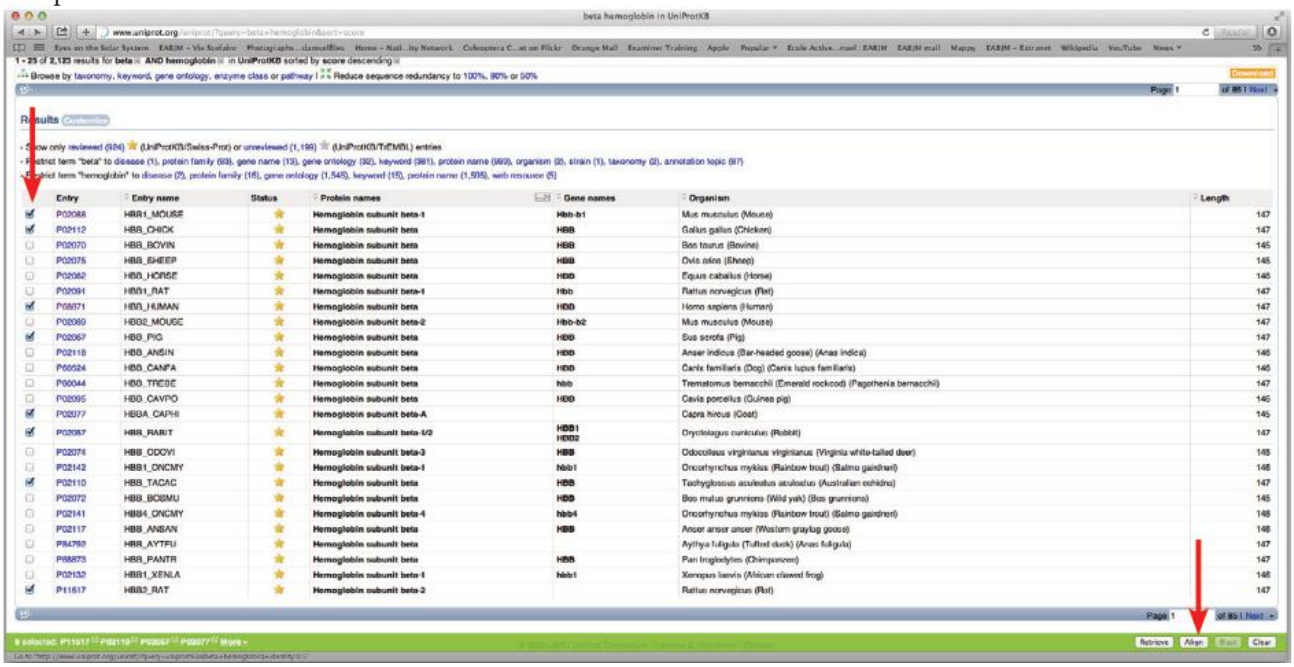
Then click on '**Search**'.




A list of species for which the sequence of amino acids is known will appear.

To see a sequence click on the '**Entry**'.

Then check off the species you wish to compare in the left hand column.

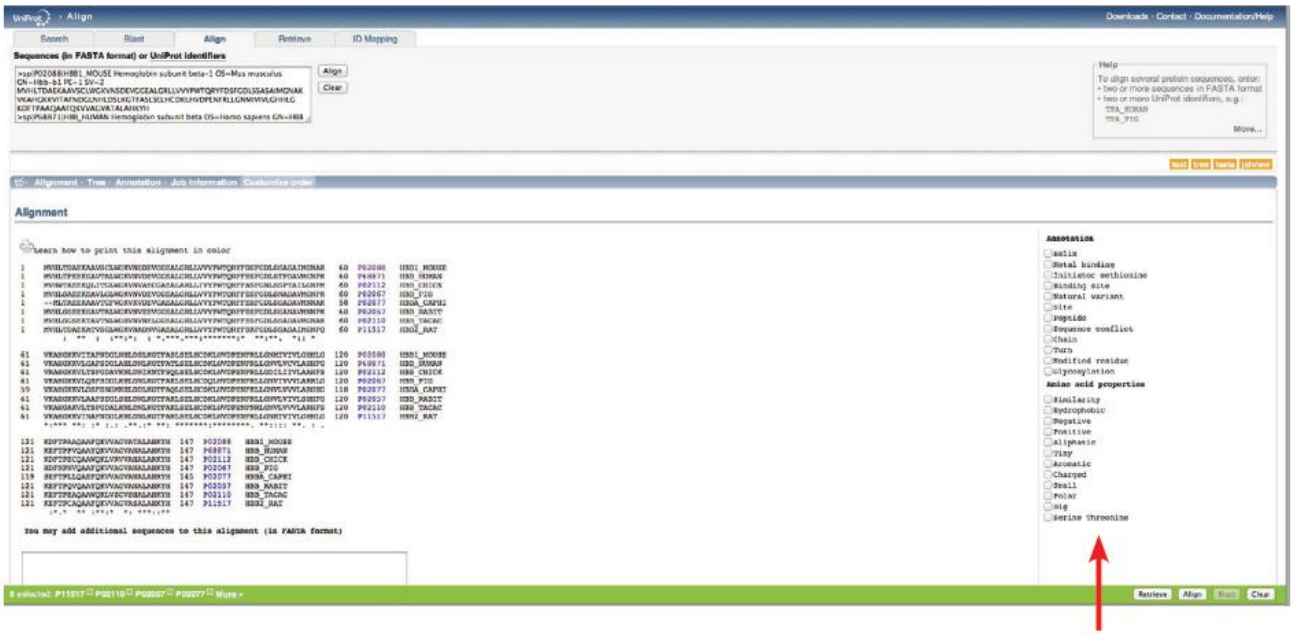



For comparison, select a series of species whose amino acid sequence for beta haemoglobin is known, for comparison by checking next to their entry. Select the house mouse (*Mus musculus*), the chicken (*Gallus gallus*), the human (*Homo sapiens*), the pig (*Sus scrofa*), the goat (*Capra hircus*), the rabbit (*Oryctolagus cuniculus*), the short-beaked echidna (*Tachyglossus aceuleatus*) and the brown rat (*Rattus norvegicus*).

Then click on '**Align**' in the bottom bar. This will compare the primary sequence.

### Studying the aligned proteins

The alignment of the amino acid sequences will take a few seconds.




When it is complete a tool bar on the right-hand side will allow you to explore the structure of the same protein in this series of animals.

**'Helix'** will show the parts that are in alpha helices.

**'Metal binding'** highlights where the iron containing heme group is bound (shown below).

## Alignment

Learn how to print this alignment in color

```
1    MVHLTDAEKAAVSCLWGKVNSDEVGGEALGRLLVVYPWTQRYFDSFGDLSSASAIMGNAK   60  P02088   HBB1_MOUSE
1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK   60  P68871   HBB_HUMAN
1    MVHWTAEEKQLITGLWGKVNVAECGAEALARLLIVYPWTQRFFASFGNLSSPTAILGNPM   60  P02112   HBB_CHICK
1    MVHLSAEEKEAVLGLWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSNADAVMGNPK   60  P02067   HBB_PIG
1    --MLTAEEKAAVTGFWGKVKVDEVGAEALGRLLVVYPWTQRFFEHFGDLSSADAVMNNAK   58  P02077   HBBA_CAPHI
1    MVHLSSEEKSAVTALWGKVNVEEVGGEALGRLLVVYPWTQRFFESFGDLSSANAVMNNPK   60  P02057   HBB_RABIT
1    MVHLSGSEKTAVTNLWGHVNVNELGGEALGRLLVVYPWTQRFFESFGDLSSADAVMGNAK   60  P02110   HBB_TACAC
1    MVHLTDAEKATVSGLWGKVNADNVGAEALGRLLVVYPWTQRYFSKFGDLSSASAIMGNPQ   60  P11517   HBB2_RAT
          :  **   :  :**:*:   : *.***.***:*******:*   **:**.   *:: *

61   VKAHGKKVITAFNDGLNHLDSLKGTFASLSELHCDKLHVDPENFRLLGNMIVIVLGHHLG   120  P02088   HBB1_MOUSE
61   VKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG   120  P68871   HBB_HUMAN
61   VRAHGKKVLTSFGDAVKNLDNIKNTFSQLSELHCDKLHVDPENFRLLGDILIIVLAAHFS   120  P02112   HBB_CHICK
61   VKAHGKKVLQSFSDGLKHLDNLKGTFAKLSELHCDQLHVDPENFRLLGNVIVVVLARRLG   120  P02067   HBB_PIG
59   VKAHGKKVLDSFSNGMKHLDDLKGTFAQLSELHCDKLHVDPENFKLLGNVLVVVLARHHG   118  P02077   HBBA_CAPHI
61   VKAHGKKVLAAFSEGLSHLDNLKGTFAKLSELHCDKLHVDPENFRLLGNVLVIVLSHHFG   120  P02057   HBB_RABIT
61   VKAHGAKVLTSFGDALKNLDNLKGTFAKLSELHCDKLHVDPENFNRLGNVLVVVLARHFS   120  P02110   HBB_TACAC
61   VKAHGKKVINAFNDGLKHLDNLKGTFAHLSELHCDKLHVDPENFRLLGNMIVIVLGHHLG   120  P11517   HBB2_RAT
     *:*** **: :* :.: .**.:* **: *******:********. **:::: **. : .

121  KDFTPAAQAAFQKVVAGVATALAHKYH  147  P02088   HBB1_MOUSE
121  KEFTPPVQAAYQKVVAGVANALAHKYH  147  P68871   HBB_HUMAN
121  KDFTPECQAAWQKLVRVVAHALARKYH  147  P02112   HBB_CHICK
121  HDFNPNVQAAFQKVVAGVANALAHKYH  147  P02067   HBB_PIG
119  SEFTPLLQAEFQKVVAGVANALAHRYH  145  P02077   HBBA_CAPHI
121  KEFTPQVQAAYQKVVAGVANALAHKYH  147  P02057   HBB_RABIT
121  KEFTPEAQAAWQKLVSGVSHALAHKYH  147  P02110   HBB_TACAC
121  KEFTPCAQAAFQKVVAGVASALAHKYH  147  P11517   HBB2_RAT
      :*.*  ** :**:*  *: ***::**

You may add additional sequences to this alignment (in FASTA format)
```

# Investigation 13D - Making a cladogram using amino acid sequences

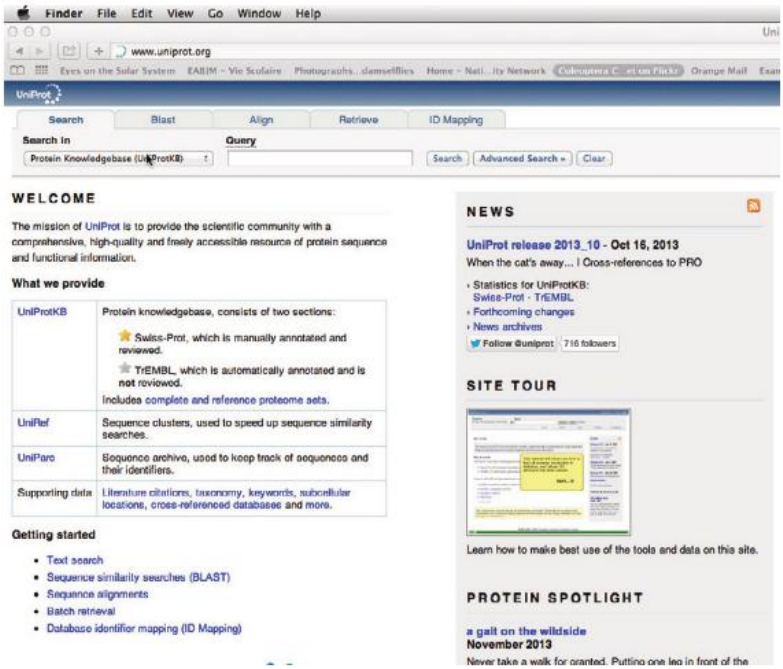


## Syllabus reference: Sub-Topic B.5 Skill

The sequence of amino acids in the same protein from different species can be used to determine how closely related species are.

Open UniProt database:

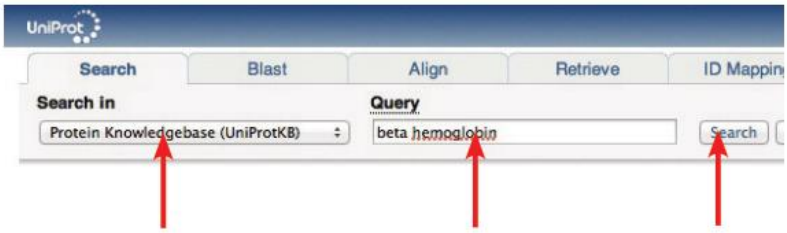
<http://www.uniprot.org>




Select '**Protein Knowledgebase':** (UniProtKB)

Type in '**Query**': (start with beta hemoglobin)

Then click on '**Search**'

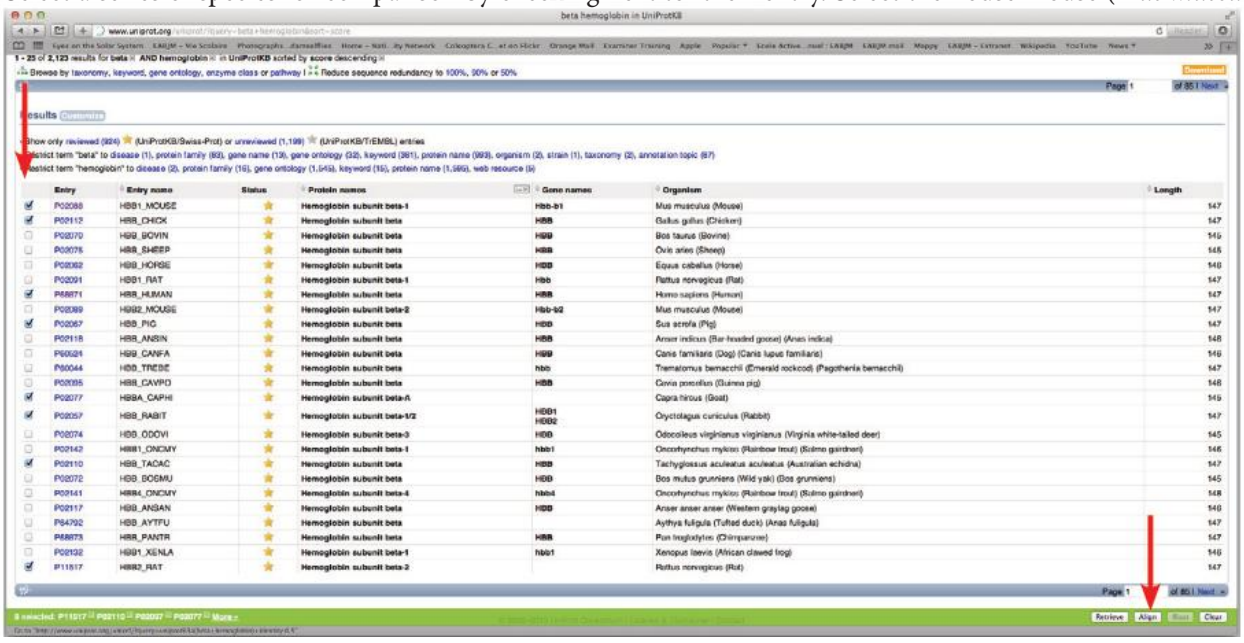



A list of species for which the sequence of amino acids is known will appear.

To see a sequence click on the '**Entry**'.

Then check off the species you wish to compare in the left hand column.

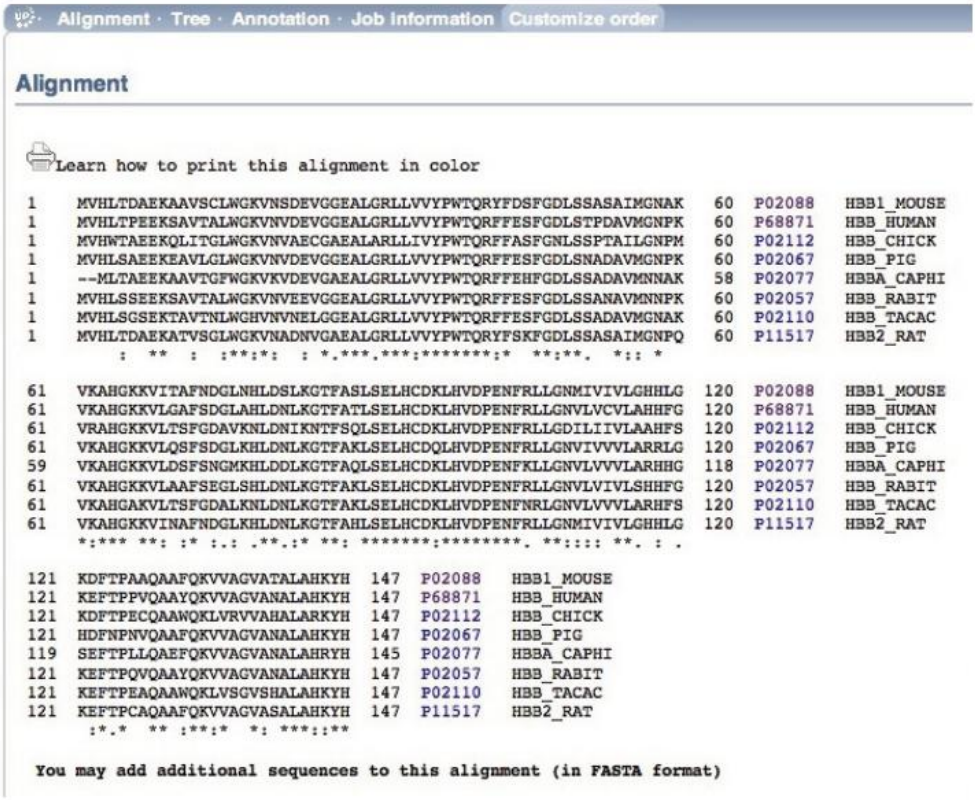
Select a series of species for comparison by checking next to their entry. Select the house mouse (*Mus musculus*),




the chicken (*Gallus gallus*), the human (*Homo sapiens*), the pig (*Sus scrofa*), the goat (*Capra hircus*), the rabbit (*Oryctolagus cuniculus*), the short-beaked echidna (*Tachyglossus aceuleatus*) and the brown rat (*Rattus norvegicus*). Then click on '**Align**' in the bottom bar.

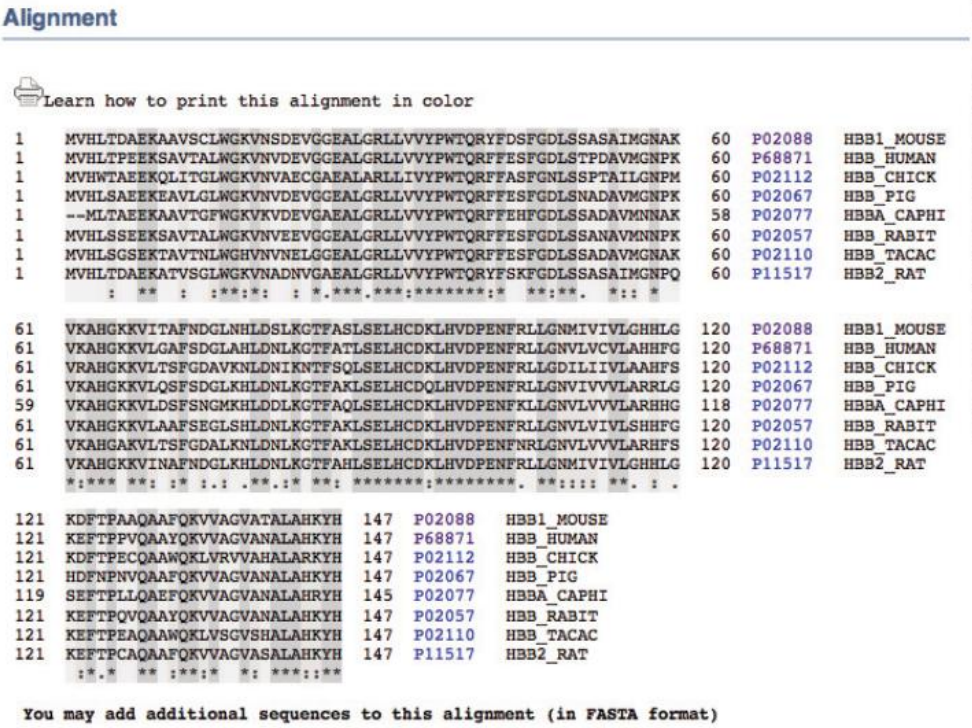
## Studying the aligned proteins

The alignment of the sequences will take a few seconds.




When it is complete a tool bar on the right-hand side will allow you to explore the structure of the same protein in this series of animals.

Under '**Amino acid properties**' select '**Similarities'.**



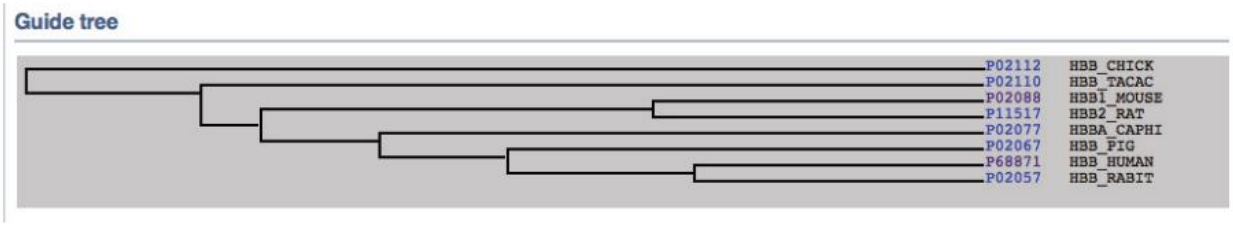

The grey areas highlight that there are many similarities but there are also differences.

## Making a cladogram from the amino acid sequences

A cladogram shows the degree of similarities between species, their phylogeny.

e.g. mouse, human, pig, rat, rabbit, goat, echidna and chicken.

A cladogram will appear below '**Alignment'** under '**Guide tree'**.




This shows the relatedness of these species for their beta hemoglobin protein.

## Analysing the cladogram

- Which animal has the most similar beta hemoglobin to us?
- Which is the most different protein from humans?
- Are there any surprising relationships?
- Do you think this cladogram represents the real relatedness?
- What would need to be done to produce a phylogram showing the period of time since different species diverged?

## Making predictions

Where do you predict a sheep (*Ovis aries*), a horse (*Equus caballus*) or a goose (*Anser anser*) would fit into the cladogram?

Go back to the UniportKB page and add them into the list, then re-run the alignment.

## Trying a different protein

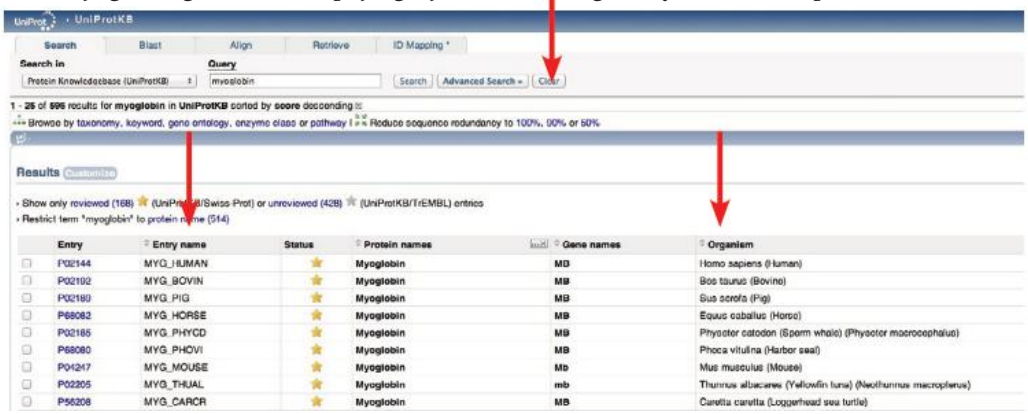
Try the same thing for a different protein e.g. myoglobin or glucagon.

First '**Clear'** the previous search.

You will find searching easier if you restrict the search to the '**protein name'.**

The database for myoglobin is quite large so it is easier to find the species you want if you know the scientific name. You can sort the names of the organisms by alphabetical order of their scientific names under '**Organism'**. You can find the scientific names for a species if you type the common name into Google ®.

*Does myoglobin give the same phylogeny as beta hemoglobin for the same species?*

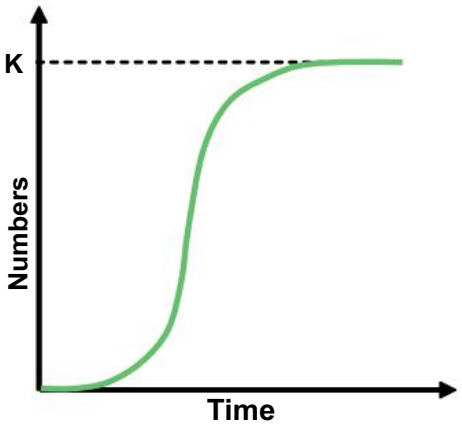


# Investigation 14C - Population growth of yeast cells



**Syllabus reference: Sub-Topic C.5 Skill**

Population growth of simple organisms in ideal conditions can be compared to a model of population growth. The predicted growth curve from these models is the sigmoid-shaped, logistic curve. If we provide a simple unicellular organism, such as baker's yeast (*Saccaromyces cerevisiae*) with ideal conditions do we get the same shaped curve?




## Materials

- microscope
- $2 \times 100cm^3$ conical flasks (sterilised)
- haemocytometer
- electronic balance
- physiological saline (0.9% NaCl)
- pasteur pipette
- $2 \times 10cm^3$ pipette + pumps
- cotton wool
- incubator at 30°C
- 10% glucose solution
- fresh baker's yeast

## Method

1. Preparation of the yeast stock suspension: Add 1g of yeast to $10cm^3$ of physiological saline solution in a conical flask. Swirl to mix and leave for 10 minutes to hydrate.
2. Swirl the yeast stock suspension to mix. Take 1 drop of suspension and add to $10cm^3$ of 10% glucose solution in a flask and mix the contents by swirling the flask. Close the flask using cotton wool. Keep this flask at 30°C for the duration of the experiment. Set up a second flask of glucose solution and a drop of yeast stock suspension in the same way.

### Use of the haemocytometer

The haemocytometer is mostly used to count blood cells but it is also useful to count small cells such as yeast cells.

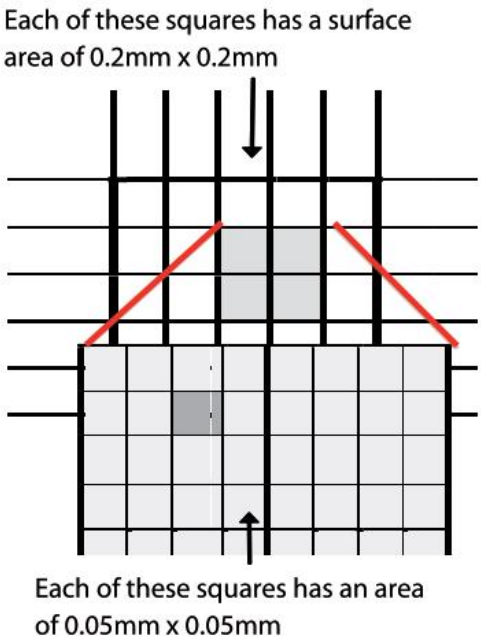
### The counting grid

The counting grid can be seen on the centre of the bar in the middle of the slide. On either side of the bar there is a groove. The central bar is slightly lower than the rest of the slide. When the cover slip is laid over it, **a gap of 0.1mm depth** is left between the bar and the cover slip. Etched onto the centre of the bar is a counting grid that appears in the form of a cross.

In the centre of the counting grid is a square (outlined in **bold** in the diagram opposite). This square has a surface of 1mm × 1mm. This square is made up of 25 smaller squares, **0.2mm × 0.2mm each**.

Each of the 25 squares is made up of 16 smaller squares. Four of these squares are shaded in the diagram above. These four squares appear in the diagram opposite. Each of the 16 squares has a surface area of **0.05mm × 0.05mm**. These squares are the smallest squares that you will see. Examine the counting grid under the ×10 objective lens of the microscope.

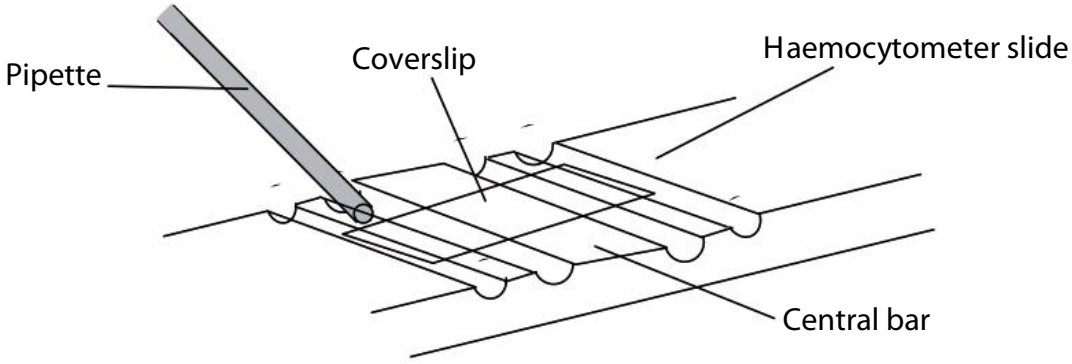
Calculate the **volumes** of the different sized squares.



## Setting up the haemocytometer

Place the coverslip on the haemocytometer.

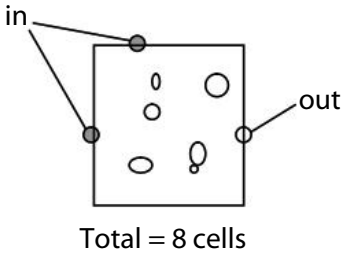
Pipette a drop of the yeast stock suspension onto the haemocytometer. Do this by holding the pipette on the edge of one of the grooves, at the edge of the central bar (see below).




Slowly push the solution out so that it travels along the groove and across the central bar. Wipe off any excess liquid.

## Running a test count to familiarise yourself with counting yeast cells

1. Count the number of yeast cells found in one of the smallest squares.
2. Yeast cells that fall on the edges of the square should be counted "in" on two of the edges. For example, the cells falling on the top and left hand side (the shaded cells). Those on the other edges are not counted (the unshaded cells) *See opposite.*
3. For the yeast cells that are budding, count the buds as individual cells.
4. Now remove the cover slip and rinse the haemocytometer and cover slip with distilled water. Dry the haemocytometer using the lens tissue.




## You are ready for your first count: Day 0

1. Take one of the prepared flasks from the oven. Swirl the flask. Using a clean Pasteur pipette take a few drops of the culture solution.
2. Set up the haemocytometer as before and count the number of yeast cells within a counting area made up of a group of four of the smallest squares.
3. Repeat the count in two other areas of the same size. Record the average count per area and the average number of cells per $mm^3$.
4. Repeat the counts using the second flask. Note that the initial dilution of the suspension is not taken into account.
5. Repeat the counts at regular times, once a day. Continue for a period of at least 15 days.
6. Represent the results in a graph of incubation time against number of yeast cells per $mm^3$.
7. Mark the different stages of growth observed on the graph and explain each of the stages observed.
8. Does the growth curve of the yeast population fit the predicted of the model?

## Simulating exponential growth in a spread sheet

Estimates for the cell cycle of Saccaromyces cerevisiae vary from 80 to 100 minutes under ideal conditions. Taking 90min as a mean value, set up a spread sheet showing generations, time and yeast cell numbers.

Type in the first two rows for generation and time and select these cells. Move the mouse to the bottom right corner of the selected cells, click left and pull down. This will give you a series for as many minutes as you wish (16 generations is 24h).

Suppose that your average count for the yeast cells at the beginning of your experiment is 8 cells per grid on the haemocytometer. Enter this in the first cell under Cell count. Then type in the formula = "click" on cell C2 then *2. Press enter and it will calculate the number you would expect per cell after one generation (90min).

Select this cell and pull down on the bottom right hand corner again to repeat the calculation.

Assuming the volume above the grid on the haemocytometer being used is 0.00025mm$^3$ (this is true for the Thoma haemocytometer). Calculate the number of yeast cells per mm$^3$ entering the equation =C2*4000 press enter and pull down on the bottom right of the cell again.

Then plot a graph of time or generations against numbers of yeast cells per mm$^3$.

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Generation | Time / min | Cell count | Cell concentration / numbers mm$^{-3}$ |
| 2 | 0 | 0 | | |
| 3 | 1 | 90 | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |
| 9 | | | | |
| 10 | | | | |

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Generation | Time / min | Cell count | Cell concentration / numbers mm$^{-3}$ |
| 2 | 0 | 0 | 8 | |
| 3 | 1 | 90 | =C2*2 | |
| 4 | 2 | 180 | | |
| 5 | 3 | 270 | | |
| 6 | 4 | 360 | | |
| 7 | 5 | 450 | | |
| 8 | 6 | 540 | | |
| 9 | 7 | 630 | | |
| 10 | 8 | 720 | | |

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Generation | Time / min | Cell count | Cell concentration / numbers mm$^{-3}$ |
| 2 | 0 | 0 | 8 | |
| 3 | 1 | 90 | 16 | |
| 4 | 2 | 180 | 32 | |
| 5 | 3 | 270 | 64 | |
| 6 | 4 | 360 | 128 | |
| 7 | 5 | 450 | 256 | |
| 8 | 6 | 540 | 512 | |
| 9 | 7 | 630 | 1024 | |
| 10 | 8 | 720 | 2048 | |

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Generation | Time / min | Cell count | Cell concentration / numbers mm$^{-3}$ |
| 2 | 0 | 0 | 8 | =C2*4000 |
| 3 | 1 | 90 | 16 | |
| 4 | 2 | 180 | 32 | |
| 5 | 3 | 270 | 64 | |
| 6 | 4 | 360 | 128 | |
| 7 | 5 | 450 | 256 | |
| 8 | 6 | 540 | 512 | |
| 9 | 7 | 630 | 1024 | |
| 10 | 8 | 720 | 2048 | |

## Some points for consideration

- Suggest various factors that might influence the rate of growth of the yeast cells.
- A similar curve is seen when plotting the average numbers of yeast cells in a counting area against the incubation time. Why were you asked to plot the number of cells per mm$^3$?
- In what other ways could the data obtained be analysed, for example, could other graphs have been drawn?

## Research

What industrial processes use information similar to that obtained in this investigation?

**Syllabus reference: Sub-Topic D.6 Application and Skill**

The proteins myoglobin and hemoglobin are present in most mammals, including humans and are involved with the storage and transport of oxygen in the body tissues. These molecules are sometimes called 'respiratory pigments'. In an experiment, the following data were obtained showing the association and dissociation of oxygen with both human myoglobin and hemoglobin.

| % Partial Pressure of $O_2$ | | 0 | 10 | 20 | 40 | 60 | 80 | 100 |
|---|---|---|---|---|---|---|---|---|
| % Saturation | Myoglobin | 0 | 80 | 87 | 93 | 96 | 96 | 96 |
| | Hemoglobin | 0 | 10 | 28 | 75 | 90 | 96 | 96 |

## Analysis

Use these data to plot the dissociation curves for both human myoglobin and hemoglobin. You may do so by hand on separate paper or with the help of computer software such as Excel.

## Some points for further consideration

- Describe the differences between these 2 molecules, as shown by these graphs.
- How can you account for the different shapes of the curves for these 2 molecules?
- What are the respective roles of these 2 molecules in the human body?
- How are their roles in the body complementary?

## Research

Use the internet or other sources to investigate one or more of the following topics, you could possibly add further lines to your graph.

- the structure and function of human fetal hemoglobin
- the structure and function of other respiratory pigments, such as hemocyanin (often found in invertebrates)
- the mechanism(s) by which humans are able to acclimatise to life at altitudes above about 3000 metres and consequently the advantages of 'altitude-training' for mountaineers and sometimes elite athletes.

# IB Biology Investigations

## Volume 2 (Higher Level)

# TEACHING NOTES

*(For use with the IB Diploma programme)*

**(Fourth edition)**

**Author: Paul Billiet**

**Series editor: David Greig**

SL

# Syllabus Correlation Table (Core)

| Core Topic | Practical Number | Sub topic | Syllabus reference | Title |
|---|---|---|---|---|
| 1 | 1A | 1.1 | **Skill:** Use of a light microscope to investigate the structure of cells and tissues, with drawing of cells. Calculation of the magnification of drawings and the actual size of structures and ultrastructures shown in drawings or micrographs. (Practical 1) | CELLS |
| | 1B | 1.4 | **Skill:** Estimation of osmolarity in tissues by bathing samples in hypotonic and hypertonic solutions. (Practical 2) | ESTIMATING THE WATER POTENTIAL OF PLANT TISSUES |
| | 1C | | **Aim**: Dialysis tubing experiments can act as a model of membrane action. Experiments with potato, beetroot or single-celled algae can be used to investigate real membranes. | MEMBRANE INTEGRITY IN CABBAGE LEAF CELLS |
| | 1D | 1.6 | **Skill:** Identification of phases of mitosis in cells viewed with a microscope or in a micrograph. | THE CELL CYCLE IN PLANT TISSUES |
| | | | **Skill:** Determination of a mitotic index from a micrograph. | |
| 2 | 2A | 2.1 | **Skill:** Identification of biochemicals such as sugars, lipids or amino acids from molecular diagrams. | DIALYSIS: SEPARATING MOLECULES BY SIZE |
| | 2B | 2.2 | **Application:** Use of water as a coolant in sweat. | WATER AS A COOLANT |
| | 2C | 2.3 | **Skill:** Use of molecular visualization software to compare cellulose, starch and glycogen. | POLYSACCHARIDE MOLECULES |
| | 2D | 2.5 | **Skill:** Experimental investigation of a factor affecting enzyme activity. (Practical 3) | MEASURING THE RATE OF REACTION OF AN ENZYME CONTROLLED REACTION |
| | 2E | 2.5, 2.8 | **Application:** Use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide in baking. | IMMOBILISING YEAST ENZYMES |
| | 2F | 2.8 | **Skill:** Analysis of results from experiments involving measurement of respiration rates in germinating seeds or invertebrates using a respirometer. | RESPIRATION RATES OF AN INVERTEBRATE |
| | 2G | 2.9 | **Skill:** Separation of photosynthetic pigments by chromatograph. (Practical 4) | LEAF PIGMENTS, THEIR EXTRACTION AND SEPARATION |
| | 2H | | **Skill:** Drawing an absorption spectrum for chlorophyll and an action spectrum for photosynthesis | USING SPECTROSCOPY TO STUDY LEAF PIGMENTS |
| | 2I | | **Skill:** Design of experiments to investigate the effect of limiting factors on photosynthesis. | METHODS TO MEASURE THE RATE OF PHOTOSYNTHESIS |
| 3 | 3A | 3.1 | **Skill:** Use of a database to determine differences in the base sequence of a gene in two species. | USING A PROTEIN DATABASE |
| | 3B | 3.5 | **Skill:** Design of an experiment to assess one factor affecting the rooting of stem-cuttings. | FACTORS AFFECTING ROOTING IN PLANTS |
| 4 | 4A | 4.1 | **Skill:** Setting up sealed mesocosms to try to establish sustainability. (Practical 5) | ECOLOGICAL SUCCESSION IN A MICROBIAL ECOSYSTEM |
| | 4B | | **Skill:** Testing for association between two species using the chi-squared test with data obtained by quadrat sampling. | PLANT ASSOCIATION TEST |
| 5 | 5A | 5.3 | **Application:** Recognition features of bryophyta, filicinophyta, coniferophyta and angiospermophyta | DICHOTOMOUS KEY FOR PLANT PHYLA |
| | | | **Skill:** Construction of dichotomous keys for use in identifying specimens. | |
| 6 | 6A | 6.1 | **Application:** Use of dialysis tubing to model absorption of digested food in the intestine. | DIALYSING TUBING GUT |
| | 6B | 6.2 | **Skill:** Recognition of the chambers and valves of the heart and the blood vessels connected to it in dissected hearts or in diagrams of heart structure. | THE ANATOMY OF THE HEART |
| | 6C | 6.4 | **Skill:** Monitoring of ventilation in humans at rest and after mild and vigorous exercise. (Practical 6) | VENTILATION AND EXERCISE |

HL

# Syllabus Correlation Table (AHL)

| AHL Topic | Practical Number | Sub topic | Syllabus reference | Title |
|---|---|---|---|---|
| 7 | 7A | 7.1 | **Skill:** Utilization of molecular visualization software to analyse the association between protein and DNA within a nucleosome. | THE NUCLEOSOME |
| 8 | 8A | 8.1 | **Skill:** Distinguishing different types of inhibition from graphs at specified substrate concentration. | INHIBITORS OF UREASE |
| 9 | 9A | 9.1 | **Skill:** Measurement of transpiration rates using potometers. (Practical 7)<br>**Skill:** Design of an experiment to test hypotheses about the effect of temperature or humidity on transpiration rates. | TRANSPIRATION IN PLANTS |
| | 9B | | **Aim:** Measurement of stomatal apertures and the distribution of stomata using leaf casts, including replicate measurements to enhance reliability are possible experiments. | STOMATA: THE SITE OF TRANSPIRATION IN PLANTS |
| | 9C | 9.1 & 9.2 | **Skill:** Drawing the structure of primary xylem vessels in sections of stems based on microscope images.<br>**Skill:** Identification of xylem and phloem in microscope images of stem and root. | CELL TYPES AND TISSUES IN THE PETIOLE OF CELERY |
| | 9D | 9.4 | **Skill:** Drawing of half-views of animal-pollinated flowers. | THE STRUCTURE AND ADAPTATIONS OF FLOWERS |
| | 9E | | **Skill:** Drawing internal structure of seeds.<br>**Skill:** Design of experiments to test hypotheses about factors affecting germination. | SEED STRUCTURE AND VIABILITY |
| 10 | 10A | 10.2 | **Skill:** Use of a chi-squared test on data from dihybrid crosses. | USING CHI$^2$ TEST TO ANALYSE INHERITANCE PATTERNS |
| 11 | 11A | 11.3 | **Application:** Consequences of dehydration and over hydration. | HOMEOSTASIS AND RED BLOOD CELLS |

SL HL

# Syllabus Correlation Table (Options)

| Option Topic | Practical Number | Sub topic | Syllabus reference | Title |
|---|---|---|---|---|
| A | 12A | A.2 | **Application:** Use of the pupil reflex to evaluate brain damage. | THE PUPIL REFLEX |
| | 12B | A.3 | **Application:** Red-green colour-blindness as a variant of normal trichromatic vision. | THE RETINA AND COLOUR VISION |
| | 12C | A.4 | **Skill:** Analysis of data from invertebrate behaviour experiments in terms of the effect on chances of survival and reproduction. | STUDYING INVERTEBRATE BEHAVIOUR |
| | 12D | A.4 | **Aim:** Data logging using an ECG sensor to analyse neuromuscular reflexes. | RECORDING AN EMG |
| B | 13A | B.1 | **Skill:** Experiments showing zone of inhibition of bacterial growth by bactericides in sterile bacterial cultures. | DO DISINFECTANTS KILL BACTERIA? |
| | 13B | | **Skill:** Gram staining of Gram-positive and Gram-negative bacteria. | THE GRAM STAIN FOR BACTERIA |
| | 13C | B.5 | **Skill:** Use of software to align two proteins. | COMPARING PROTEINS FROM DIFFERENT SPECIES |
| | 13D | | **Skill:** Use of software to construct simple cladograms and phylograms of related organisms using DNA sequences | MAKING A CLADOGRAM FROM PROTEIN SEQUENCES |
| C | 14A | C.1 | **Skill:** Use of a transect to correlate the distribution of plant or animal species with an abiotic variable. | USING INVERTEBRATE PITFALL TRAPS ON A LINE TRANSECT |
| | 14B | C.2 &4 | **Skill:** Investigation into the effect of an environmental disturbance on an ecosystem.<br>Skill: Analysis of the biodiversity of two local communities using Simpson's reciprocal index of diversity. | MEASUREMENT OF A DIVERSITY INDEX AND A BIOTIC INDEX |
| | 14C | C.5 | **Skill:** Modelling the growth curve using a simple organism such as yeast or species of *Lemna*. | POPULATION GROWTH OF YEAST CELLS |
| D | 15A | D.1 | **Skill:** Determination of the energy content of food by combustion. | ENERGY FROM FOOD |
| | 15B | D.4 | **Skill:** Measurement and interpretation of the heart rate under different conditions. | HEART RATE AND BLOOD PRESSURE |
| | | | **Skill:** Interpretation of systolic and diastolic blood pressure measurements. | |
| | 15C | | **Skill:** Mapping of the cardiac cycle to a normal electrocardiogram (ECG) trace. | TAKING AND READING AN ELECTROCARDIOGRAM |
| | 15D | D.6 | **Skill**: Analysis of dissociation curves for hemoglobin and myoglobin.<br>**Application**: Consequences of high altitude for gas exchange. | MYOGLOBIN AND HEMOGLOBIN |

| | | | | |
|---|---|---|---|---|
| APPENDICES | 1 | 6, 11 & D | The IB animal experimentation policy and the biology course safety guidelines. | INFORMED CONSENT FORM |
| | 2 | 4.1 | **Guidance:** Sampling should be based on random numbers. In each quadrat the presence or absence of the chosen species should be recorded. | TABLE OF RANDOM NUMBERS |
| | 3 | | | USING A TI CALCULATOR TO GENERATE RANDOM NUMBERS |

# 1A CELLS

## Syllabus reference: Topic 1.1

**Skill**: Use of a light microscope to investigate the structure of cells and tissues, with drawing of cells. Calculation of the magnification of drawings and the actual size of structures and ultrastructures shown in drawings or micrographs. (Practical 1)

**Required knowledge**: Use of the microscope, preparation of a temporary mount, characteristics of animal and plant cells, the test for starch using iodine solution.

## 1A.1 ANIMAL CELLS

Time: 1 hour

### Materials

**The bleach**: This could be a household cleaning product containing sodium hypochlorite.

e.g. *Domestos* or sodium hypochlorite at 1% (1g per 100cm$^3$ of distilled water)

**Methylene blue stain**: 1%

1g + 0.6g NaCl in 100cm$^3$ distilled water

Other sources of epithelial cells could be:

- Moulted skin from a frog
- Cells removed with self-adhesive from the inside of the wrist

### Data

An average cell size of 20µm may be measured.

Liver cells have a tendency to be bi-nucleate. This could encourage a discussion on the limits of the cell theory.

## 1A.2 PLANT CELLS

Time: 1.5 hours

### Materials

For good results the *Elodea* should be freshly cut. The cut *Elodea* branches may be kept in an aquarium for a few days. The young leaves surrounding the terminal bud at the end of the branch give good results. If *Elodea* is not available the leaf scales of moss plants and leafy liverworts are usually sufficiently transparent though cyclosis may not be observable.

Avoid taking onion scales from the outside of the bulb, inner scales provide better results.

The banana should be ripe for the observation of starch grains, however, as the fruit ripens the cell wall is partially digested and becomes less visible.

One red onion and one banana should provide enough material for six groups.

Other plant material suitable for observation might include: potatoes (*Solanum tuberosum*) or rice (*Oryza*) for starch grains and tomatoes (*Solanum lycopersicon*) for pigmented chromoplasts.

The rice grains need to be soaked in water for a few minutes before crushing.

**Iodine solution:** Dissolve 1g of potassium iodide in a small amount of distilled water, mix whilst adding 0.5g of iodine crystals. Make the solution up to 100cm$^3$ with distilled water. Store the solution in dark brown bottles.

**Sucrose solution:** 20g of sucrose for 100cm$^3$ of distilled water. This is sufficient to shrink the vacuole by osmosis.

## Method

As the *Elodea* leaf is several cells thick, the students may be confused over the limits of individual cells and their contents. This provides a good exercise in using the fine focusing control.

To observe the sap vacuole clearly it is possible to make it visible using fresh 0.1% aqueous neutral red stain. Transparent, colourless tissues such as the inner epithelium of onion scales or from leeks can be used.

Make sure the pH of the water used to make up the neutral red is 7 to 7.5. If it is slightly acidic, the stain will not be taken up. *Evian* water could be used here as it has a pH of 7.4.

Squares 1cm × 1cm squares can be cut from an onion scale or a leek leaf. Using fine forceps, peal off the epidermis from the inside surface of each square. Place the squares of epidermal tissue in a Petri dish containing neutral red stain at pH 7.4. Leave the tissues to soak for 3 minutes. Remove the squares of epidermis and transfer them to a Petri dish of buffer at pH 7.4. Wash them thoroughly four times in clean buffer. Mount a square of tissue on a microscope slide in a drop of the buffer. The sap vacuoles should stain up brick red.

# 1A.3 DRAWINGS

All drawings must be done in pencil and on drawing paper. They must have a title and be fully labelled. Neatness is very important. Avoid excess detail. The magnification used must be stated below the drawing (e.g. ×400).

## Observations

The students should be encouraged to pose questions on observations made for each tissue looked at.

**For example:**

**Canadian pond weed (*Elodea*):** The Elodea leaf provides good observations of chloroplasts and their cytoplasmic streaming (cyclosis).

- Why are the chloroplasts so abundant?
- Why do the chloroplasts move?

**Onion (*Allium*):** The red onion epidermal cells should be fully turgid in the distilled water and partly plasmolysed in the sucrose solution. The plasmolysed cells will permit the observation of the plasma membrane and the cell wall as separate structures.

- Why are nuclei not seen in each cell?
- Why are no chloroplasts observed here?
- Why was sucrose used to shrink the cell vacuole?

Students do not need to know about osmosis for this, though they may question the phenomenon they are observing. The point is to get them to distinguish between the cell wall and the plasma membrane which are usually stuck together..

- Banana (Musa): The banana cells contain starch grains which are clearly visible and they stain black with the iodine solution:
- Why are the starch grains so abundant here?

## Some points for consideration

Table comparing the structures seen in animal and plant cells as seen with the light microscope.

| Structure | Animal Cell | Plant cell |
|---|---|---|
| Cell wall | - | * |
| Cell surface membrane | * | * |
| Cytoplasm | * | * |
| Cell sap vacuole | - | * |
| Tonoplast | - | * |
| Chloroplasts | - | * |
| Starch grains | - | * |
| Nucleus | * | * |
| Nuclear envelope | * | * |
| Nucleolus | * | * |

NB Plasmodesmata may be observed clearly in red pepper epidermal cells.

## To investigate further

- A project could be carried out comparing starch grains in different tissues: e.g. potato (*Solanum tuberosum*), rice (*Oryza*), banana (*Musa*) and maize (*Zea*).
- What determines the speed of movement of chloroplast cyclosis? This could be investigated using a micrometer eye piece and stop watch whilst varying environmental conditions (e.g. temperature, light intensity, light colour/ wavelength or the composition of the solution bathing the leaf).
- The changes in the appearance of cells in ripening bananas.
- The effect of a range of sucrose solutions on red onion epithelial cells.

# 1A.4 THE MICROMETER EYEPIECE

This accessory will turn the microscope into a quantitative measuring tool.

## Materials

### Micrometer eyepieces

Various models of micrometer eyepieces exist. Eyepieces with built-in scales are the easiest to use. Graticules can be purchased and inserted into the standard eyepiece of the microscope. However, be careful, these graticules may not lie exactly in the plane of focus, they may fall in upside down and are liable to get fingerprints on them.

### Micrometer slides

These are expensive. A class set should not be necessary. One slide per two or three microscopes is convenient. These slides are easily confused with ordinary slides. To avoid this confusion, a coloured self-adhesive label may be stuck to the slide. This has the advantage of informing the student which way up the slide is. A common error by students is to place the micrometer slide on the microscope stage upside down. The graduations cannot be brought into focus on high power when the slide is like this.

### Microscopes

Numbering the microscopes in the laboratory is useful. The calibration of the eyepiece will not have to be repeated if the student uses the same microscope in subsequent investigations.

# 1B ESTIMATING THE WATER POTENTIAL OF PLANT TISSUES

Time: 1 hour for preparation + 1 hour for measurement.

## Syllabus reference: Topic 1.4

**Skill**: Estimation of osmolarity in tissues by bathing samples in hypotonic and hypertonic solutions. (Practical 2)

**Required knowledge:** Osmosis, plant cell structure.

## Materials

An electronic balance sensitive to 0.1g

Any plant tissue which is easily cut into chips or cylinders.

A potato chip cutter significantly speeds up the preparation time.

Flat bottomed specimen tubes (25mm diameter) rather than test tubes are very useful for this investigation.

Fruits (apple) or other roots, (carrot, swede, sweet potato, yam, cassava etc.) could also be tried.

## Method

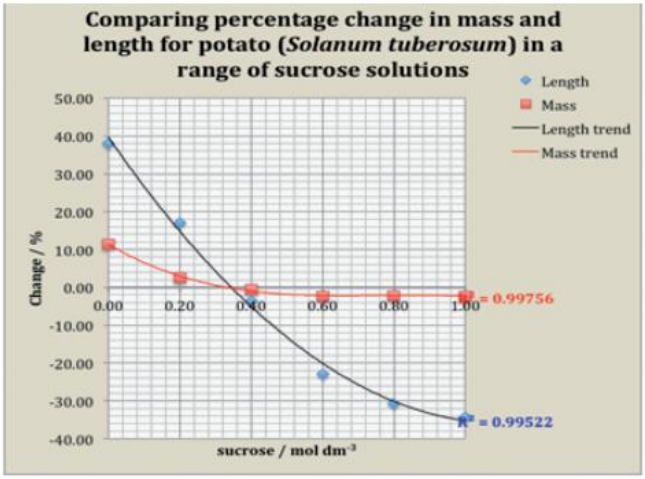
The strips should be left for at least several hours in the solutions before re-measuring. The tubes can be left for up to one or two days in a refrigerator and then re-measured.

For the method involving changes in density of the liquid results can be obtained in as little as 20 minutes.

## Data

The following results were obtained for apple fruit, potato tuber and sweet potato tissues:

| Sucrose solution /mol dm$^{-3}$ | Potato | | | Apple | | |
|---|---|---|---|---|---|---|
| | % change in mass | % change in length | Movement of methylene blue | % change in mass | % change in length | Movement of methylene blue |
| 0 | 18.2 | 19.0 | Up | 16.0 | 5.0 | Up |
| 0.2 | 4.1 | 8.0 | Up | 15.4 | 8.0 | Up |
| 0.4 | -13.0 | -1.0 | Down | 15.0 | 3.5 | Up |
| 0.6 | -28.6 | -6.0 | Down | 4.1 | -0.5 | Stable |
| 0.8 | -34.3 | -8.0 | Down | -4.6 | -2.5 | Down |
| 1.0 | -36.8 | -10.0 | Down | -16.6 | -3.5 | Down |



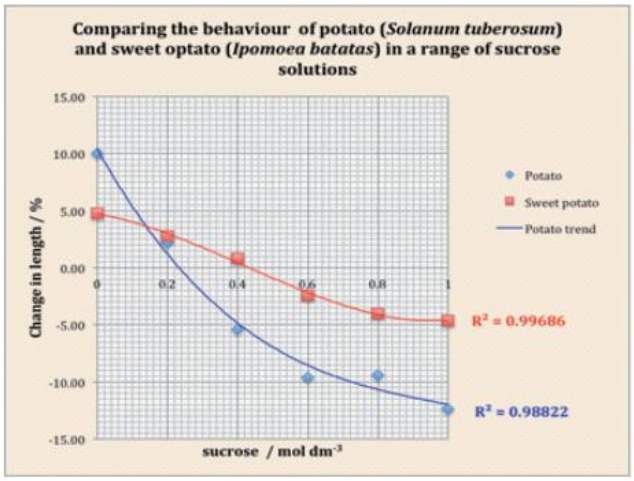



Table to show isotonic sucrose solution found with various plant tissues:

| Tissue | Isotonic Sucrose Solution /mol $dm^{-3}$ |
|---|---|
| Potato | 0.19 |
| Apple (Granny Smith) | 0.35 - 0.55 |
| Apple (Golden Delicious) | 0.53 |
| Celeriac | 0.32 |
| Sweet potato | 0.50 |
| Taro or Dasheen | 0.20 |
| Turnip | 0.35 - 0.45 |

## Evaluation of the three methods

- The gravimetric method is the easiest to carry out and produces results that are least likely to be affected by experimental error. The pieces of tissue, however, must be blotted dry to remove surplus liquid.
- The method involving a change in dimension is likely to produce errors due to unequal cutting or inaccurate measurement of the lengths of the pieces of tissue. The tissues used must be homogeneous in texture to ensure even expansion or shrinkage. Preparing the tissues is time consuming.
- The method involving a change in density (Chardokov's method) is rapid and easy to perform but the results are not always clear. They are less quantitative. A series of solutions with smaller increments would be necessary to pinpoint the isotonic solution.

## Some points for consideration

- An increase in length or mass indicates that the cells in the tissue absorbed water by diffusion to become more turgid. The cells are in a solution that has a higher water potential than their cell sap (hypotonic). If the drop of methylene blue rises, the liquid bathing the tissue has become denser and water has been absorbed by the tissue.
- A decrease in length or mass indicates the opposite. The cells have become flaccid and may even be plasmolysed. These cells are in a solution which has a lower water potential than their cell sap (hypertonic). If the drop of methylene blue falls, the liquid bathing the tissues has become less dense and water has been lost by the tissues.
- In an isotonic solution the strips remain the same length or mass because there is no movement of water in or out of the cells. The drop of methylene blue will stay at the same level. The solution surrounding the cells has the same water potential as the cell sap.
- In the isotonic solutions no changes in length or mass will be seen and the drop of methylene blue will stay at the same level. The isotonic solution for the length and mass could also be found if the results are analysed graphically (sucrose concentration against change in length or mass), the isotonic solution for the tissue will be the point where the curve is equivalent to the original length or mass.

## Other changes

- When placing the tissues in the solution, the pieces of potato tend to float in the denser sucrose solutions with a lower water potential. They sink in the solutions with a higher water potential.
- The tissues become flaccid in the solutions with a lower water potential and firmer (turgid) in the solutions which have a higher water potential. The degree of flexibility could be used as a measure of water uptake, or water loss.

# 1C MEMBRANE INTEGRITY IN CABBAGE LEAF CELLS

Time 1.5 hours

## Syllabus reference: Topic 1.4

**Aim**: Dialysis tubing experiments can act as a model of membrane action. Experiments with potato, beetroot or single-celled algae can be used to investigate real membranes.

**Required knowledge:** The chemical composition of cell membranes. The chemical properties of lipids and proteins.

## Materials

1. Raw beetroot can be used instead of raw red cabbage. The cylinders of beetroot must then be cut into discs about 3mm thick. This will make them more uniform than the red cabbage discs but the preparation is longer. Beetroot also has the advantage of producing more intense colour. Other plant tissues containing anthocyanins could be tried.
2. To cut the discs, a cork borer should be used of a diameter a bit smaller than the test tubes.
3. To wash the discs, they are best placed in a sieve and dipped in a bowl of water. Change the water periodically until it stops changing colour (about 3 hours). Discs can be washed the day before and left in soak over night. If they must be left over the weekend then leave them soaking in a refrigerator.

## Method

The leaf discs tend to float in water. So when the students are heating them in the water bath make sure that the water in the water bath is high enough to cover the leaf discs in the tubes. The water level will drop as the tubes are successively removed.

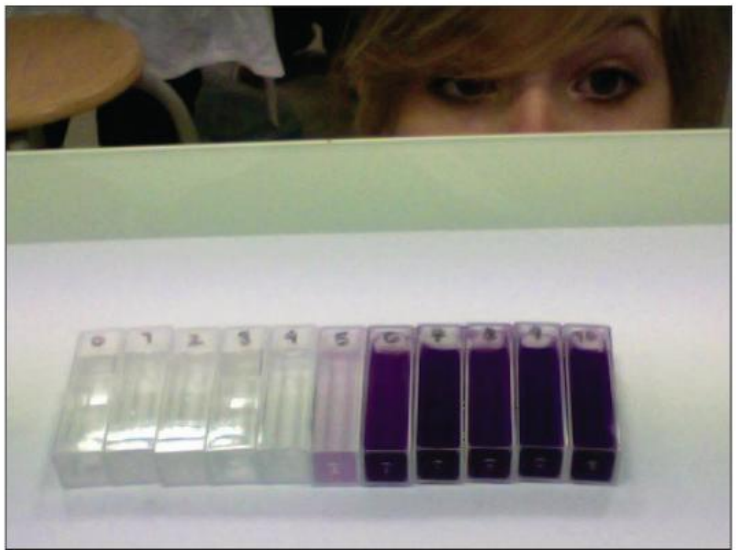
To make qualitative observations the colour depth of the tubes the students should place them, altogether, in front of a white piece of paper, in a uniformly illuminated part of their bench. Photographs should be taken.

## Results

### Observation and explanation

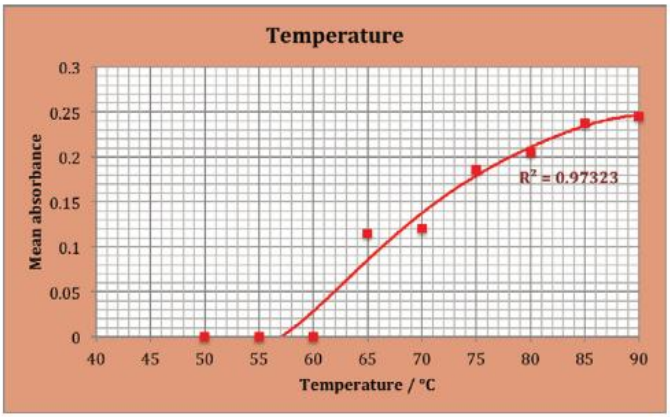
No leakage at 40°C. Slight leakage of pigment above 55°C. Maximum leakage from 65°C. The membranes are damaged by high temperatures. The proteins of the cell membranes are being denatured.

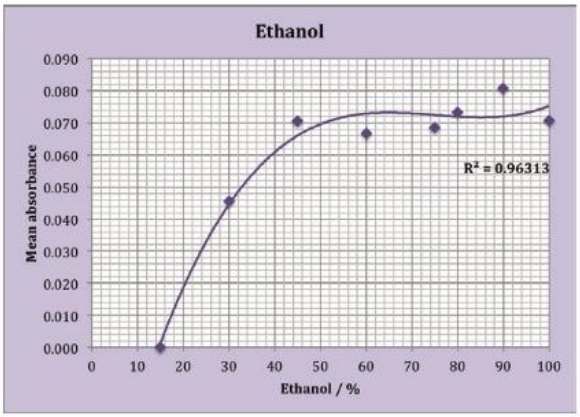
Ethanol dissolves the phospholipid bilayer and precipitates proteins. In ethanol the pigment leaks and gathers at the bottom of the tube.



*A series of colorimeter cuvettes showing the leakage over the range of temperatures.*

**The effect of temperature and ethanol on the permeability of pigment from leaf epidermal cells of red cabbage (*Brassica oleracea var. capitata f. rubra*). Absorbance at 565nm**




## To investigate further

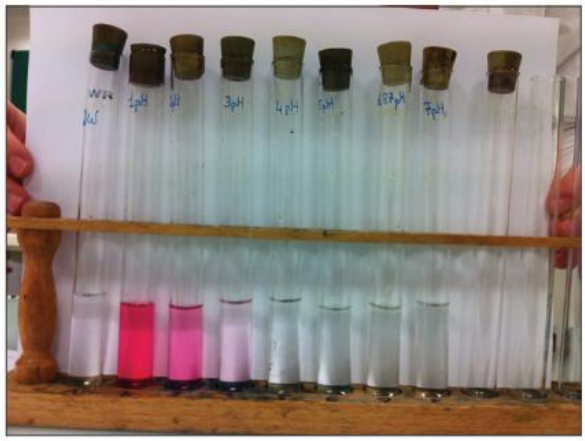
- A range of buffers at different pH values can be tried

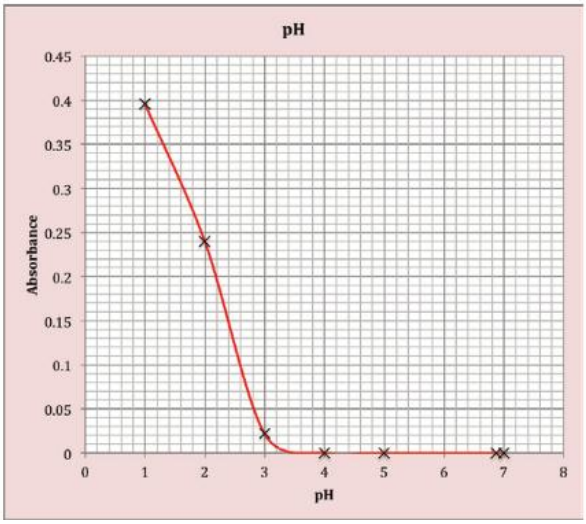
In buffers below pH7 the pigment leaks and changes colour: from purple to bright red. A colorimeter set on a green or yellow light would be appropriate.

Above pH 7 the colour of the pigment becomes yellow and a different colour should be used in the colorimeter.

At pH 1 extreme pH damages the cell membranes, they leak freely. The proteins of the cell membranes are denatured. This pigment (anthocyanin) is also a natural pH indicator.

- Other organic solvents: e.g. propanone (acetone). Unfortunately acetone dissolves the plastic of plastic colorimeter cuvettes, glass ones will be needed. Benzene, toluene (methyl benzene) or aniline (phenylamine) should be avoided because of their carcinogenic nature.
- Alcohols have an increasing effect on membrane permeability as their polarity decreases. The series methanol, ethanol, propan-1-ol and butan-1-ol can be tried.
- The effect of freezing and thawing the leaf discs.





# 1D THE CELL CYCLE IN PLANT TISSUES

Time: 1 hour

## Syllabus reference: Topic 1.6

**Skill**: Identification of phases of mitosis in cells viewed with a microscope or in a micrograph.

**Skill**: Determination of a mitotic index from a micrograph.

## Syllabus reference: Topic 3.2

**Aim**: Staining root tip squashes and microscope examination of chromosomes is recommended but not obligatory.

## Materials

Prepared slides of root tips all from the same species of plant.

If computers linked to internet are available, a shared class bulletin board can be set up which the students can access. A pre-prepared spread sheet will show data collection in real time.

A spreadsheet can be set up in Google Drive or in Dropbox. For Google Drive, it is useful if the students all have gmail addresses, this is not necessary in Dropbox.

Other online share sites exist in the Cloud, most have a small volume starter pack that is free.

## Method

Systematic sampling is best. This can be discussed with the students after an initial observation of the slide. Either all the cells in one field of view, or as the cells come in lines, counting along lines in a field of view. This way the students will not be 'attracted' to cells with mitotic figures inside.

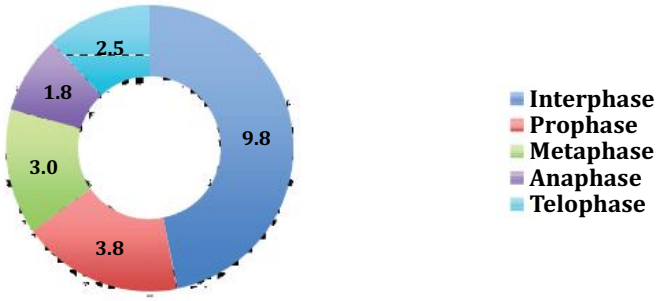
## Data

Data from a class observing prepared slides of onion root tips (*Allium cepa*). Where research online indicated the average length of the cell cycle was 21h.

| Stage | Number of Cells per 100 cells | | | | | | | | | | Total | % | 21h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Interphase | 20 | 56 | 35 | 30 | 88 | 48 | 48 | 37 | 51 | 68 | 481 | 46.9 | 9.8 |
| Prophase | 24 | 8 | 15 | 35 | 5 | 32 | 30 | 17 | 16 | 6 | 188 | 18.3 | 3.8 |
| Metaphase | 20 | 4 | 15 | 25 | 2 | 11 | 8 | 17 | 19 | 24 | 145 | 14.1 | 3.0 |
| Anaphase | 9 | 14 | 18 | 18 | 2 | 6 | 3 | 11 | 9 | 0 | 90 | 8.8 | 1.8 |
| Telophase | 27 | 18 | 17 | 17 | 3 | 3 | 11 | 18 | 6 | 2 | 122 | 11.9 | 2.5 |
| Total | 100 | 100 | 100 | 125 | 100 | 100 | 100 | 100 | 101 | 100 | 1026 | 100 | |

The above data presented as a ring chart as follows overpage:

**The phases of the cell cycle in onion *(Allium cepa)* root tips. Figures show estimated duration in hours.**




The **Mitotic Index** for these cells = 545/1026 = 0.53 or 53%

Problems encountered are mainly the identification of the different stages. For example, distinguishing between Interphase and Prophase. This will influence the calculation of the mitotic index.

### Using freshly prepared root tip cells

It is possible to carry out the same exercise on freshly prepared slides of root tip squashes. The following method is recommended.

## Materials

A microscope eyepiece with a pointer is a very useful instrument for this practical.

**Fixative**: 99 parts of 70% ethanol to 1 part of pure ethanoic acid.

### Stains for chromosomes include

- **Acetocarmine stain:** Add 1g of carmine stain to 45cm$^3$ of pure ethanoic acid. Mix and add 55cm$^3$ of distilled water. Boil, cool and filter.
- **Toluidine blue:** 0.05% aqueous
- **Iodine green:** 1% aqueous
- **Acetic orcein:** Dissolve 3.3g of orcein in 100cm$^3$ of pure ethanoic acid. Reflux for six hours in a water-cooled condenser. Filter to obtain a stock solution. For use in staining, dilute 10cm$^3$ of stock solution with 12cm$^3$ of distilled water.

*Warning: Care should be taken whenever using reagents that react specifically with nucleic acids. Avoid contact with the skin, wear gloves.*


## Method

Grow the roots to 1.5 cm long, cut off the last 0.5 cm from the growing tip and fix them in a mixture of ethanol and ethanoic acid (fixative) for at least two hours.

The development of the root tips is very important. The root should not be longer than 1.5cm. The length of time for the bulbs to sprout roots seems to vary a lot, depending on the variety of the bulb and the condition that it is in. Furthermore, some suppliers of onions and garlic seem to be using inhibitors to prevent the bulbs from producing roots. If roots have not appeared in three days, throw them away and try another source. The author has found garlic bulbs are more reliable than onion bulbs.

Other bulbs, such as shallot, tulip and hyacinth can be tried, but hyacinth is known to provoke allergies. Suspend the bulb over a beaker of water so that the bottom of the bulb is just touching the water. The roots should start growing in 24 hours. Garlic will produce roots of the desired length in 2 to 3 days. One bulb will produce more than enough roots for a class, but to be sure of success, two or three bulbs should be prepared.

The root tips can be cut off using scissors and dropped into freshly prepared fixative. They should be left, soaking in the fixative in a stoppered flask, for at least two hours. Once they are fixed, the root tips can be kept for several days, or even weeks, in the refrigerator.

Before observation, the root tips are hydrolysed to soften them in 1 mol hydrochloric acid for 6 to 7 minutes at 60°C. The acid is pipetted off and distilled water added to rinse the root tips. Do this twice. The root tips are then poured out into a Petri dish placed on the square of black paper. This helps to see the root tips easily.

The fixing and the hydrolysis in the acid make the root tips semi-transparent. The area of dividing cells can be observed as a dense yellow-green patch near the end of the root tip.

To increase the chances of success several slides can be prepared at once. It is better not to put more than one or two root tips on each slide. Too much tissue produces uneven staining of the cells.

One root tip is placed on a clean slide and one or two drops of stain added. This should be done on the piece of black paper. The root tip tissues are teased apart using two needles, then covered with a coverslip. The coverslip is tapped with the handle of the needle to spread the tissues out underneath.


*Warning: Wear gloves whilst staining nuclei*

The slide is covered with two layers of filter paper and firmly squashed using the thumb on the coverslip.

*N.B. Pressure should be applied vertically, any side-ways movement of the coverslip will rupture the cells.*

The cells may be observed under medium power on the microscope. The dividing cells are cube-shaped with a relatively large, conspicuous nucleus (as shown above). The colour of the chromosomes will depend upon the stain used. They can then be observed and counted under high power.

## To investigate further

- Mitosis is a dynamic process, a fact which is easily forgotten when looking at these fixed cells. This practical investigation could be accompanied by a film or video showing the process.
- Investigating the cell cycle. It has been suggested that the incidence of mitotic divisions vary during the day. Some sources suggest 01.30 hours is a good time to harvest root tips. Other sources suggest harvesting after 15.00 hours.
- Forcing the bulbs has also been suggested, placing the bulbs in the refrigerator for 24 hours as soon as the roots reach the desired length. Then place the bulbs into an incubator at 30°C for half an hour.
- Using a micrometer eye piece it is possible to count the cells at know distances from the root tip. The mitotic index of the cell sample will fall as the distance increases from the root tip.

## Websites

This process of mitosis is dynamic and viewing it using time-lapse photography of living cells and phase-contrast microscopy reveals this:

Plant cells are large and quite easy to film:
<http://www.youtube.com/watch?v=SlgV_zoHQxE>
Growing root tip marked with fluorescent stain:
<http://www.youtube.com/watch?v=o_dGBDjWW-A>

Animals cells are smaller and so the behaviour of the chromosomes is a little more difficult to observe:
<http://www.youtube.com/watch?v=YJP-egujAm0>
<http://www.youtube.com/watch?v=9A3jZYnzlpQ>
<http://www.youtube.com/watch?v=NVfqzSKa_Bg>

# 2A DIALYSIS

Time 1 hour

## Syllabus reference: Topic 1.4

**Aim**: Dialysis tubing experiments can act as a model of membrane action. Experiments with potato, beetroot or single-celled algae can be used to investigate real membranes.

## Syllabus reference: Topic 2.1

**Skill**: Identification of biochemicals such as sugars, lipids or amino acids from molecular diagrams.

## Materials

5% starch, 5% glucose and 5% sodium chloride. 'Soluble' starch should be avoided, it tends to leak easily, ordinary laundry starch is best.

Dialysing tubing pore size: equivalent to a protein MW 12000.

### Fehling's solution

**Solution 1.** Dissolve 34.6g of hydrated copper (II) sulphate ($CuSO_4$. 5 $H_2O$) in distilled water to give a final volume of 500$cm^{3.}$ Add a drop of concentrated sulphuric acid if the solution remains cloudy.

**Solution 2.** Dissolve 77g of sodium hydroxide together with 175g of sodium potassium tartrate in distilled water to give a final volume of 500$cm^3$.

Keep solution 1 and 2 separately for storage. For use, mix equal volumes of solutions 1 and 2.

Benedict's solution can be used instead of Fehling's solution.

### Benedict's solution

Dissolve 173g of hydrated sodium citrate together with 100g of hydrated sodium carbonate in 800$cm^3$ of warm distilled water. Filter and add distilled water to a final volume of 850cm. Separately, dissolve 17.3g of hydrated copper (II) sulphate in 100$cm^3$ of cold distilled water. Add the copper (II) sulphate solution to the citrate-carbonate solution, stirring constantly. Add distilled water to a final volume of 1$dm^3$.

### Iodine solution

Dissolve 1g potassium iodide in a little water. Mix whilst adding 0.5g iodine crystals. Make up to 100$cm^3$. Store in dark brown bottles.

### Silver nitrate

0.1 mol $dm^{-3}$ $AgNO_3$

## Method

Completely wet the dialysis tubing may help to open it up. There may be problems with leaking dialysing tubing. Insist that the knot in the bottom of the tube is tied tightly but be careful not to tear the tubing with long fingernails. Use enough tubing so that the upper end is well clear of the water (as shown in the diagram on the student's guide).

Make sure that the results for time zero are all negative, though the chlorides may appear extremely quickly. If glucose and/or starch appear in the first samples it would be best to start again.

There should be enough distilled water surrounding the dialysing tubing to last for six samples but warn the students not to take large samples. Furthermore, the samples should not be "drowned" with reagent when being tested.

Tests for substances which have already appeared outside the dialysing tubing can be discontinued.

## Data

| | |
|---|---|
| Chloride | 0 to 5 min |
| Glucose | 10 to 20 min |
| Starch | Should not diffuse out of the tubing. |
| Iodide | Diffuses into the tube quickly, within 5 min the "sausage" of dialysing tubing should be black (*photograph opposite*). |

## Some points for consideration

The bigger the particle is, the slower its rate of diffusion through the dialysing membrane.

N.B. For the sake of simplicity, solutions of 5% concentration have been used, as the tests used are qualitative. If necessary, however, a mixture containing sodium chloride, glucose and starch of the same molarity could be used to give similar results.

**From the additional information**: Starch molecules are too big to diffuse through the pores in the membrane. Therefore, these pores must be less than 17500 nm wide. Glucose just manages to pass through. Therefore, the pores must be greater than 70 nm wide.

The addition of the iodine solution shows that diffusion of different particles can proceed in both directions at once. Diffusion depends upon the concentration gradient of each particle dissolved in the water. Furthermore, adding the iodine solution acts as a control; it shows that the starch is still present, trapped inside the membrane. The direction of the diffusion of a molecule or ion depends upon its relative concentration on either side of the membrane.

Errors may be encountered due to leaking membranes, badly tied knots or insufficient washing of the tube after filling. Beware of sharp rings and fingernails damaging the membrane.

## To investigate further

Other molecules of different sizes could be tried:

- **Fructose, galactose, maltose, lactose.** These are all reducing sugars that will react with Fehlings reagent or Benedicts reagent.
- **Glycogen**. This turns chocolate brown with iodine solution.
- **Protein**. Proteins and peptides turn purple with the biuret test.

# 2B WATER AS A COOLANT

Time: 1 hour

This investigation is a relatively simple and quick one that will give the students experience in data logging.

## Syllabus reference: Topic 2.2

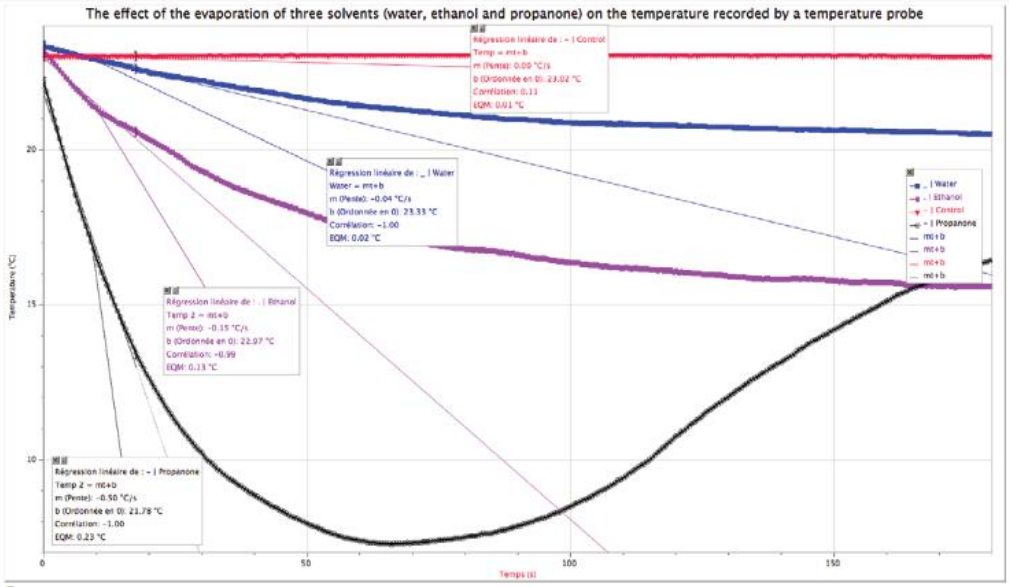
**Application**: Use of water as a coolant in sweat.

## Materials

The material used for the trials is from Vernier but all other data logging systems have temperature probes that could be used.

## Method

Air currents in the room need to be controlled or at least the temperature probes should be too far apart from one another and positioned in the same way, such that both the experimental and control probe are affected to the same degree.




## Data

The graph above shows the cooling effect of the three solvents. The linear analysis is carried out to estimate the initial rate at which the temperature drops over the first 20s.

Water = 0.4 °C $s^{-1}$

Ethanol = 0.15 °C $s^{-1}$

Propanone = 0.50 °C $s^{-1}$

## Some points for consideration

The temperature drop will depend upon the ambient temperature in the room but water should only drop by about 3°C, ethanol by 7 to 8°C and propanone by over 20°C. The propanone curve is interesting in that it rises back up again. This solvent is so volatile that all of it evaporates from the filter paper and the cooling effect is lost.

Water ($H_2O$) is the smallest molecule (MW 18), ethanol ($CH_3CH_2OH$) the next (MW 46) and propanone {$(CH_3)_2CO$} is the largest (MW 58). Curiously in this experiment the larger the molecular mass the faster the rate of evaporation cooling the temperature probes. There must be another reason.

Water has a very high polarity compared to ethanol and propanone and so it forms hydrogen bonds easily. Ethanol's alcohol group (-OH) gives it some polarity so it takes more energy to evaporate ethanol than propanone, which has no -OH group. Propanone's carbonyl group (=O) gives it a weak polarity.

Water is usually abundant in the environment or in the bodies of animals and plants. Having a high polarity it takes a lot of heat energy to evaporate a little water (compared to ethanol for example) so it is quite efficient as a thermal regulator. Ethanol and propanone are toxic to living systems because both are organic solvents that will disrupt cell membranes.

## Adaptations include

**Physiological** e.g. reduced subcutaneous insulation, increased radiation of heat through increased blood flow to body surface, conservation of water released through urine or breath.

**Morphological** e.g. taller and/or slimmer body shape has an increased surface area to volume ratio.

**Behavioural** e.g. searching for shade, inactivity during hot parts of the day or year (aestivation).

# 2C POLYSACCHARIDE MOLECULES

Time: 30 min

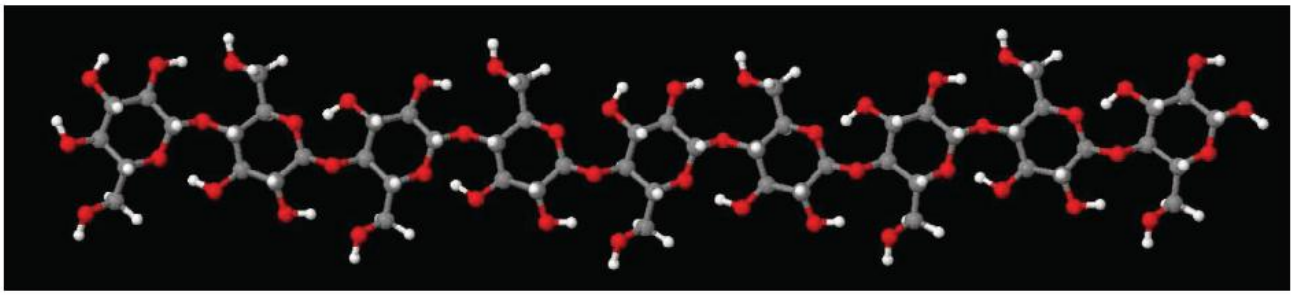
## Syllabus reference: Topic 2.3

**Skill**: Use of molecular visualisation software to compare cellulose, starch and glycogen.

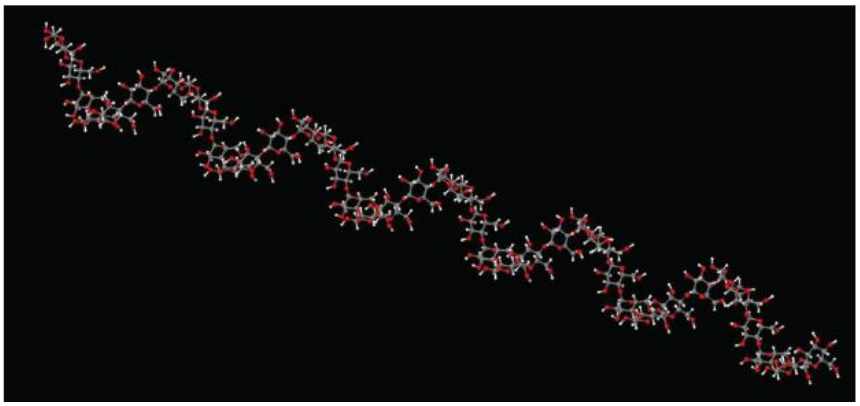
## Materials

The JMol applet will need to be installed on the computer.

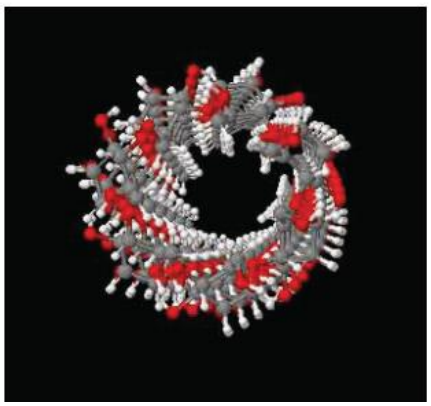

## Images



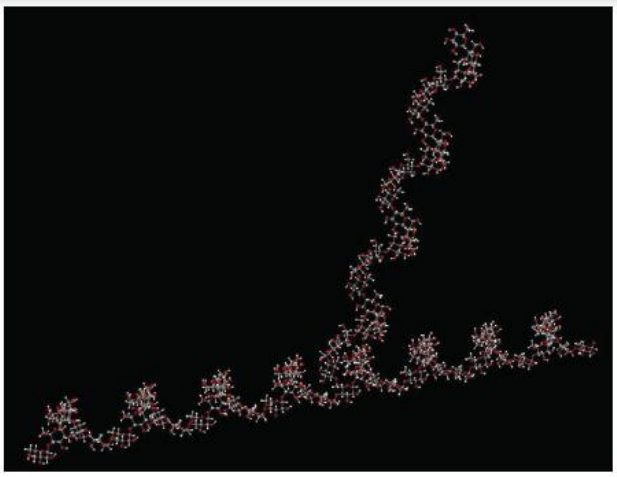
*Cellulose*



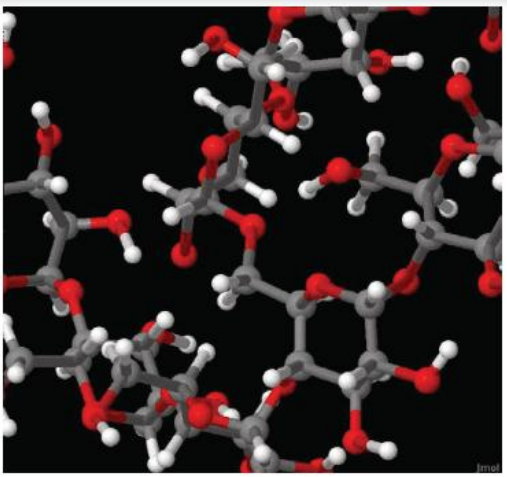
***Amylose*** *Side view*



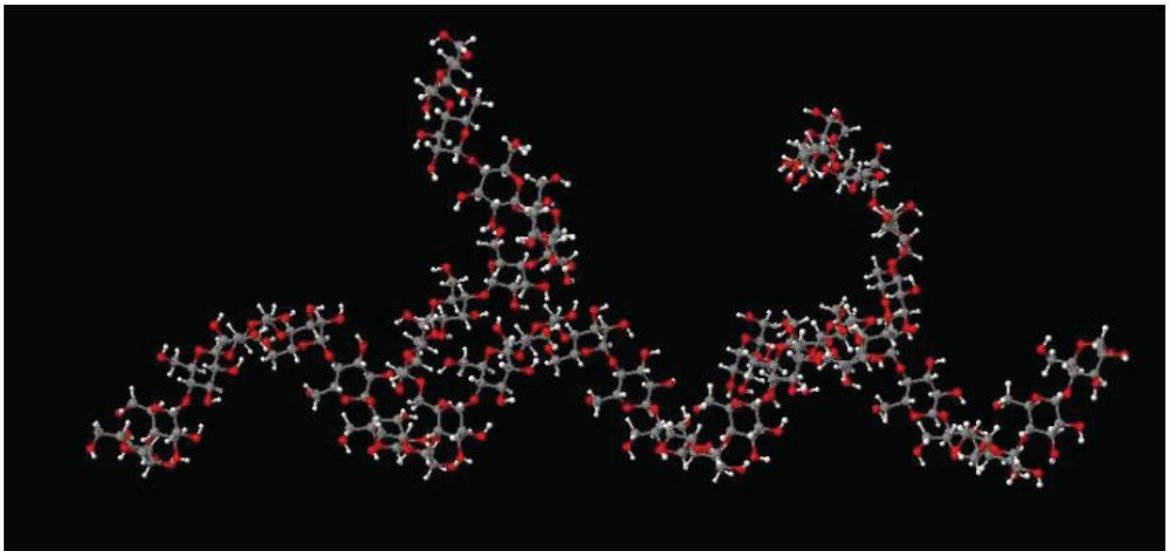
*End view*



*Amylopectin showing its branched structure*



*Close up view of the branch point in amylopectin*



*Glycogen showing it is more highly branched than amylopectin*

## Observations

- All of the molecules shown are made of glucose monomer units.
- Cellulose and amylose are unbranched molecules.
- Amylopectin has a branch in it (in fact the complete molecule is highly branched and much bigger, 2 000 to 200 000 glucose monomers), glycogen has more branches (again the complete molecule is bigger, 2000 to 600 000 glucose monomers, very highly branched).

## Some points for consideration

- Glycogen and the starch are used as energy stores. They are easily built up from glucose monomers and broken down again when needed. The more branched the molecule is the more compact the storage. So animals, being motile, use the branched glycogen molecule as an energy store that is more compact.
- Weight for weight, fats contain twice the energy of polysaccharides. Animals being motile need an energy store that is compact and light. The same is true for the seeds of many plants. They often use oils as an energy store for the future seedling, as fats are less bulky than carbohydrate making dispersal easier.
- The simple sugars are soluble in water. A build up of these in membrane bound structures would create osmotic problems. Polysaccharides, being insoluble, can be stored in large quantities without osmotic problems.
- Cellulose is a long unbranched chain of glucose monomer units. The image here only shows a short chain but a cellulose molecule is usually made of 2 000 -14 000 glucose monomers. They align themselves into microfibrils held together by hydrogen bonds.

# 2D MEASURING THE RATE OF REACTION OF AN ENZYME CONTROLLED REACTION

Time 1.5 hours

## Syllabus reference: Topic 2.5

**Skill**: Experimental investigation of a factor affecting enzyme activity. (Practical 3)

## Materials

Small kitchen blenders are very useful as they can homogenise a sample of tissue rapidly.

Hydrogen peroxide concentrations are measured in a number of different ways. The term 'volumes' is used here.

**To covert:**

10 vol = 3.03 % = 0.83 mol $dm^{-3}$

Sterile water: Any bottled mineral water can be used. However if sterile water needs to be made, place distilled water heater in an autoclave to 120°C for 20 min.

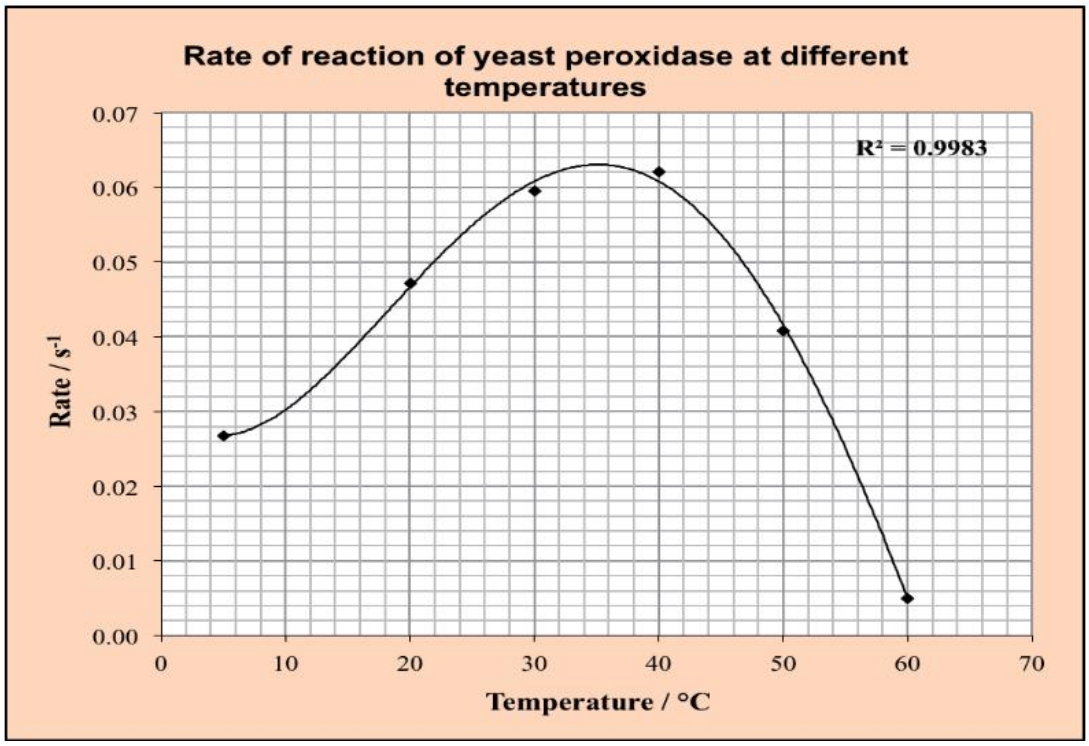
## Method A: Using flotation to measure enzyme activity

This method could be adapted to investigate the influence of all the factors that affect the activity of catalase.

## Materials

Large test tubes or 100 ml measuring cylinders are useful reaction chambers.

### Example data



# Method B: Using foam production to measure the enzyme activity

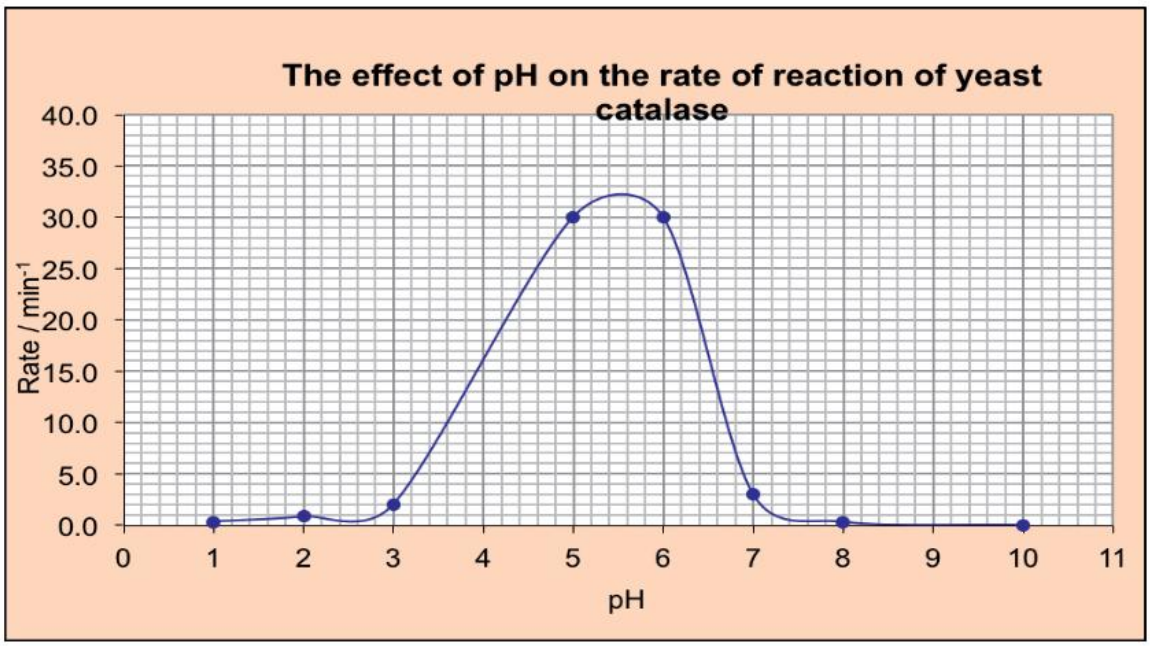
This method could be used for all factors that influence enzyme activity, except temperature.

## Materials

A web cam or even the camera on a portable computer can be used to record the rate. To avoid parallax error, the camera needs to be positioned with care in front of the measuring cylinder at the level of the meniscus.

The measuring cylinder needs to be sufficiently large to be able to distinguish the graduations on it clearly.

### Example data

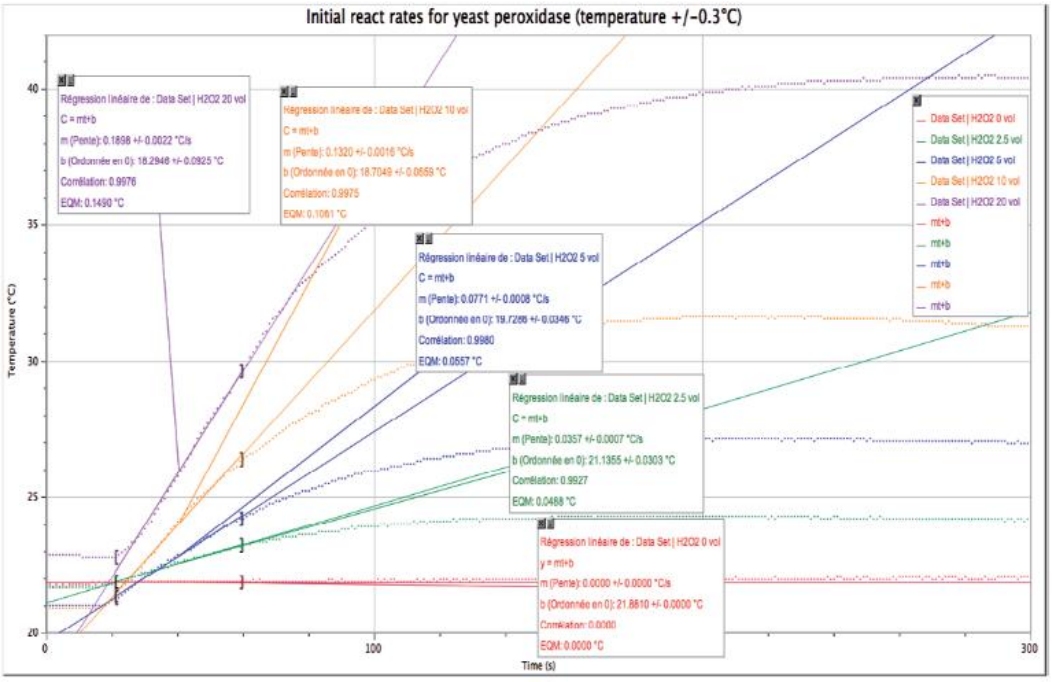



# Methods C and D: Using the heat released as an indicator of the reaction

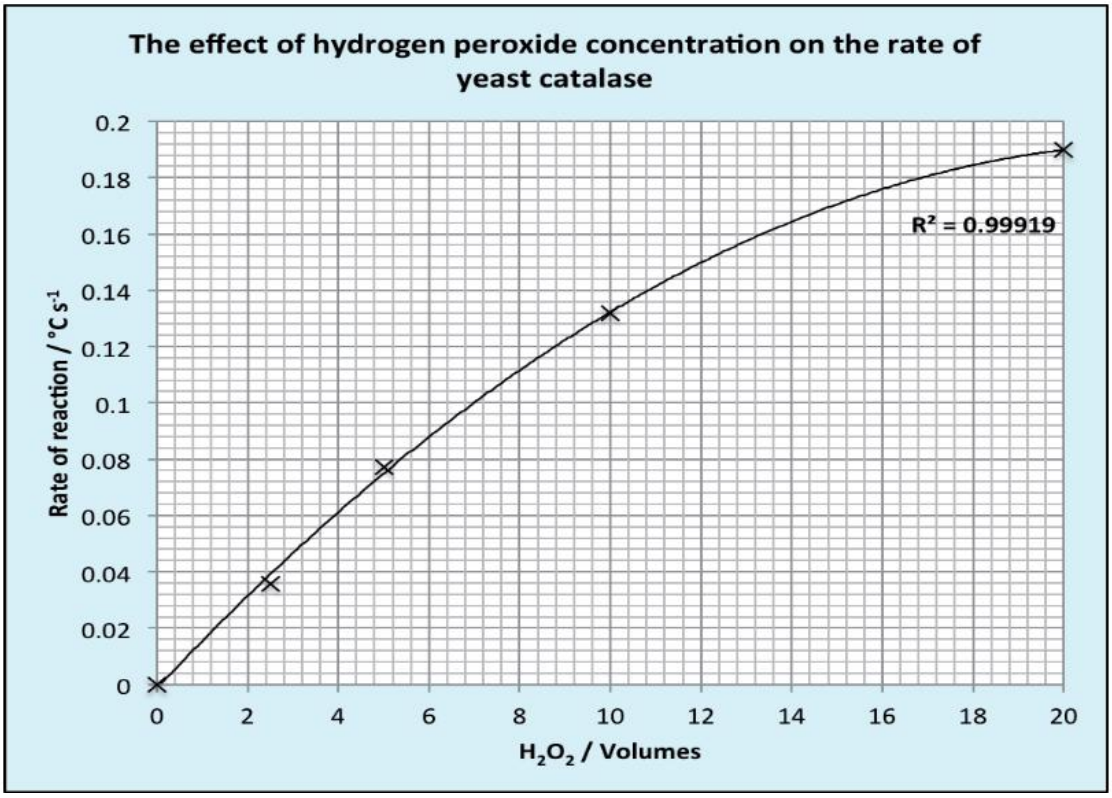
Temperature would be a difficult factor to investigate using these methods. At low temperatures the exothermic nature of the reaction itself would raise the temperature of the reaction mixture. At high temperatures the rise in temperature due to the reaction would not be recorded.

## Example data

Calculating the initial reaction rates from the raw data.

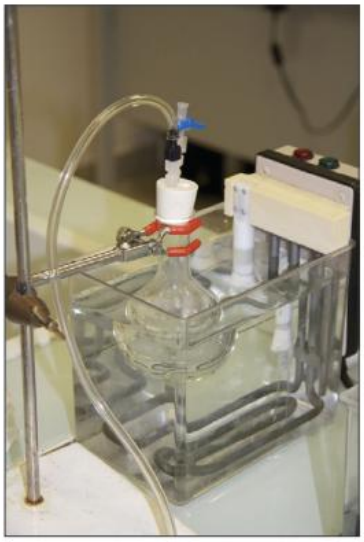



Plotting the reaction rates against the concentration of hydrogen peroxide approximates to the typical Michaelis-Menten curve.



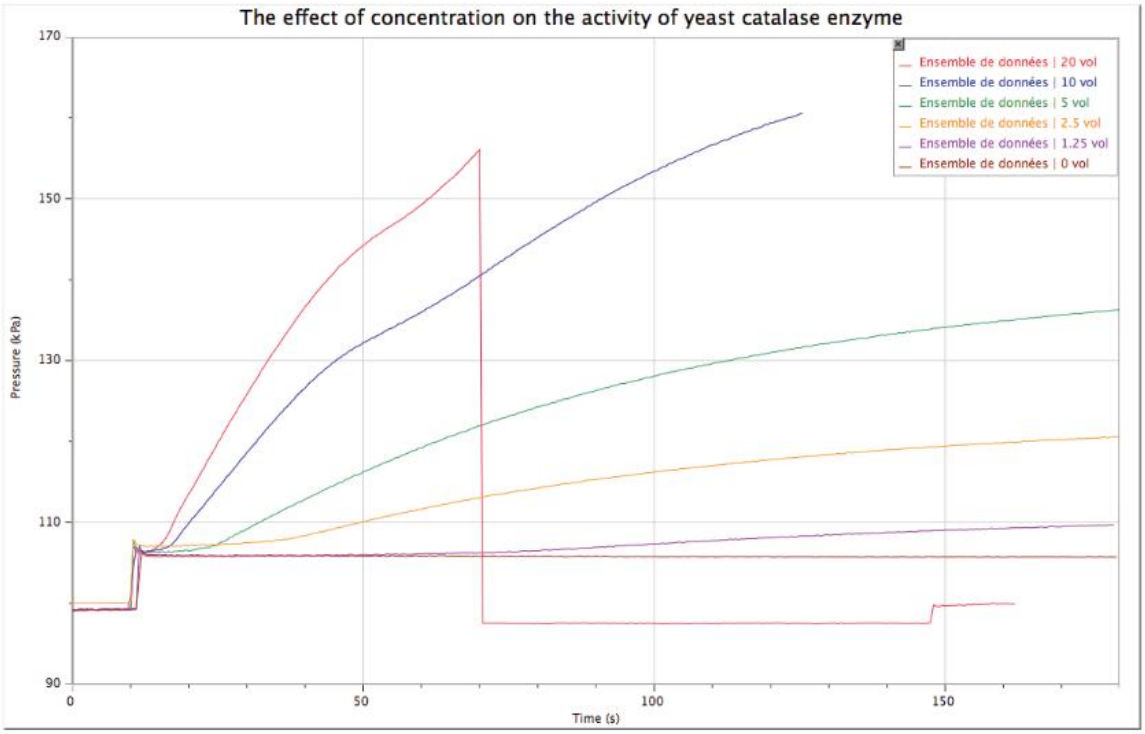
# Method E: Using data logging and a pressure sensor

It is worth having the reaction chamber in a water bath to reduce fluctuations in the ambient temperature.

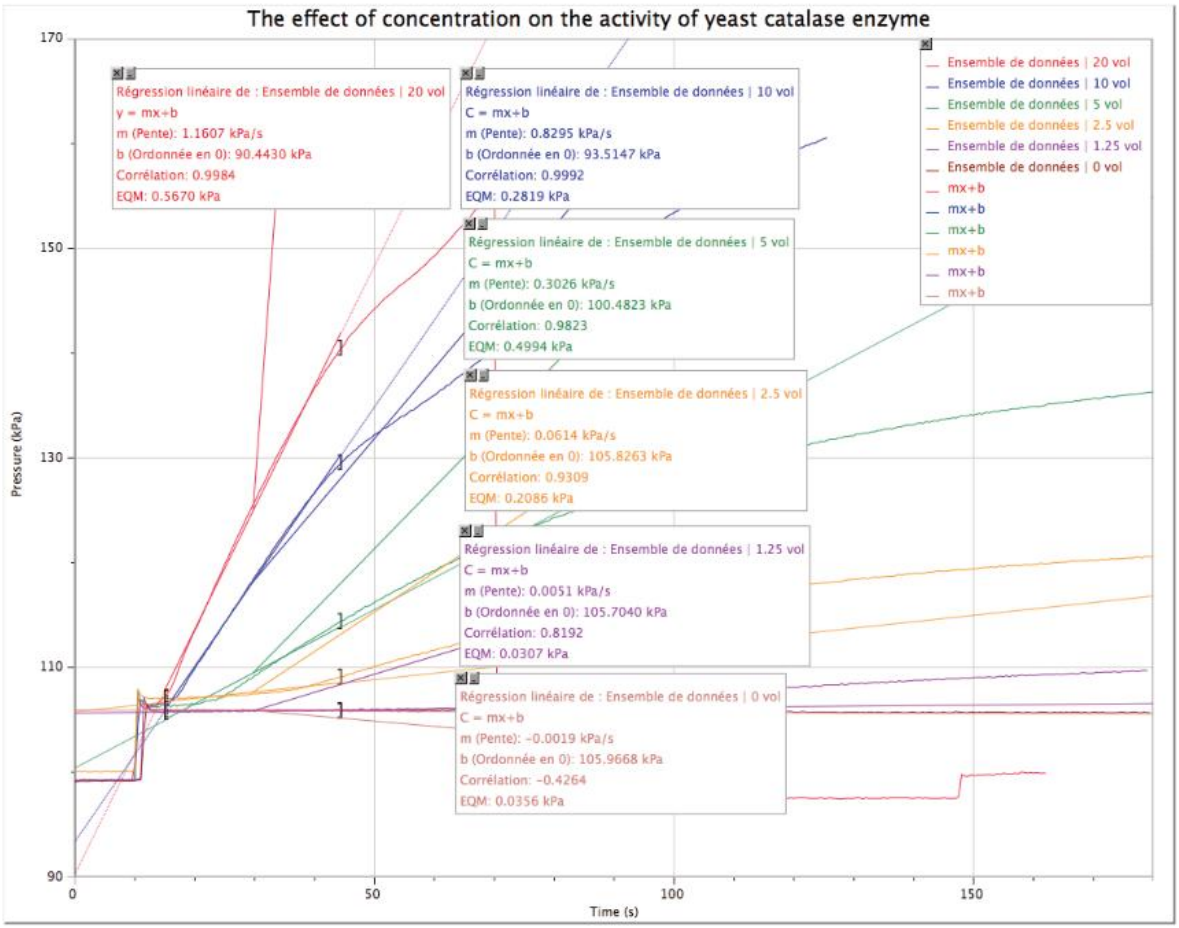


## Example data

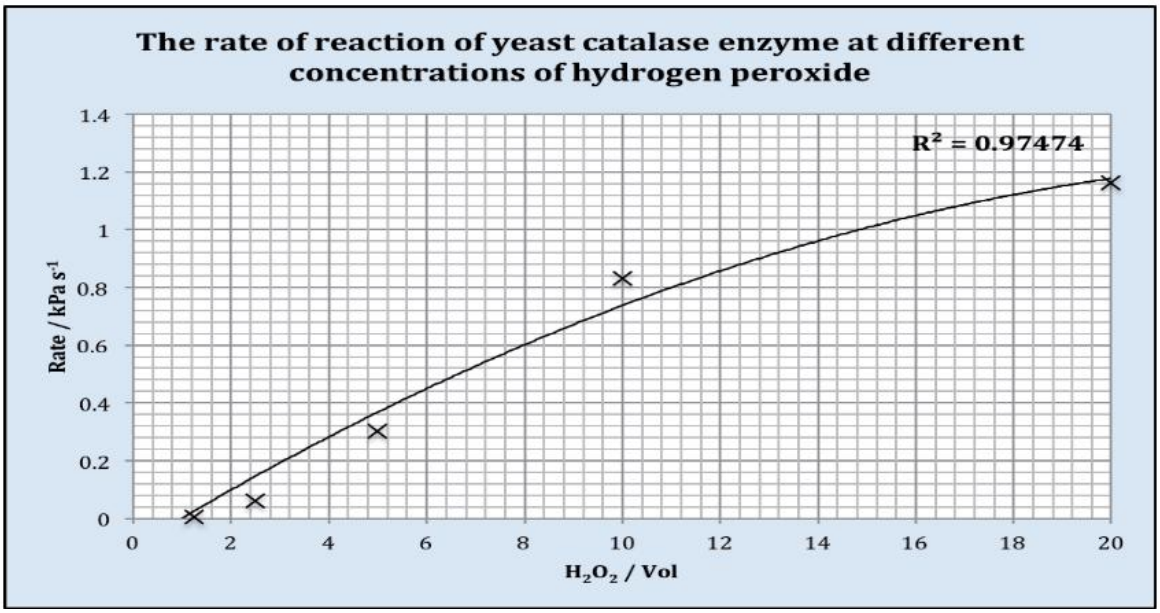
The sudden drop for the 20 vol solution is where the bung popped off the flask under the excess pressure. Nevertheless, the initial reaction rate can still be determined.



Using linear plot, the initial reaction rates are calculated.




Plotting the reaction rates against the concentration of hydrogen peroxide, the curve approaches the expected Michaelis-Menten curve.



# 2E USING SODIUM ALGINATE TO IMMOBILISE YEAST ENZYMES

Time: 2 hours

## Syllabus reference: Topic 2.5

**Understandings**: Immobilised enzymes are widely used in industry.

## Syllabus reference: Topic 2.8

**Application**: Use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide in baking.

**Required knowledge:** A basic understanding of the properties of enzymes, a knowledge of the different types of microbes available is useful.

## Materials

Fresh yeast works best. Dried yeast needs to be mixed with water first to allow it to rehydrate. Otherwise, it forms lumps in the alginate.

The number of glucose strips that are consumed can be reduced by cutting the plastic strips lengthwise.

Adding a needle to the syringe can make pellets of different size. Investigate surface area to volume effect.

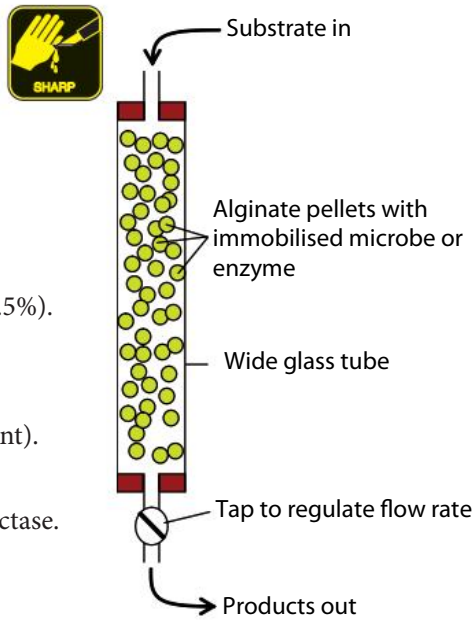
*Warning: Sharp instrument, use with care*

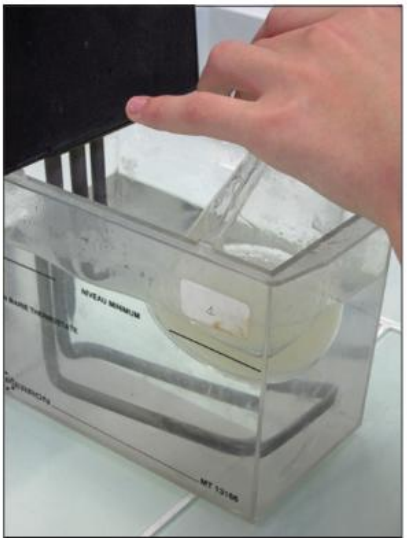
## Method

Large-scale production of pellets can be made using a burette.

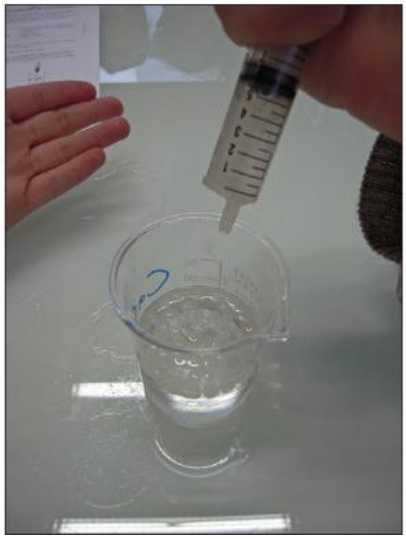
## To investigate further

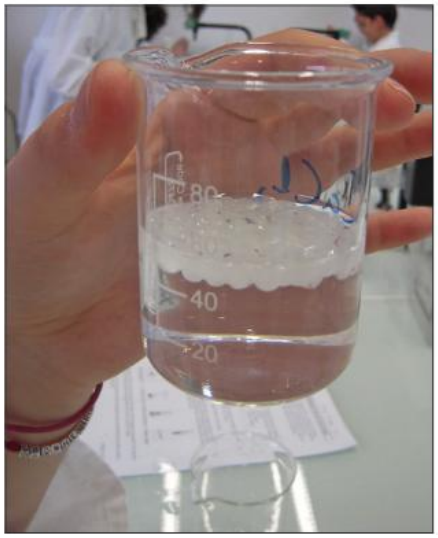
- Compare pellets of different sizes from different nozzle diameters.
- Investigate surface area to volume ratio effect.
- Compare pellets of different concentrations of alginate (try 1% and 0.5%).
- Compare different concentrations of yeast suspension.
- Compare yeast immobilised in pellets with yeast free in suspension.
- Try algae-alginate for nitrate or phosphate absorption (water treatment).
- Try homogenised liver for catalase activity.
- Try enzymes instead of yeast cells e.g. amylase, sucrase (invertase), lactase.
- Set up pellet columns with through-flow of substrate. (*See opposite*)



*Mixing alginate and yeast*





*Producing pellets by flocculating alginate in calcium chloride solution*

# 2F RESPIRATION RATES OF INVERTEBRATES

Time: 1.5 hours

## Syllabus reference: Topic 2.8

**Skill:** Analysis of results from experiments involving measurement of respiration rates in germinating seeds or invertebrates using a respirometer.

## Materials

**Soda lime:** Calcium oxide with sodium hydroxide.

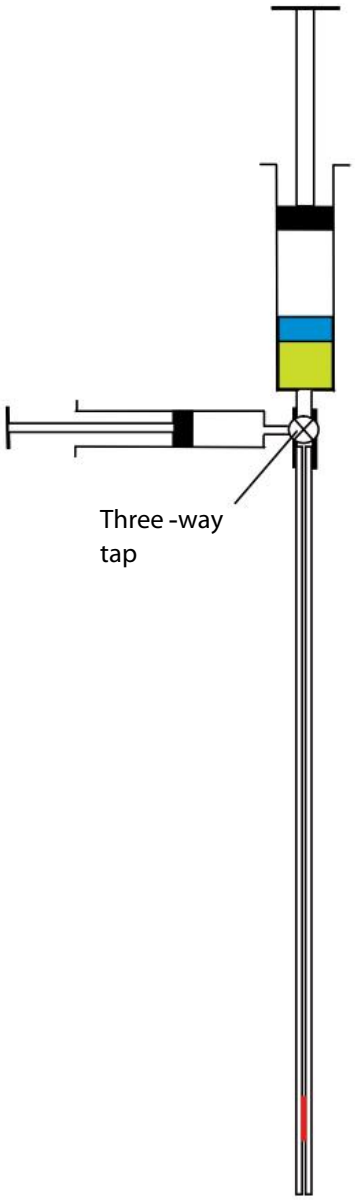
Sodium hydroxide or potassium hydroxide could be used instead of soda lime. However, these compounds are hygroscopic and very caustic, especially in their solid form, and therefore, present an additional safety risk.

The piece of **sponge** should be carefully cut so that it is not so loose that the animals can burrow through or around it, nor too tight so that it prevents airflow.

A **three-way tap** can be introduced into the plastic tubing to "start" and "stop" the experiment when desired. A second, smaller, syringe can be attached to this tap to push the coloured liquid back down the capillary tube.

The **capillary tubing** needs to be kept clean. A build up of lime scale in hard water regions can lead to irregular results, the liquid does not flow smoothly in the tube.

Ensure that the **animals** are fresh and in good health.




## Method

A respirometer without the mealworms permits the calculation of any change in volume due to other factors such as temperature or air pressure. It is properly called a **thermobarometer**. The apparatus without animals is a suitable control. The result of the control respirometer should be added or subtracted from the results for the respirometer that contains the mealworms.

After 10 to 15min the apparatus may fail to give further results. It is possible that the animals are no longer respiring aerobically, or their metabolism is slowing down. Changing the animals for fresh specimens can solve this problem.

## Data

Average readings of about 12mm$^3$ $O_2$ g$^{-1}$ min$^{-1}$ have been observed for mealworms. Variations in results can be quite large however. Example results obtained in a class are given below:

| Mass of meal worms / g ±0.01g | Movement of bubble / mm min$^{-1}$ ±0.05 mm min$^{-1}$ | Volume of oxygen absorbed / mm$^3$ g$^{-1}$ min$^{-1}$ |
|---|---|---|
| 3.28 | 60.0 | 14.34 |
| 5.00 | 93.5 | 14.70 |
| 3.16 | 76.5 | 19.06 |
| 3.04 | 36.5 | 9.40 |
| 3.54 | 36.5 | 8.05 |

These results are corrected for thermo-barometric fluctuations. They were taken using a 1mm bore capillary tube and the students were all working on the same batch of insects.

### Alternative material

A data logger can be used with gaseous $CO_2$ or $O_2$ probes. Though they are expensive, they do extend the possibilities with additional safety.

## Some points for consideration

- If this apparatus were used to measure the rate of respiration of a green plant it would need to be kept in the dark to inhibit photosynthesis. Photosynthesis produces oxygen and absorbs carbon dioxide.
- If the metabolism of the animal drops there may be no change recorded. If the animal's metabolism increases the rate of oxygen consumption will increase.
- If the organisms undergo anaerobic metabolism, then no movement will be recorded in the capillary tube.

## Research

- The Respiratory Quotient (RQ) is the ratio of the amount of carbon dioxide given out per unit time divided by the amount of oxygen taken in per unit time. It allows you to determine the metabolites being used for respiration and to see if the organism is respiring aerobically or anaerobically.
- The above apparatus gives the rates of oxygen consumption only. The RQ could be calculated if it is assumed that only carbohydrates are being consumed.
- Using similar apparatus with animals (but without the soda lime) will permit the measurement of the difference between the volume of oxygen absorbed and the volume of the $CO_2$ produced. From this the Respiratory Quotient can be calculated.
- For carbohydrate consumption, where the amount of oxygen consumed is the same as the amount of carbon dioxide being given out, then the RQ = 1.
- If proteins or fats are metabolised, then the volume of oxygen consumed and the carbon dioxide being given out will differ. RQ for lipids = 0.7 and RQ for proteins is variable but below 1.0.

## To investigate further

- If available, try comparing the respiration rates of mealworm larvae, pupae and adults, other invertebrates (e.g. blowfly larvae, woodlice) or germinating seeds.
- Using the above apparatus for measuring the RQ
- Design an apparatus to overcome the problems of the variation of temperature throughout the experiment.
- Assuming mealworms use only carbohydrate as their respiratory substrate, it may be possible to estimate how much carbohydrate they consume.

### For example

If 14.34$mm^3$ $g^{-1}$ $min^{-1}$ of oxygen is being consumed.

1 mole of oxygen occupies 22.4$dm^3$ at stp

Using the equation for respiration, it is known that 6 moles of oxygen (6 × 22.4$dm^3$) are consumed for every mole (180g) of glucose consumed.

14.34 × 1 / 1000000 = 1.434 ×$10^{-5}$$dm^3$ $g^{-1}$ $min^{-1}$ of oxygen

We know that for every 6 × 22.4$dm^3$ of oxygen 180g of glucose are consumed.

So, for 1.434 × $10^{-5}$$dm^3$ of oxygen(1.434 × $10^{-5}$/134.4) × 180 = 0.0192mg of glucose $g^{-1}$ $min^{-1}$ was used.

# 2G LEAF PIGMENTS, THEIR EXTRACTION AND SEPARATION

Time: 2 hours.

## Syllabus reference: Topic 2.9

**Skill**: Separation of photosynthetic pigments by chromatograph. (Practical 4)

**Required knowledge:** A basic knowledge of photosynthesis, absorption and action spectra.

## Materials

Solvent = 90% petroleum ether + 10% acetone

It is best to use leaves that are dark green and that are not too hard.

Alternative source of leaf material: spinach beet

A large test tube could be used instead of a measuring cylinder.

## Method

As fine a spot of pigment as possible should be made, at the most 3-4mm in diameter.

Thin layer chromatography give results faster and the sport of colour remain more condensed. In paper chromatography the spots do tend to smear on the paper.

## Some points for consideration

- The leaves were ground to release the chlorophyll from the cells and to allow the pigments to dissolve in the alcohol. Some pigments may not be soluble in the alcohol.
- Four pigments are seen with this method. If parsley is used two traces of carotene may be seen.
- The different pigments separate out due to their different molecular masses, the affinity for the solvent and the affinity for the water molecules adsorbed to the paper.
- Some pigments may not be seen, due to being too dilute, or a similar colour to the paper, or have not separated out sufficiently to be observed. In the latter case, two-way paper chromatography could be used.
- Rf values are good if the chromatogram has been set up under the same conditions as those given in the table used. (e.g. same extraction, same solvent, same paper). Colour is misleading, as the way in which colour is perceived and described can vary greatly, but, it gives a simple guide.

## Research

- The leaf is green despite the orange and yellow pigments that it contains. Each pigment absorbs all the wavelengths of light except that corresponding to their colour. There is more chlorophyll present than the other pigments. The chlorophyll masks the other pigments. This is not always the case: e.g. brown seaweeds, red seaweeds and copper beech.
- Plants have different pigments to absorb different parts of visible spectrum and maximise light energy absorbed.

# 2H USING A SPECTROSCOPE TO OBSERVE LEAF PIGMENTS

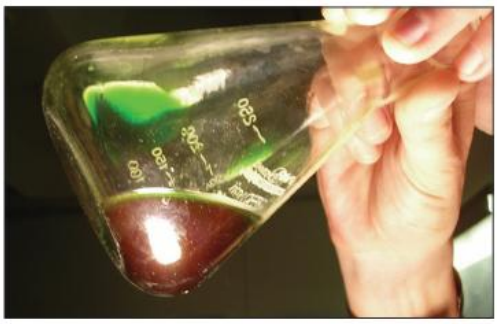
Time: 1 hour

## Syllabus reference: Topic 2.9

**Skill**: Drawing an absorption spectrum for chlorophyll and an action spectrum for photosynthesis.

## Materials

The author has successfully used a diffraction grating of 140 line $mm^{-1}$ others may be suitable. Diffraction gratings greater than 500 line $mm^{-1}$ give wider spectra that are not intense enough for use on an OHP.



**Plant material:** Parsley extract works particularly well for the fluorescence part of the investigation. Spinach may give a turbid solution which hinders the reaction. Different species of plants, or leaves of the same plant in different states, can be compared (as shown in the results from the spectrometer below).

**Fluorescence**: This can be observed with greater intensity if a UV light of 365nm frequency is used.

*Warning: UV light is harmful, wear protective spectacles.*


## Data

### Absorption spectrum

The blue and the red ends of the spectrum are seen to be absorbed by the chlorophyll solution.

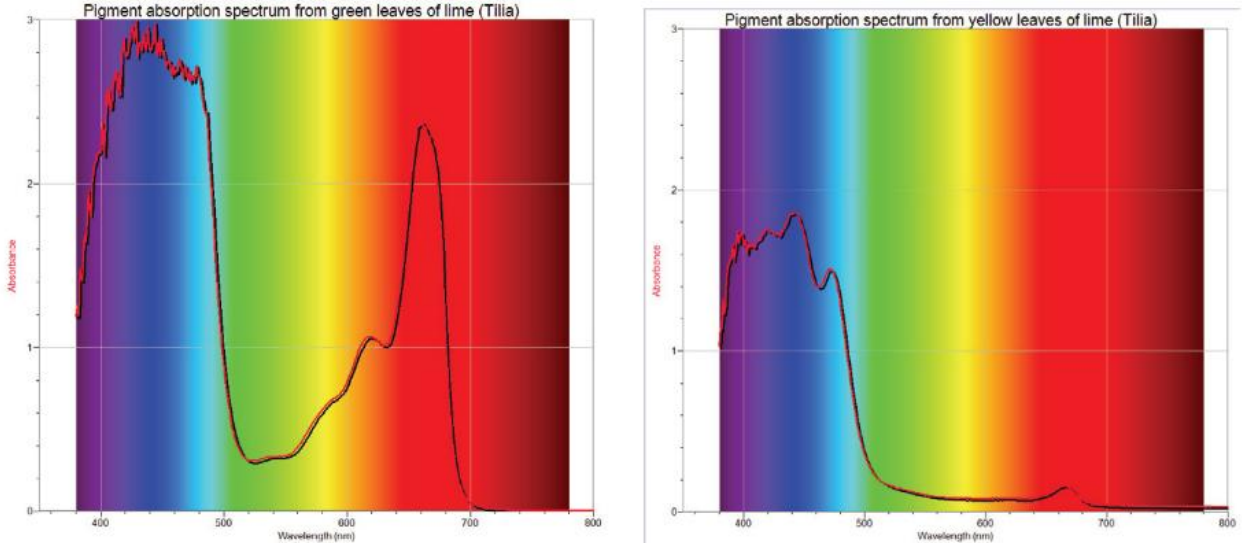
### Fluorescence

Under bright light the chlorophyll extract gives out red light. In the absence of electron acceptors, the electrons are exited out of their orbitals by the light. They fall back to their lowest energy levels, giving out light energy.

## Some points for consideration

The leaf pigments trap light energy. They absorb certain wavelengths (parts of light). This could be called the absorption spectrum.

To see if all parts of light absorbed are used in photosynthesis, different wavelengths of light are used and starch or oxygen production is measured accordingly. This gives the action spectrum.




*Absorption spectra of autumnal leaves of lime (Tilia)*

# 2I METHODS TO MEASURE THE RATE OF PHOTOSYNTHESIS

Time 1.5 hours

## Syllabus reference: Topic 2.9

**Skill**: Design of experiments to investigate the effect of limiting factors on photosynthesis.

### Materials

**Water enriched with carbon dioxide:** Diluted carbonated water can be used if the concentration of the dissolved carbon dioxide is not critical. Alternatively, a 0.1 mol $dm^{-3}$ solution of $NaHCO_3$ can be used. A serial dilution of this would produce an independent variable for dissolved carbon dioxide.

**Lamp with 60 Watt bulb:** A lamp with LEDs is best, they give the same intensity of light but they do not give out a lot of heat energy so the temperature can be controlled more easily.

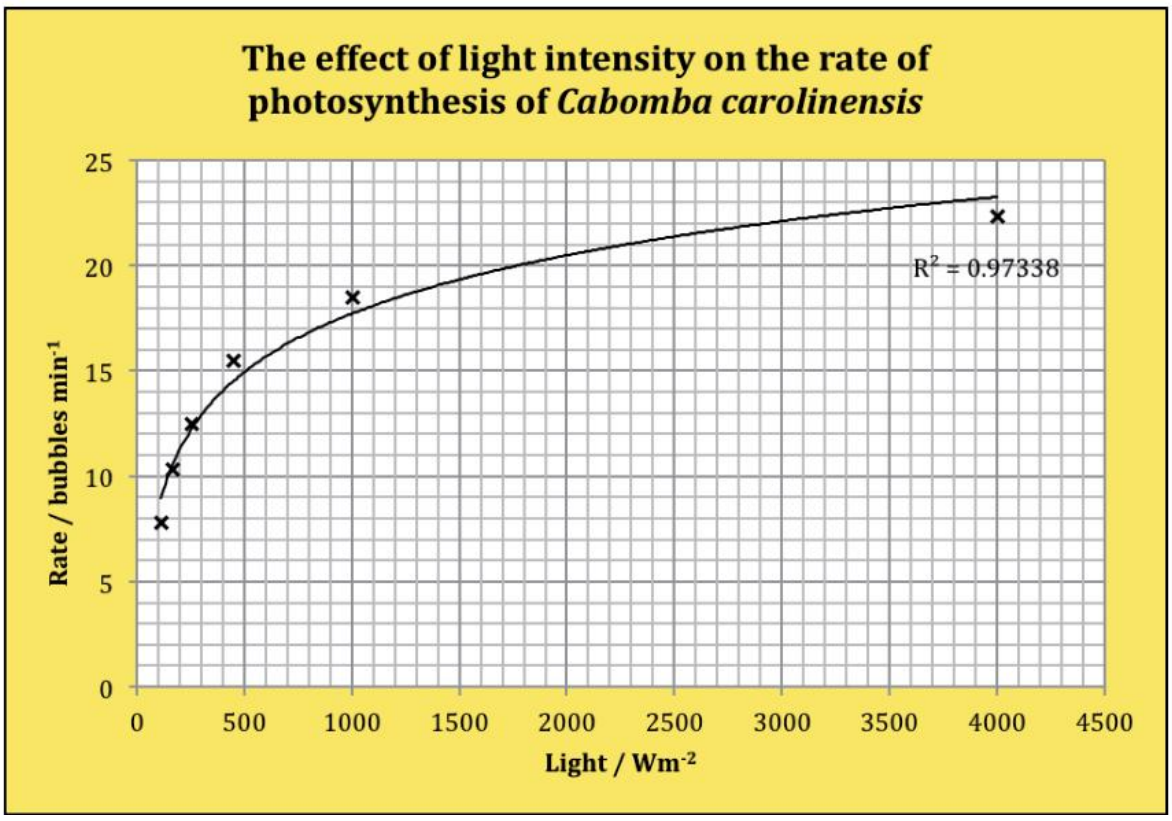
**Water plant:** Many species are possible, *Elodea, Cabomba, Mryiophyllum*. However, a number of these species are regarded as invasive aliens in certain parts of the world suitable alternatives should be used.

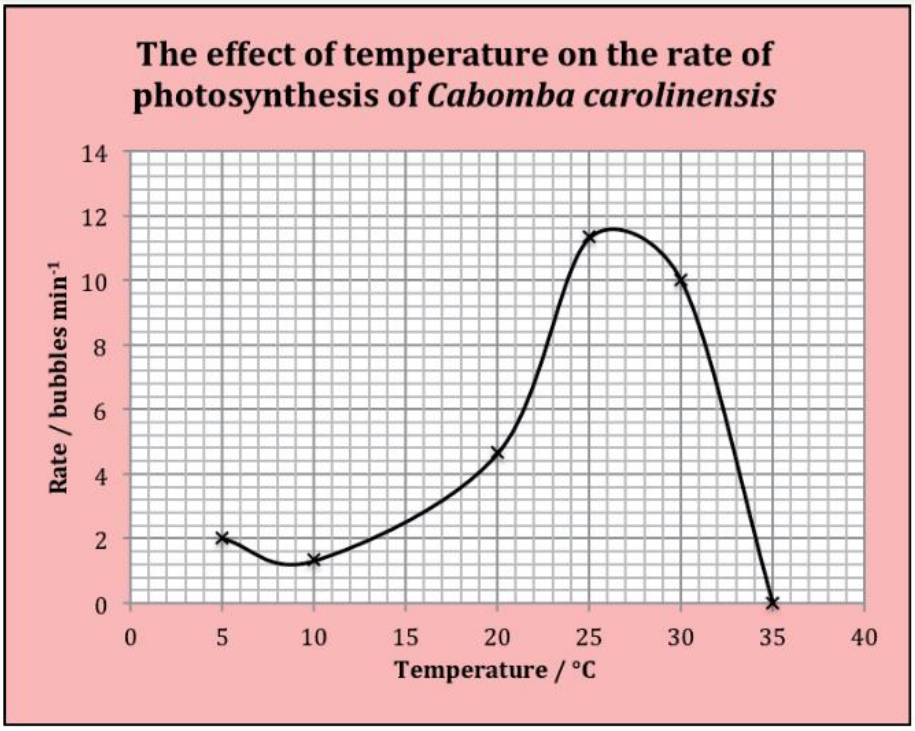
Using a water bath to control temperature is best but it may be possible for the plant to operate at room temperature. Nevertheless the room temperature should be monitored to see if it varies significantly, especially if a bench lamp is close to the apparatus.

## Method A: Determining oxygen production in a water plant directly

This could be used to explore the effects of dissolved carbon dioxide, light intensity, light wavelength and, with a water bath, temperature.

### Example Data






## Method B: Determining oxygen production by the displacement of a liquid

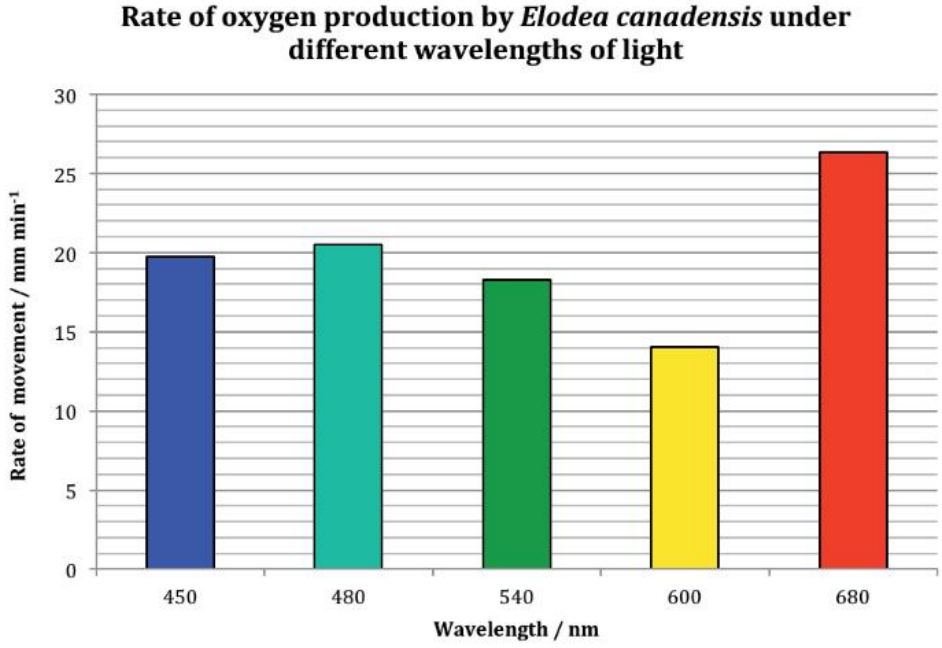
The temperature of this apparatus cannot be adjusted in a controlled way but it can be used to investigate the effect of anything dissolved in the water, the effect of light intensity or the effect of light quality.

A second apparatus will need to be set up as a control (a thermobarometer) as small changes in ambient temperature will tend to cause large changes in the level of the liquid in the capillary tube.

### Materials

The capillary tubing needs to be kept clean, a build up of lime scale in hard water regions can lead to irregular results because the liquid does not flow smoothly in the tube.

### Example Data

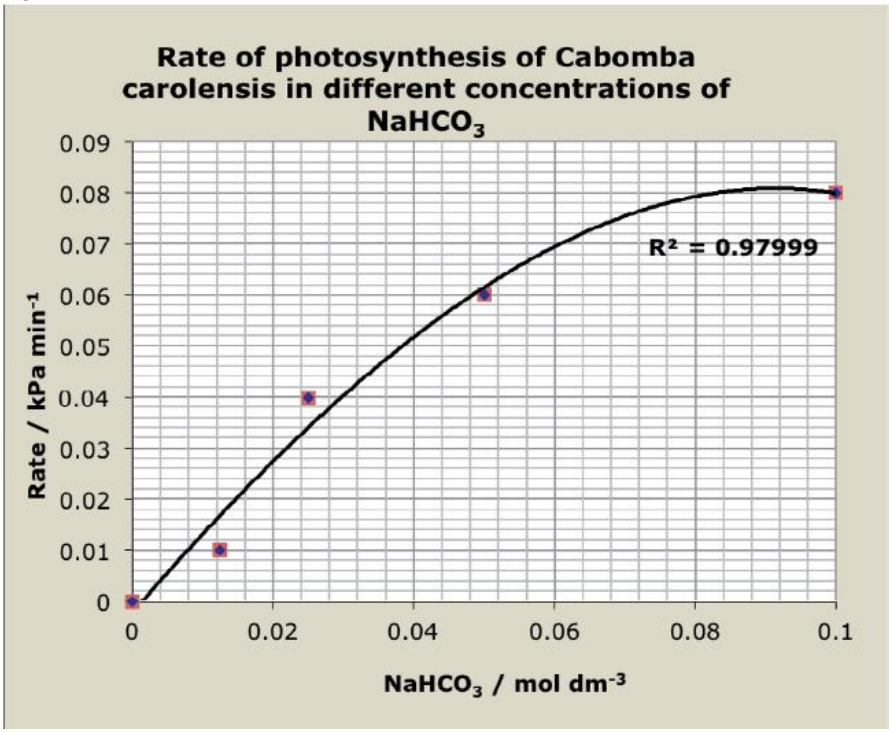


## Method C: Determining oxygen production using a pressure sensor

This apparatus is useful for investigations involving the effect of light intensity, light quality or levels of dissolved carbon dioxide.

Varying temperature of this apparatus is not really a practical option as this would result in a change in pressure inside the reaction chamber.

### Example Data




## Method D: Determining the consumption of carbon dioxide by a water plant using a pH indicator

This method only provides a qualitative impression of the changes due to photosynthesis. It is possible to combine it with the use of a colourimeter, sampling the liquid around the plant periodically. Alternatively, it can be used as a visual check on the progression of the reaction with a pH probe. However, the change in colour of the indicator may have an impact on the wavelength of light reaching the plant.

## Method E: Determining carbon dioxide absorption using a data logger and pH probe

This apparatus could not be used to investigate the effect of different levels of dissolved carbon dioxide as it would interfere with the dependent variable.

Other probes can be exploited for the measurement of photosynthesis. Dissolved oxygen probes for water plants and gaseous carbon dioxide or gaseous oxygen probes for terrestrial plants. If terrestrial plants are to be used, they should not be planted in soil as the microbial activity in the soil will influence the results.

# 3A USING A PROTEIN DATABASE

Time: 1 hour

## Syllabus reference: Topic 3.1

Skill: Use of a database to determine differences in the base sequence of a gene in two species.

UniProt database <http:/www.uniprot.org> is an open access site.

It operates on most servers, however it would be advisable to trial the use of the site on the computers that are to be used for the exercise in case some of the programs are incompatible.

## The amino acid sequence

Search programs using FAST-A (A for all)) can compare proteins by aligning them side-by-side. This permits searches for homology. Here, the FAST-A format is uses the amino acid sequence (sometimes referred to as FAST-P, P for protein). It uses the single letter abbreviations for the amino acids.

| Amino acid | Abbreviation |
|---|---|
| Alanine | A |
| Arginine | R |
| Asparagine | N |
| Aspartate | D |
| Cysteine | C |
| Glutamate | E |
| Glutamine | Q |
| Glycine | G |
| Histidine | H |
| Isoleucine | I |
| Leucine | L |
| Lysine | K |
| Methionine | M |
| Phenylalanine | F |
| Proline | P |
| Serine | S |
| Threonine | T |
| Tryptophan | W |
| Tyrosine | Y |
| Valine | V |

## The Nucleotide Sequence

This also uses FAST-A format, though this time it is the nucleotide sequence of the four letters, A, T, G and C (sometimes called FAST-N, N for nucleotide).

# 3B FACTORS AFFECTING ROOTING IN PLANTS

Time: 1 hour to set up and 3 weeks to run

## Syllabus reference: Topic 3.5

**Skill**: Design of an experiment to assess one factor affecting the rooting of stem-cuttings.

## Liquid media

Although covering the flask with aluminium foil will prevent growth of algae, it will not prevent other microbes from growing. These may infect the medium. To reduce this risk it is best to make up the media with sterile water.

### Investigating the effect of mineral deficiency on rooting using Sachs Solution

Showing the composition of the complete medium and the media deficient in one of the elements. In some cases the compound used in the complete medium (in blue) needs to be replaced by another compound (in red).

| | The amount of mineral / g $dm^{-3}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| **Mineral content** | **Complete** | **Minus Ca** | **Minus Fe** | **Minus N** | **Minus P** | **Minus S** | **Minus K** |
| $KNO_3$ | 0.70 | 0.70 | 0.70 | KCl 0.52 | 0.70 | 0.70 | $NaNO_3$ 0.59 |
| $Ca_3H_4(PO_4)_2$ | 0.25 | $NaH_2PO_4.2H_2O$ 0.71 | 0.25 | 0.25 | $CaNO_3.4H_2O$ 0.16 | 0.25 | 0.25 |
| $MgSO_4.7H_2O$ | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | $MgCl_2.6H_2O$ 0.17 | 0.25 |
| $CaSO_4$ | 0.25 | $K_2SO_4$ 0.2 | 0.25 | 0.25 | 0.25 | $CaCl_2$ 0.16 | 0.25 |
| NaCl | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| $FeCl_3$ $6H_20$ | 0.005 | 0.005 | 0 | 0.005 | 0.005 | 0.005 | 0.005 |

## Safety

- Potassium nitrate (V): an oxidising agent and dangerous with some metals and flammable substances
- Iron (III) chloride-6-water: harmful as a solid
- Calcium nitrate (V)-4-water: an oxidising agent and an irritant
- Calcium chloride: irritant as a solid
- Sodium nitrate (V): an oxidising agent and harmful as solid. Dangerous with some metals and flammable materials.

# 4A LIFE IN AN ENCLOSED ECOSYSTEM

Time: About 1 hour to set up and clean up. At least 6 weeks for observations.

## Syllabus reference: Topic 4.1

**Skill**: Setting up sealed mesocosms to try to establish sustainability. (Practical 5)

**Required knowledge:** An understanding of the principles of ecological succession is helpful but not essential.

## Materials

**Straw**: Straw used for the bedding material of pet animals is a useful source. Shredded filter paper can be used instead of straw.

**Mud**: Ensure that the source is not contaminated with pathogens, e.g. sewage effluent. Always treat with care, use rubber gloves.

**Water**: Neutral or slightly alkaline pond water should be used. The pH could be adjusted by adding sodium hydrogencarbonate. Fresh tap water is not to be used, it is chlorinated. It should be left to stand for 24 hours.

**Jar**: The ideal jar would be a food preserving jar (e.g. Kilner jar) but any tall jar (> 20cm) with a tight fitting lid would do.

## Method

Mapping the progressive changes in the microbial community can be carried out by using an acetate sheet wrapped around the jar. The limits of the colonies and their colours can be indicated using fine indelible marker pens.

### Useful information

<http://en.wikipedia.org/wiki/Sergei_Winogradsky>

<http://en.wikipedia.org/wiki/Winogradsky_column>

# 4B THE ASSOCIATION BETWEEN TWO PLANT SPECIES

Time: 45 min

## Syllabus reference: Topic 4.1

**Skill**: Testing for association between two species using the chi-squared test with data obtained by quadrat sampling.

## Materials

### Quadrats

These can be made of a number of different materials. Wood, metal or plastic frames can be constructed. They should be robust. Metal frames should be solid enough so they do not distort; wooden frames will need their corners reinforcing with metal right-angle brackets.

Large quadrat frames should preferably be collapsible. One tried and tested model can be made from 1.5cm or 2cm diameter plastic piping with right-angle joints for the corners. This could be made into 1m × 1m quadrat frames. One right angle joint should be firmly glued onto each 1m sidepiece. Four of these can then be slotted together in the field.

Small quadrats can be drawn or scratched on plastic Petri dishes.

## Method

Work in the field has its risks. The teacher should have visited the site first, obtained permission from the owner to work there and verified any safety risks. The weather forecast for the day needs to be consulted. Adequate protection from cold or wet conditions must be provided for. Excessive sunlight and heat must be also considered.

## Data

It is perhaps easy to count up for small sample sizes but using a spread sheet, such as MSExcel, the data can be filtered to give the totals. The example below is for a field of pasture grazed by cattle. It was sampled 30 times using 1×1m quadrats randomly placed. The two species recorded were Yarrow, *Achillea millefolium*, and Dandelion, *Taraxacum officinale*. They are recorded as 1 (present) or 0 (absent) on an MSExcel spread sheet. The 'Filter' function for the columns is selected from the Data menu.

The filters are adjusted to select 1 for both the Yarrow column and the Dandelion column. It will reveal the samples where both are present.

| | A | B | C |
|---|---|---|---|
| 1 | Quadrats | Yarrow | Dandelion |
| 2 | 1 | 1 | 1 |
| 3 | 2 | 0 | 1 |
| 4 | 3 | 0 | 1 |
| 5 | 4 | 1 | 1 |
| 6 | 5 | 1 | 1 |
| 7 | 6 | 0 | 1 |
| 8 | 7 | 1 | 1 |
| 9 | 8 | 0 | 1 |
| 10 | 9 | 0 | 0 |
| 11 | 10 | 0 | 1 |
| 12 | 11 | 1 | 1 |
| 13 | 12 | 1 | 1 |
| 14 | 13 | 0 | 1 |
| 15 | 14 | 0 | 1 |
| 16 | 15 | 1 | 1 |
| 17 | 16 | 1 | 1 |
| 18 | 17 | 0 | 1 |
| 19 | 18 | 1 | 1 |
| 20 | 19 | 0 | 1 |
| 21 | 20 | 1 | 1 |
| 22 | 21 | 1 | 1 |
| 23 | 22 | 1 | 1 |
| 24 | 23 | 1 | 1 |
| 25 | 24 | 1 | 1 |
| 26 | 25 | 1 | 1 |
| 27 | 26 | 1 | 0 |
| 28 | 27 | 1 | 1 |
| 29 | 28 | 1 | 1 |
| 30 | 29 | 0 | 1 |
| 31 | 30 | 1 | 1 |

| | A | B | C |
|---|---|---|---|
| 1 | Quadrats | Yarrow | Dandelio |
| 2 | 1 | 1 | 1 |
| 5 | 4 | 1 | 1 |
| 6 | 5 | 1 | 1 |
| 8 | 7 | 1 | 1 |
| 12 | 11 | 1 | 1 |
| 13 | 12 | 1 | 1 |
| 16 | 15 | 1 | 1 |
| 17 | 16 | 1 | 1 |
| 19 | 18 | 1 | 1 |
| 21 | 20 | 1 | 1 |
| 22 | 21 | 1 | 1 |
| 23 | 22 | 1 | 1 |
| 24 | 23 | 1 | 1 |
| 25 | 24 | 1 | 1 |
| 26 | 25 | 1 | 1 |
| 28 | 27 | 1 | 1 |
| 29 | 28 | 1 | 1 |
| 31 | 30 | 1 | 1 |
| 35 | Total Both | 18 | |
| 36 | | | |

**Finally,** return the filters to select all cells for both columns and the results required for the 2×2 table of the observed frequencies (O) will be shown.

| | A | B | C |
|---|---|---|---|
| 1 | Quadrats | Yarrow | Dandelio |
| 2 | 1 | 1 | 1 |
| 3 | 2 | 0 | 1 |
| 4 | 3 | 0 | 1 |
| 5 | 4 | 1 | 1 |
| 6 | 5 | 1 | 1 |
| 7 | 6 | 0 | 1 |
| 8 | 7 | 1 | 1 |
| 9 | 8 | 0 | 1 |
| 10 | 9 | 0 | 0 |
| 11 | 10 | 0 | 1 |
| 12 | 11 | 1 | 1 |
| 13 | 12 | 1 | 1 |
| 14 | 13 | 0 | 1 |
| 15 | 14 | 0 | 1 |
| 16 | 15 | 1 | 1 |
| 17 | 16 | 1 | 1 |
| 18 | 17 | 0 | 1 |
| 19 | 18 | 1 | 1 |
| 20 | 19 | 0 | 1 |
| 21 | 20 | 1 | 1 |
| 22 | 21 | 1 | 1 |
| 23 | 22 | 1 | 1 |
| 24 | 23 | 1 | 1 |
| 25 | 24 | 1 | 1 |
| 26 | 25 | 1 | 1 |
| 27 | 26 | 1 | 0 |
| 28 | 27 | 1 | 1 |
| 29 | 28 | 1 | 1 |
| 30 | 29 | 0 | 1 |
| 31 | 30 | 1 | 1 |
| 32 | Total Both | 19 | |
| 33 | Total Yarrow not Dandelion | 1 | |
| 34 | Total Dandelion not Yarrow | | 10 |
| 35 | Total neither | 1 | |
| 36 | | | |

Then the filters are changed to 1 for Yarrow and 0 for Dandelion.

| | A | B | C |
|---|---|---|---|
| 1 | Quadrats | Yarrow | Dandelio |
| 27 | 26 | 1 | 0 |
| 34 | Total Yarrow not dandelion | 1 | |
| 35 | | | |

**Next,** 0 for Yarrow and 1 for Dandelion.

| | A | B | C |
|---|---|---|---|
| 1 | Quadrats | Yarrow | Dandelio |
| 3 | 2 | 0 | 1 |
| 4 | 3 | 0 | 1 |
| 7 | 6 | 0 | 1 |
| 9 | 8 | 0 | 1 |
| 11 | 10 | 0 | 1 |
| 14 | 13 | 0 | 1 |
| 15 | 14 | 0 | 1 |
| 18 | 17 | 0 | 1 |
| 20 | 19 | 0 | 1 |
| 30 | 29 | 0 | 1 |
| 35 | Total Dandelion not Yarrow | | 10 |
| 36 | | | |

**Next,** 0 for both filters.

| | A | B | C |
|---|---|---|---|
| 1 | Quadrats | Yarrow | Dandelio |
| 10 | 9 | 0 | 0 |
| 36 | Total neither | 1 | |
| 37 | | | |

### Observed frequencies (O)

| | | Dandelion | | |
|---|---|---|---|---|
| | | Present | Absent | Row total |
| Yarrow | Present | 19 | 1 | 20 |
| | Absent | 10 | 1 | 11 |
| | Column total | 29 | 2 | 31 |

### Calculating the $\chi^2$ value

| | O | E | (O-E) | (O-E)2 | (O-E)2/E |
|---|---|---|---|---|---|
| Yarrow present Dandelion present | 19 | 18.71 | 0.29 | 0.0841 | 0.0045 |
| Yarrow present Dandelion absent | 1 | 1.29 | -0.29 | 0.0841 | 0.0651 |
| Yarrow absent Dandelion present | 10 | 10.29 | -0.29 | 0.0841 | 0.0082 |
| Yarrow absent Dandelion absent | 1 | 0.71 | 0.29 | 0.0841 | 0.1185 |
| | | | | Total ($\chi$2) | 0.1963 |

The calculated $\chi$2 value is $p<0.5$. This is well below the critical value of $p=0.05$.

Therefore, there is no association between these species.

## Some points for consideration

- Do the species show any particular adaptations that may help them to compete easily with other species?
- Growth forms may vary from tall vertical stems to rosettes at ground level. Tall plants will rise above competitors and shade them out but low growing forms will perform better where there are grazers.
- Criticise this method of estimating the association between two species of plant in a habitat.

(i) Would it make any difference if the species were clumped, uniform or random in their distribution?

*Clumped species tend to develop from asexual propagation through runners or rhizomes. This can be a very competitive strategy.*

(ii) Would it make any difference if the species chosen were large or small?

*Large species will tend to out compete smaller species.*

# 5A CONSTRUCTING A DICHOTOMOUS KEY FOR PLANT PHYLA

Time 1 hour

## Syllabus reference: Topic 5.3

**Application**: Recognition features of Bryophyta, Filicinophyta, Coniferophyta and Angiospermophyta

**Skill**: Construction of dichotomous keys for use in identifying specimens.

## Materials

### Environmental impact

If the specimens are taken from the wild, care must be taken not to harvest protected or vulnerable species. In any case, the number of specimens must be limited to a minimum. For large organisms e.g. conifer trees specimens should be cut from the tree, preferably using secateurs, to limit wounding.

### Plant classification

This is greatly simplified below. The Tree of Life Project <http://tolweb.org/tree/phylogeny.html> has been set up online to provide information from international sources about biodiversity and the characteristics of the different taxonomic groups. This will provide much more detailed classification.

| PHYLA | CLASSES | Characteristics |
|---|---|---|
| Bryophyta | | Mosses. Small terrestrial plants restricted to damp habitats. Erect stems with leaf scales +/- rhizoids<br>Show an alternation of generations between a haploid gametophyte and a diploid sporophyte.<br>The gametophyte is the dominant generation in the life cycle.<br>Water is needed for fertilisation.<br>The sporophyte generation is represented by the spore capsule. |
| **Vascular plants**<br>The sporophyte generation is dominant in the life cycle. It is differentiated into: leaves, stem, roots and rhizome. Lignified tissue (wood) may be present. | | |
| Filicinophyta | | Water is needed for fertilisation. |
| | Pteridopsida | Ferns. Leaves (fronds) often open by uncurling. |
| | Sphenopsida | Horsetails. Needle-like leaves radiating from a single stem. |
| **Seed plants**<br>The sporophyte generation dominates, it is the plant.<br>Water is no longer needed for fertilisation.<br>The gametophyte generation is very reduced.<br>The female gametophyte becomes a seed that contains a food store and protective coat. | | |
| Coniferophyta | | Conifers. Naked seeds born in cones. |
| Angiospermophyta | | Flowering plants. Seeds protected by an ovary which becomes a fruit |
| | Dicotyledons | Plants with two seed leaves. |
| | Monocotyledons | Plants with one seed leaf. |

## Summary

| Feature | Bryophyta | Filicinophyta | Coniferophyta | Angiospermophyta |
|---|---|---|---|---|
| Common name | Mosses | Ferns | Conifers | Flowering plants |
| Leaves | No, scales | Yes | Yes | Yes |
| Roots | No rhizoids | Yes | Yes | Yes |
| Vascular system | No | Yes | Yes | Yes |
| Woody tissue | No | Yes | Yes | Yes |
| Waxy cuticle | No | Yes | Yes | Yes |
| Water for fertilisation | Yes | Yes | No | No |
| Seeds | No | No | Yes | Yes |
| Cones | No | No | Yes | No |
| Fruit | No | No | No | Yes |

# 6A THE DIGESTION OF STARCH IN A MODEL GUT

Time 1 hour

## Syllabus reference: Topic 6.1

**Application**: Use of dialysis tubing to model absorption of digested food in the intestine.

## Materials

Dialysing tubing pore size: equivalent to a protein MW 12000.

### Fehling's reagent

Solution 1. Dissolve 34.6g of hydrated copper (II) sulphate ($CuSO_4$. 5 $H_2O$) in distilled water to give a final volume of 500cm$^3$. Add a drop of concentrated sulphuric acid if the solution remains cloudy.

Solution 2. Dissolve 77g of sodium hydroxide together with 175g of sodium potassium tartrate in distilled water to give a final volume of 500cm$^3$.

Keep solution 1 and 2 separately for storage. For use, mix equal volumes of solutions 1 and 2.

Benedict's solution can be used instead of Fehling's solution.

### Benedict's reagent

Dissolve 173g of hydrated sodium citrate together with 100g of hydrated sodium carbonate in 800cm$^3$ of warm distilled water. Filter and add distilled water to a final volume of 850cm. Separately, dissolve 17.3g of hydrated copper (II) sulphate in 100cm$^3$ of cold distilled water. Add the copper (II) sulphate solution to the citrate-carbonate solution, stirring constantly. Add distilled water to a final volume of 1dm$^3$.

### Iodine solution

Dissolve 1g potassium iodide in a little water. Mix whilst adding 0.5g iodine crystals. Make up to 100cm$^3$. Store in dark brown bottles.

### Starch solution

'Soluble' starch should be avoided, it tends to leak through the pores easily, ordinary laundry starch is best.

### Amylase solution

Commercial preparations of amylase are available. Salivary amylase should NOT be used.

## Method

Completely wetting the dialysis tubing may help to open it up. There may be problems with leaking dialysing tubing. Insist that the knot in the bottom of the tube is tied tightly but be careful not to tear the tubing with long fingernails. Use enough tubing so that the upper end is well clear of the water (as shown in the diagram on the student's guide).

There should be enough distilled water surrounding the dialysing tubing to last for six samples but warn the students not to take large samples. Furthermore, the samples should not be "drowned" with Fehling's solution when being tested.

## Some points for consideration

- *What are the sources of error that you may have encountered?*

The tests for glucose are not specific, they are general tests for reducing sugars. It is possible the maltose molecules are giving positive results.

The red precipitate indicating a positive test may not be easy to see in the small samples when the concentration is quite low.

- *How does the model resemble the process of digestion?*

Food is processed in the lumen of the digestive system before being absorbed by the wall of the gut, principally the small intestines. The dialysing tubing represents the digestive system and the distilled water surrounding the tube represents the blood.

- *How could the model digestive system be improved upon?*

Glucose uptake will be by active transport, it would be difficult to represent this in a non-living model.

The blood carries the glucose away (to the liver), this could be simulated by periodically circulating the blood or changing the water surrounding the tubing.

Food is a mixture of molecules, perhaps proteins and proteases, such as trypsin, could be added to the mixture.

The pH of the gut is controlled by secretions of acid or alkali digestive juices. Amylase works in the small intestines where the conditions are alkaline. These alkaline conditions could be simulated by adding sodium hydrogencarbonate to buffer the gut contents.

# 6B ANATOMY OF THE HEART AND ITS PHYSIOLOGY

Time: 2 hours

## Syllabus reference: Topic 6.2

**Skill**: Recognition of the chambers and valves of the heart and the blood vessels connected to it in dissected hearts or in diagrams of heart structure.

**Required Knowledge:** The characteristics of muscle tissues, the basic anatomy of the circulatory system, use of dissecting instruments.

## Materials

A lamb's heart is smaller than a human heart (it weighs approx. 150 g compared with 300 g for a human heart).

A butcher should be able to supply whole hearts which are not cut and with the vessels still attached. If not, this can be a major problem in the activities to follow.

It would be interesting to observe an intact heart-lung set from a lamb or a pig to show the relationship between these organs.

## Part A: Observations on the heart and the vessels attached to it

1. The heart appears as a conical muscle, red where it is uncovered and yellowish or white where it is covered with fat.
2. The atria are reddish brown and they have an uneven surface covered in ridges of muscles.
3. Blood vessels (the coronary arteries and coronary veins) are visible in this groove that marks the division between the two ventricles. A second set of coronary vessels are found on the dorsal side of the heart.

## Part B: Examination of the inside of the heart

The heart is a hollow muscle with elastic walls. It contains four cavities which are connected two-by-two. In each part (the lefthand side of the heart and the righthand side of the heart) the atrium is connected to a ventricle via the atrioventricular valve. There is no communication between the ventricles or the atria.

The wall of the left ventricle forms the apex of the heart.

## Part C: The circulation of blood in the heart

During the experiment the water replaces the blood which normally circulates in this organ. A sheep's heart is better than a pig's heart for this. It is small and fills with water more rapidly.

1. When water is injected into the right atrium, it escapes from the pulmonary artery via the right ventricle. When water is injected into the left atrium it escapes from the aorta via the left ventricle.
2. When the water is introduced into the arteries under pressure, it tends to be rejected back out as the valves close. Some water does enter the ventricles and the atria due to leaking valves. The arterial valves in the arteries operate very well even in a dead heart. These may be observed closing to stop the back flow of water when it is poured into the arteries. The water will leak from the ventricle to the atrium however, because the flaps of the atrioventricular valves are no longer held taught by the fibres which are attached to dead cardiac muscle.

*NB For the valves to close well the pressure of water flowing into the vessels needs to be increased gently or the water spurts back up. It might be a good idea to demonstrate this before the students try it by themselves.*

# Part D: Looking for the structures which direct the blood flow through the heart

- Both sides of the heart have the same basic structure (atrium, ventricle and valves).
- The wall of the left ventricle is much thicker than the wall of the right ventricle (two to three times thicker).
- The tricuspid valve, on the right, has three flaps and the bicuspid valve, on the left, has two flaps.
- The aorta has a thicker wall than the pulmonary artery.
- The vena cava (if attached) has a much bigger internal diameter (lumen) than the pulmonary vein.

(*NB The volume of the two ventricles is the same*).

The contraction of the atria and then the ventricles pushes the blood into the arteries. The atrioventricular valves and the arterial valves stop the back flow of the blood into the cavity that it has just left.

Therefore, the blood flows into the heart through the veins and collects in the atria. From there, it flows into the ventricles and out via the arterial valves into the arteries.

The students should conclude that each side of the heart has a one-way circulation of the blood.

Probing the coronary artery shows how it forms a ring around the heart between the atria and the ventricles. Branches of the artery can be seen spreading over the ventricles, especially down the groove between the left and the right sides. The coronary arteries distribute oxygen and nutrients to the cardiac muscle cells.

## Some points for consideration

The right ventricle pumps blood towards the nearby lungs which surround the heart. The left ventricle must send the blood, via the aorta and its branches, to all the organs, including the most distant, in the head, as well as to the ends of the limbs. Thus, the muscular wall of the left ventricle is much thicker than that on the right in order to produce enough force to propel the blood all through the body. The relative thickness of the aorta can be explained by the high pressure which it must contain after each contraction, (about five times more pressure on the left than the pressure exerted by the right ventricle).

## Research

- The blood leaving the right ventricle is deoxygenated the blood leaving the left ventricle is oxygenated.
- Arteries carry high pressure, high velocity blood away from the heart and veins carry low pressure, slow velocity blood towards the heart.

## To investigate further

- A transverse cut above the apex of the heart, carried out at the end of the dissection, allows the different thickness of the ventricular walls to be observed clearly.
- Investigating the heart sounds (corresponding to the movement of the valves) using a stethoscope.
- Investigating the circulation of the blood in the vessels. Measuring the pulse and measuring the blood pressure using a sphygmomanometer after or during different activities, or in different groups of people, or at different times of the day.

# 6C ANALYSING BREATHING USING A SPIROMETER

Time: 1.5 hours

## Syllabus reference: Topic 6.4

**Skill**: Monitoring of ventilation in humans at rest and after mild and vigorous exercise. (Practical 6)

## Materials

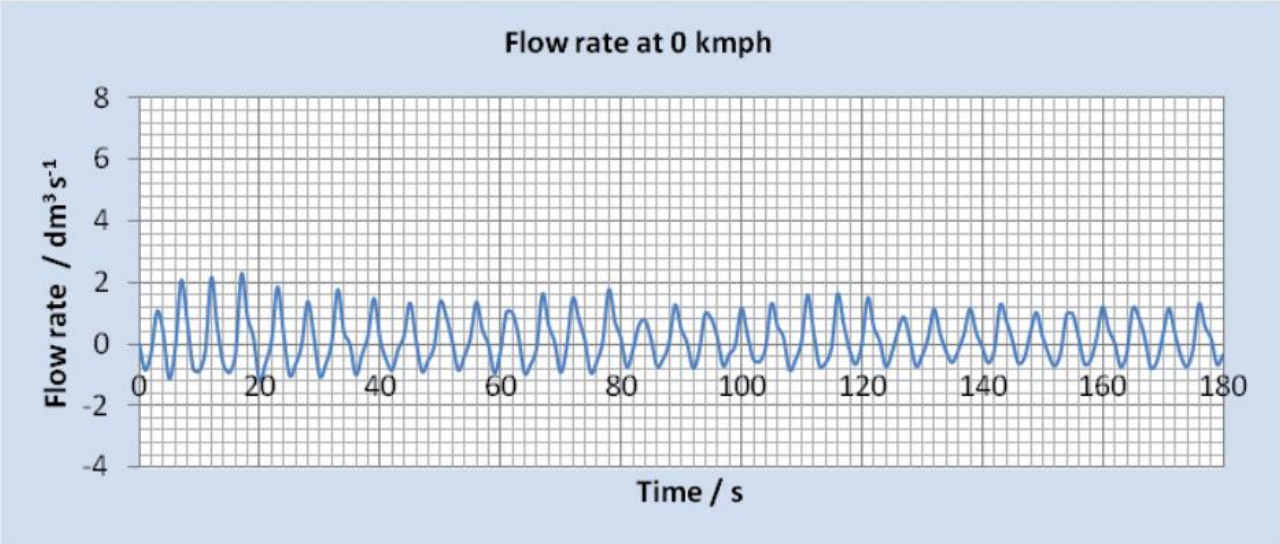
The data recorded here was obtained using Vernier ® data logging materials.

## Data

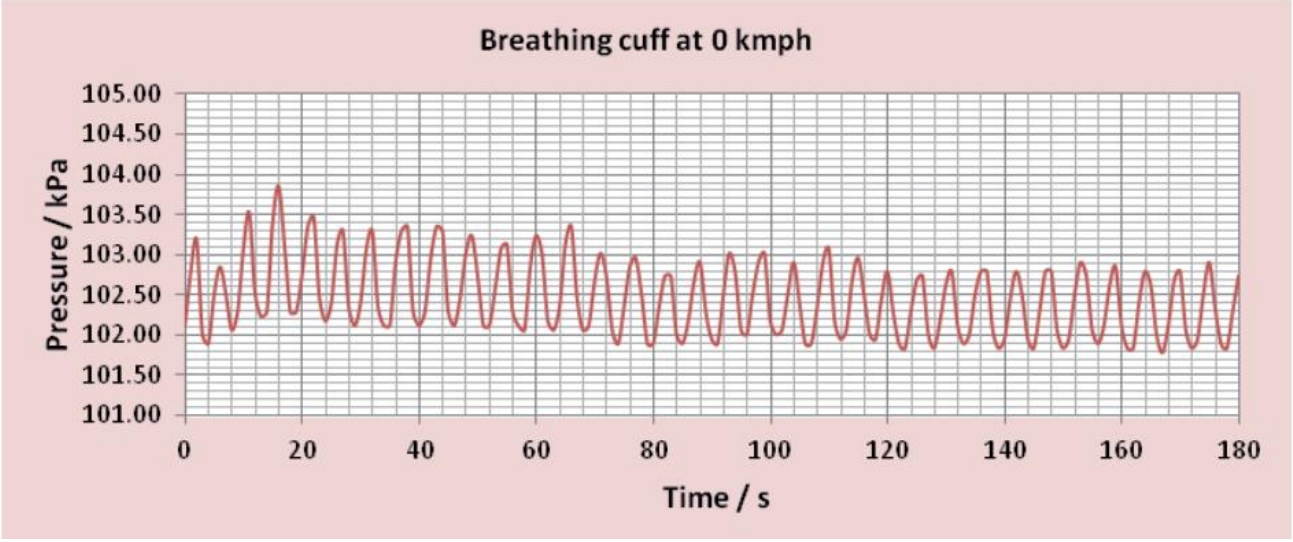
Using the breathing cuff attached to a pressure sensor, the frequency of the breathing can be determined and a relative measurement of the volume breathed is possible.

Using the spirometer the frequency and the volume of the breaths can be measured.

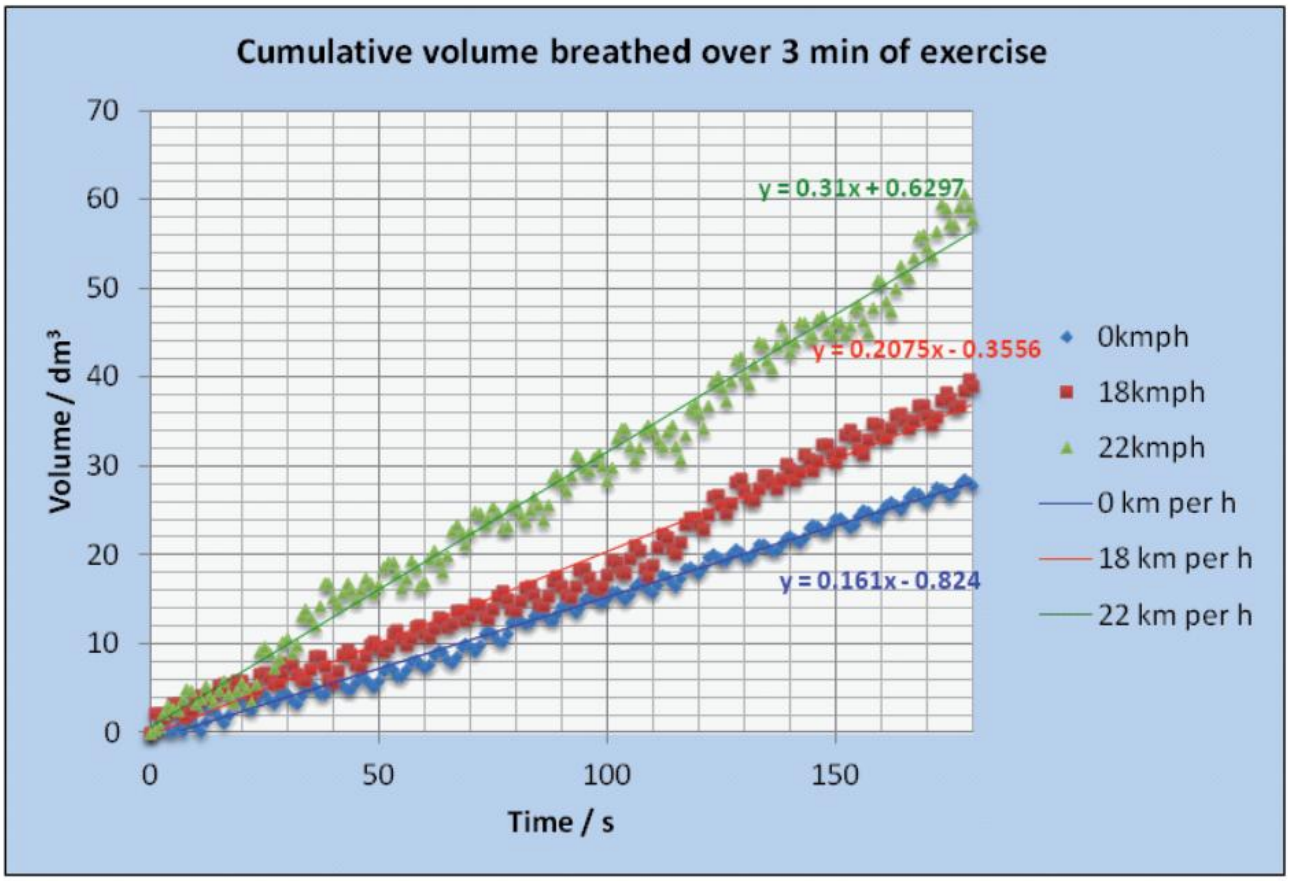
### Spirometer data



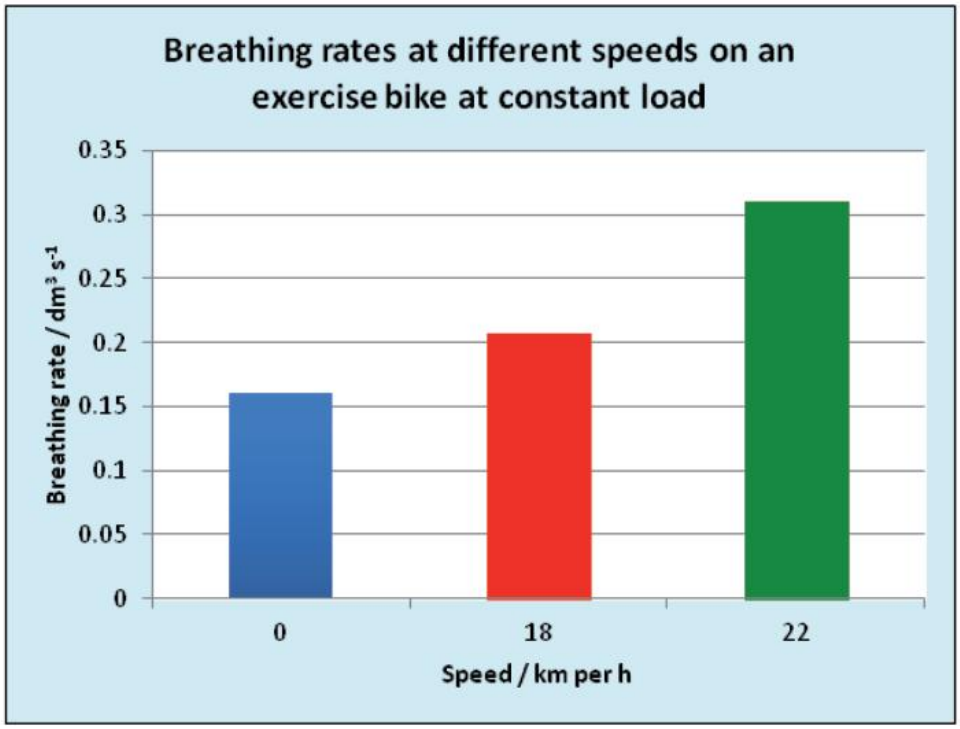

### Breathing cuff data (on the same person at the same time)



With the spirometer, it is also possible to calculate the cumulative volume of air breathed. This can give a clear idea of the changes with the increase in activity.




Giving the following breathing rates:



# OPTIONS

## 12A THE PUPIL REFLEX

Time: 1 hour

**Syllabus reference: Option A.2**

**Application**: Use of the pupil reflex to evaluate brain damage.

### Method

The electric circuit set up will give adequate results, however, a pen light will give a clearer response and it is easier to manipulate if a subject is used.

*Warning: The light should be placed below the line of sight and the subjects should not stare at it for long periods of time.*
*LEDs should not be used as a light source for this investigation.*


*UNDER NO CIRCUMSTANCES SHOULD A LASER POINTER BE USED*


### Observations

#### Direct reflex

The irises of both eyes should behave in the same way. Constriction of the pupil in bright light and dilation in the dark.

When the light intensity is constant, careful observation will reveal a small oscillation of the pupil diameter, even if the eye is perfectly steady. What is being observed is the effect of negative feedback from the brain.

The control of the pupil diameter is regulated by two pathways. One, using the parasympathetic system constricts the pupil. The other, from the sympathetic system dilates the pupil. The iris is a sphincter muscle with circular and radial muscles. The radial muscles pull the pupil open when they contract. The circular muscles pull the pupil shut when they contract.

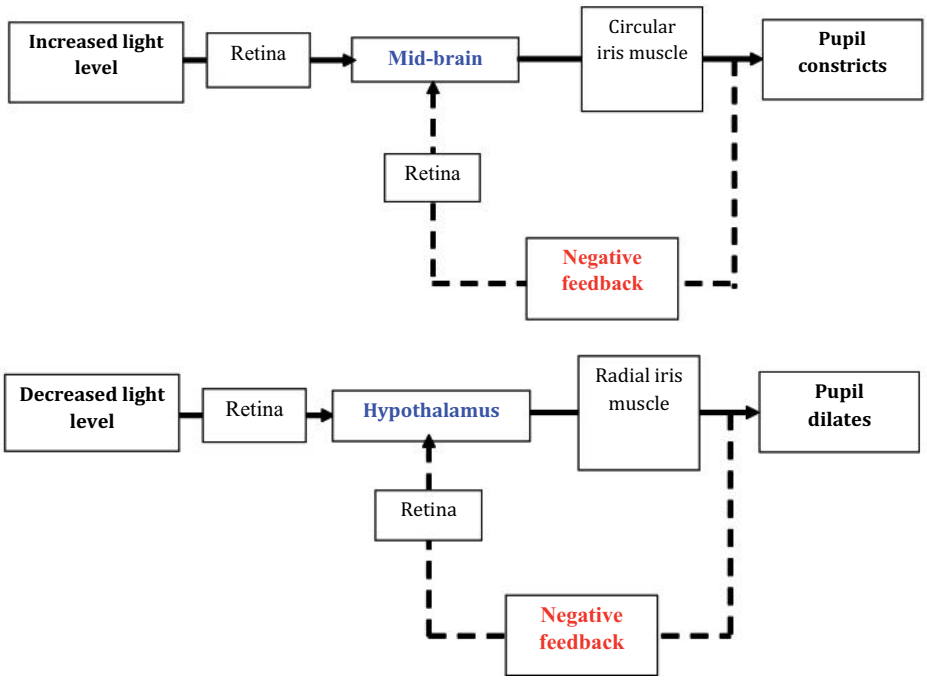
Note: Pupil diameter will also vary with gender, eye colour, emotional state, myopia and the distance of an object being observed from the eye.

#### The consensual reflex

When one eye is illuminated, both pupils constrict.

## Some points for consideration

How can the response of the iris be explained in terms of negative feedback?




**In fact, there are two reflexes that interact with one another.**

**The pupil light reflex** that operates when there is too much light, causing the pupil to constrict by contracting the circular muscles until the signals from the retina are reduced and the pupil stops constricting further.

**The pupil dark reflex** operates when the light levels are too low. It opens the pupil by contracting the radial muscles.

When the pupil dilates to the point where the signals from the retina arrive at the brain, the pupil constriction reflex with stop the pupil down. In this way, eyes observed under low light conditions tend to oscillate the size of the pupil a little.

- How can you explain the response of the shaded eye?

The pupil reflex applies to both eyes. Some neurones from the left eye pass to the midbrain on the right and vice versa. The information from the left visual field of the left eye passes to the left visual cortex as does the left visual field of the right eye and vice versa. So, the corresponding part of the visual cortex should be receiving information from both eyes.

## Research

- Find out which sensory and motor nerves are involved in the pupil reflex. Draw a diagram of the reflex arch involved in this reflex.

### The pupillary light reflex is a parasympathetic reflex

Signals from the retina pass to the optic nerve and to centres in the midbrain, then via the occulomotor nerve back to the pupil, constricting the circular muscles.

### The pupillary dark reflex is a sympathetic reflex

Some of the neurones of the optic nerve innervate the hypothalamus, this takes a signal down the spinal cord to sympathetic ganglia at the level of the lungs in the thorax. A sympathetic nerve then returns to the head through the neck and innervates the radial muscles passing beside the trigeminal nerve.

# 12B THE RETINA AND COLOUR VISION

Time: 1 hour

## Syllabus reference: Option A.3

**Application**: Red-green colour-blindness as a variant of normal trichromatic vision.

### The structure of the retina

What kind of cell will be used in daylight vision or low light night vision?

Cone cells are used in daylight vision; the rod cells operate in low light.

### Afterimages and complementary colours

Lamps with LEDs give a more intense light. Care must be taken not to look directly at the LEDs.

## Data

When a white object is observed all three types of cones are being stimulated whereas a black object stimulates none of the cone cells.

As the complementary colour chart indicates:

- A red cross should give a cyan (turquoise) afterimage. L cones are mostly stimulated and bleached, so when the white sheet is observed the M and S cones are the only ones working giving a mixture of green and blue-violet while the L cones are regenerating.
- A blue cross gives a yellow afterimage. The L and M cones being the ones that are not bleached.
- A green cross green gives magenta. The L and S cones are not bleached.

### Afterimages from complementary colours

- Predict what you will get if you stare at yellow, cyan and magenta crosses for a minute. You might not get quite what you expect.

The classic yellow highlighter usually gives a violet afterimage though blue would be logical. The reason being the nature of the pigments and the wavelength of the light source used.

### Colourblindness simulations

Example images from the Colblindor simulator Coblis:


# 13A DO DISINFECTANTS KILL BACTERIA?

Time: 1 hour to set up and 30 min for observations

## Syllabus reference: Topic B.1

**Skill**: Experiments showing zone of inhibition of bacterial growth by bactericides in sterile bacterial cultures.

## Materials

**Bleach solution**: Use commercial strength bleach.

## Method

### Filter paper discs

The discs used here can be cut using a hole punch. Do not fold the paper to cut several disks at once. This tends to make the discs stick together and they are difficult to separate. Wrap the discs in an envelope made from aluminium foil and sterilise them in an autoclave.

### Using the autoclave

All glass material and growth media to be used should be sterilised for 20 minutes at 120°C.

All open material should be either plugged with cotton wool and the cotton wool plug covered with aluminium paper before sterilisation (e.g. test tubes, conical flasks); or, the openings should be covered with aluminium foil before sterilisation (e.g. beakers).

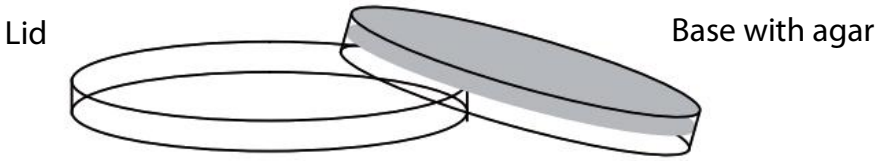
After sterilisation the material should be transferred to a working zone and used as soon as possible.

If material is to be kept, ensure that plugs and or aluminium paper covers are not removed and that the material is kept in a dry place.

### Preparing media for growth of the micro-organisms

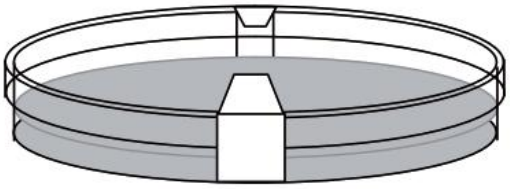
Different organisms have different growth medium requirements. Whichever medium is being used, it must be sterilised in the autoclave before use. Liquid media can be sterilised in closed conical flasks or in closed test tubes. For a medium that is to be solidified after sterilisation in plastic Petri dishes, the medium can be poured into opened dishes within the sterile zone. The dishes used should come from an unopened pack. The medium must be left to cool in the sterile zone with the lids on. Once the agar has set, the dishes can be opened and turned upside down to allow any condensation to dry for about 30 minutes.

The dishes should then be closed and kept upside down, until use, in a dry place (the solid medium upward).

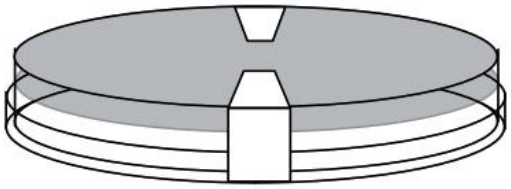


Use self-adhesive tape to hold on the lid of the Petri dish. Do not seal the dish completely as this may encourage the growth of anaerobic bacteria.

Use self-adhesive tape to hold on the lid of the Petri dish



Transfer the dish to the oven and leave for the required growth time and at the required temperature. The Petri dishes must be placed upside down (the solid medium towards the top)



The Petri dishes must be placed upsidedown

## Examination of the micro-organisms

*Warning: Do not open the dishes containing cultured microbes*


It is preferable to keep microscopic investigation and staining to media that already contain bacteria which are not pathogenic, for example bacteria already present in fresh yoghurt. Even so when organisms are grown, the dishes must not be opened.

## Disposing of material at the end of manipulation

Ideally, all the material should be autoclaved at 120° C for 20 minutes. Including plastic Petri dishes, which will melt in the process, so they must be placed in a suitable container before autoclaving. For any material that is to be thrown away after autoclaving, autoclavable plastic bags can be used, these should be tied before autoclaving. All material to be kept, such as glassware and slides, should be autoclaved and then washed in the normal way.

An alternative is to leave all the material (except metal tools) to soak in bleach for 24 hours.

Clean the working area once again with bleach. Wash your hands again after handling any of the experimental materials.

# 13B THE GRAM STAIN FOR BACTERIA

Time: 1 hour

## Syllabus reference: Topic B.1

**Skill**: Gram staining of Gram-positive and Gram-negative bacteria.

## Materials

**Crystal violet stain** (0.5% aqueous) Add 0.5g crystal violet to 100cm$^3$ distilled water. Dissolve and filter.

**Lugol's iodine** Add 1g iodine to 2g potassium iodide in 25cm$^3$ distilled water. Dissolve this mixture then add 75cm$^3$ distilled water.

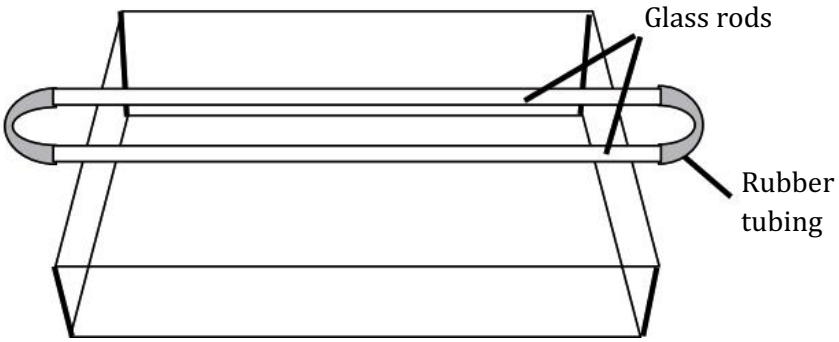
**Acetone-alcohol** (50% acetone + 50% pure ethanol) Mix 50cm$^3$ acetone with 50cm$^3$ 100% ethanol.

**Safranin** (1% aqueous) or Add 1g safranin to 100cm$^3$ distilled water. Dissolve and filter.

**Basic fuchsin** (an alternative to Safranin). Dissolve 0.5 g basic fuchsin in 20 cm$^3$ 95% ethanol. Then dilute to 100 cm$^3$ with distilled water and filter the solution.

### Making a staining rack

This can be made quite simply from two 0.7cm diameter glass rods about 30cm joined by two pieces of rubber tubing about 15cm long.




### Cleaning off immersion oil

Immersion oil should always be cleaned off the lens after use. If necessary, the lens can be cleaned using a lens tissue soaked in ethanol.

Some oils recommend using solvents such as xylene for cleaning. These can be very toxic. Check the safety procedures before using them.

## Research

What is the relationship between the Gram staining characteristics of a bacterium and its virulence when it has infected a patient?

Gram stain will help to identify bacteria. Even though more precise molecular techniques for identifying pathogens, (such as PCR), are used, Gram stain is often the first step. The cell wall characteristics of Gram negative bacteria is often associated with the presence of an endotoxin that stimulates an immune reaction that causes inflammation.

# 14A STUDYING THE CHANGES IN A PLANT COMMUNITY USING A BELT TRANSECT

Time: 3 hours in the field

## Syllabus reference: Option C.1

**Skill**: Use of a transect to correlate the distribution of plant or animal species with an abiotic variable.

**Required knowledge:** Use of identification guide or key if it is to be used.

## Materials

**Quadrats:** See investigation 4B.

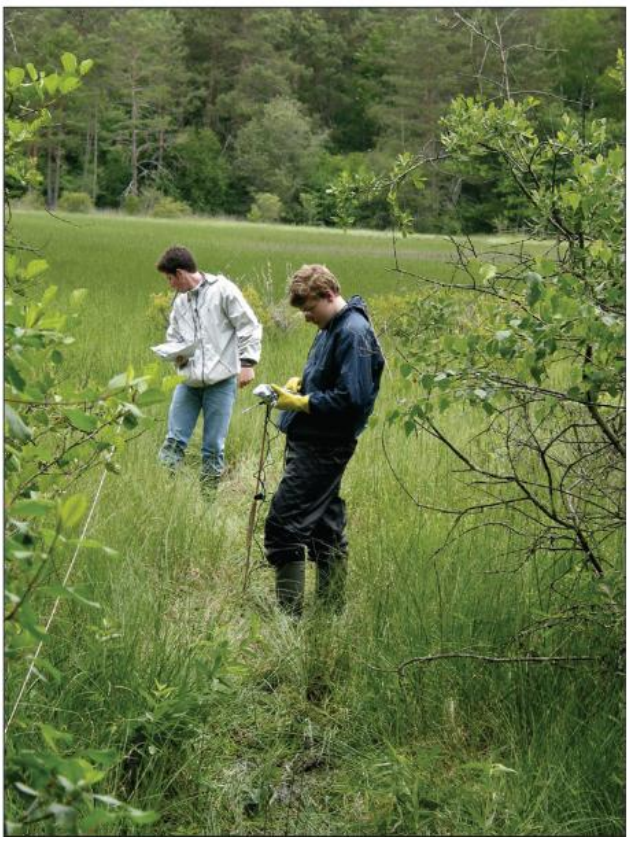
**Ranging poles:** These can be made cheaply from 2m lengths of wood marked at 10cm intervals using bright insulating tape.

**Data sheet:** An example of a data sheet is given (see following page) which was used by students studying a lake-woodland transect in Europe.

The abiotic factors temperature, light intensity and humidity were recorded directly on a data logger. Height change can be recorded on a graphic calculator which can give a readout of the profile as the survey progresses.

## Method

This method presents an interrupted belt transect over 25 metres. Shorter distances could use a continuous transect or even smaller units (e.g. 0.25 $m^2$). To get an idea of any changes in the transition zone, at least 10 results would be needed along a transect.



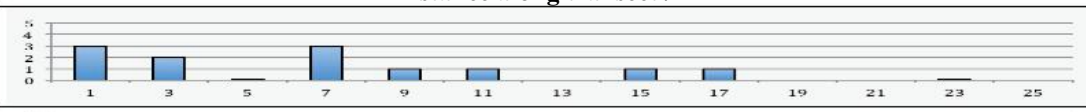
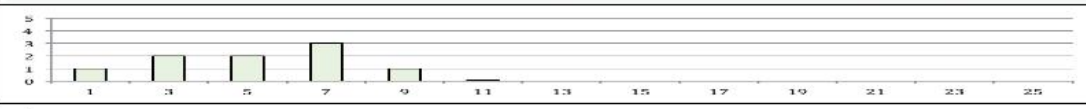
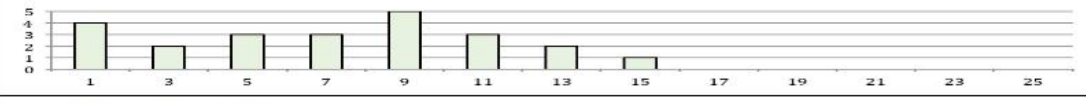
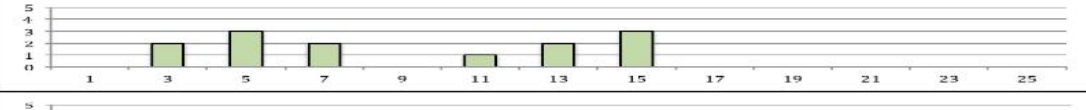
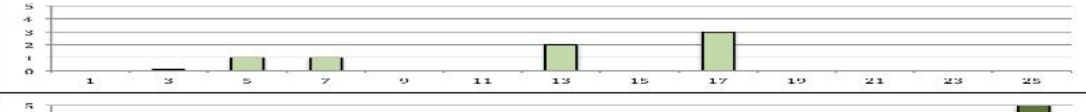
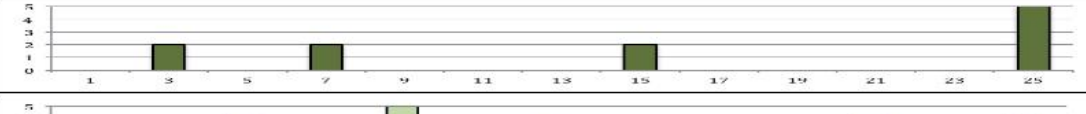
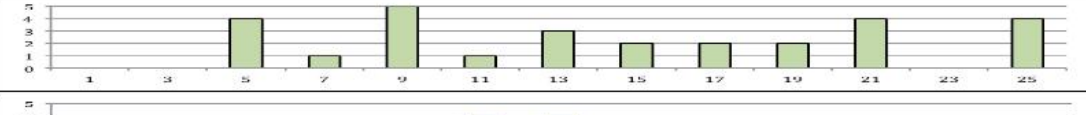
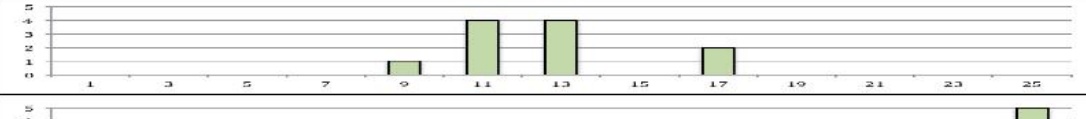
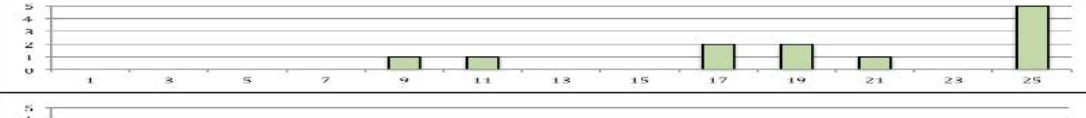
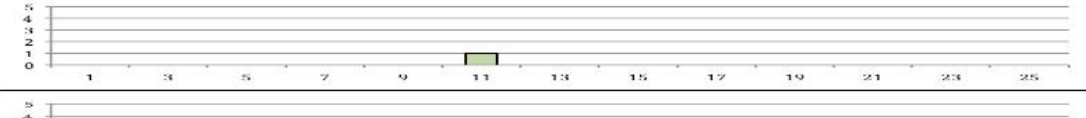
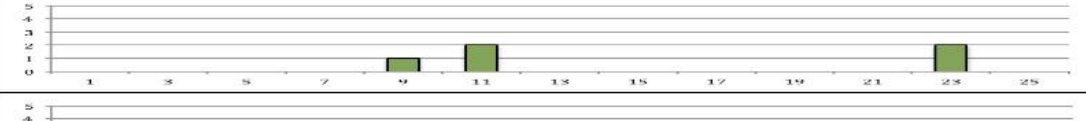
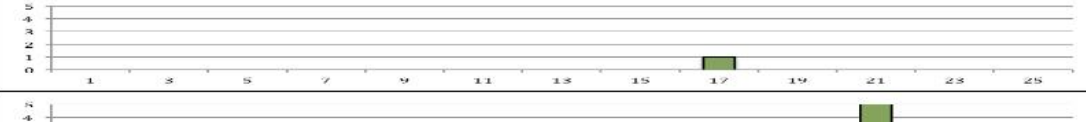
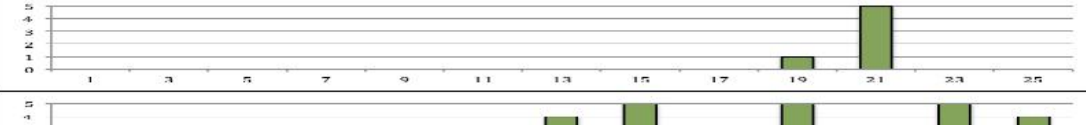
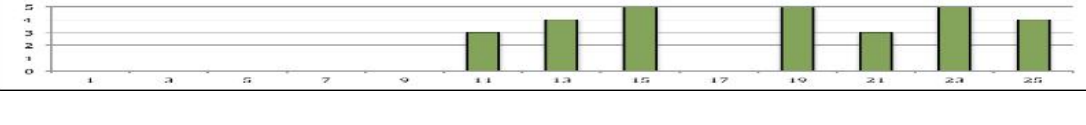
The problem of identifying species can be simplified by providing the students with a species check list which has been established for the area being investigated. This, of course, requires some prior investigation by the teacher. An example of a species checklist for a N.W European lake-woodland boundary is given.

Any transition zone could be studied using this method: lake margins, field to forest boundaries, seashore, sand dunes.

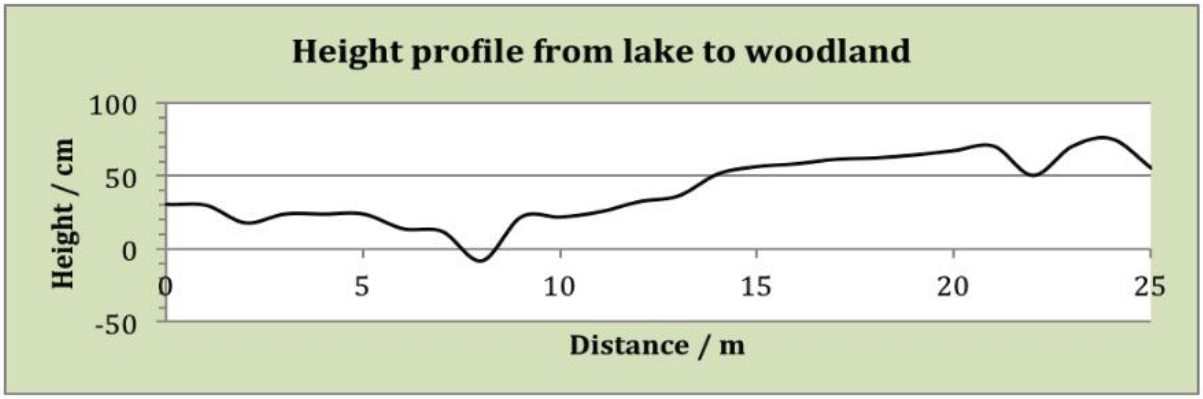
Rocky seashore habitats, where there are sessile animals such as barnacles and molluscs as well as algae (sea weeds) can use this method too.

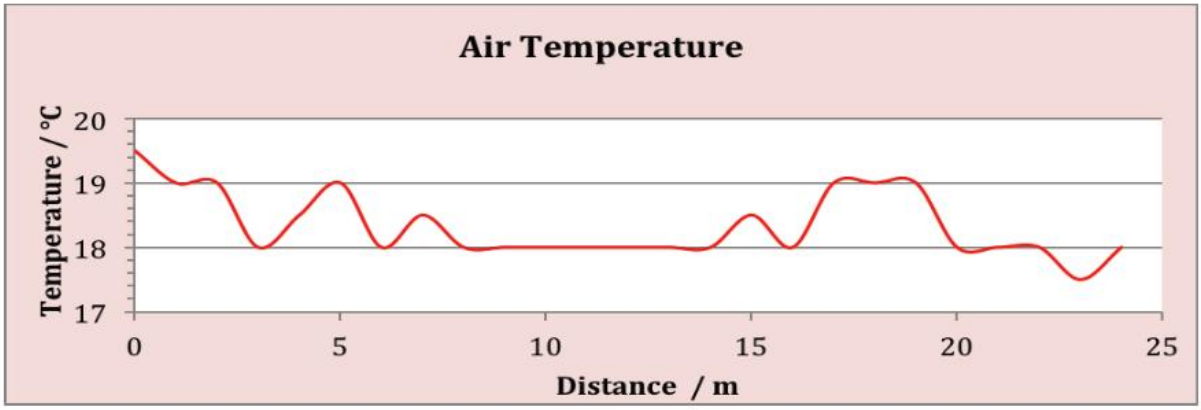
## ZONATION OF LAKE SHORE VEGETATION – DATA SHEET

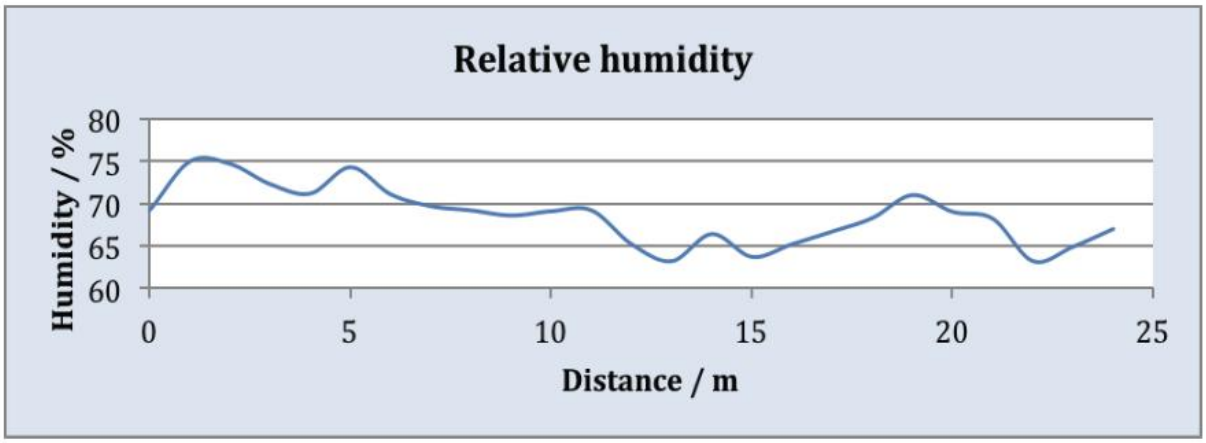
| | **QUADRAT**/m along transect | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SPECIES** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
| Open water | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Equisetum palustre* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Carex panicea* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Lycopus europeus* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Hydrocotyle vulgaris* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Galium palustre* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Filipendula ulmaria* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Lotus corniculatus* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Lysimachia vulgaris* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Epilobium palustre* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Aethus cynapium* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Viola palustris* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Poa serotina* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Molinea caerulea* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Betula pubescense* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Alnus glutinosa* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Salix alba* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pinus sylvestris* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Juncus inflexus* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *J. conglomerata* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Spagnum spp.* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Stellaria sp.* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Mysotis sylvatica* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Urtica dioica* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Rubus fruticosus* | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Cirsium sp.* | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | |

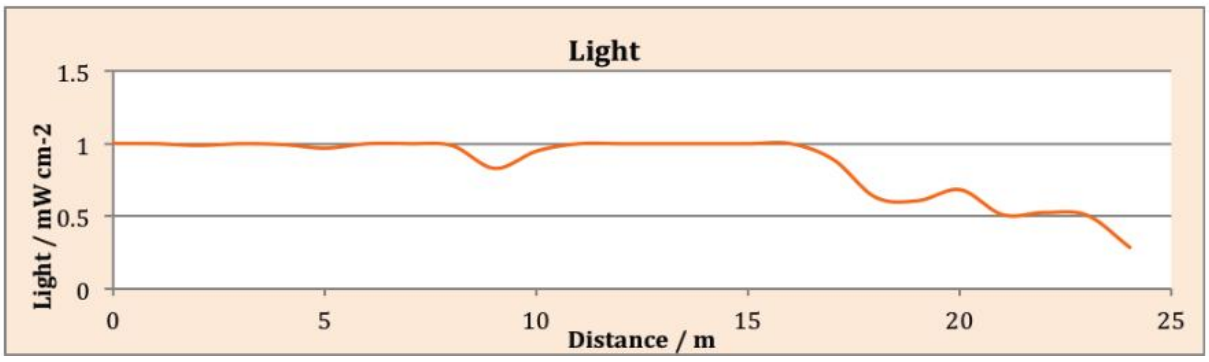
| Species | Distance along transect / m |
|---|---|
| Open water |  |
| *Equisetum palustre* |  |
| *Carex panicea* |  |
| *Filipendula ulmaria* |  |
| *Comarum palustre* |  |
| *Lysimachia vulgaris* |  |
| *Salix alba* |  |
| *Sphagnum spp* |  |
| *Epilobium palustre* |  |
| *Viola palustris* |  |
| *Lycopus europeus* |  |
| *Angelic silvestris* |  |
| *Lotus corniculatus* |  |
| *Anthyrium filix-femina* |  |
| *Poa serotina* |  |
| *Molinia caerulea* |  |

## Abiotic data



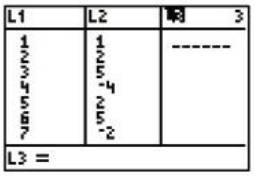




It is now possible to see if there are any trends that suggest a relationship. For example, a relationship with light intensity can indicate shade tolerant species. However, as the abundance scale is a very approximate scale of the importance of a species (it is only 0-5 scale), a scatter plot is not very realistic.

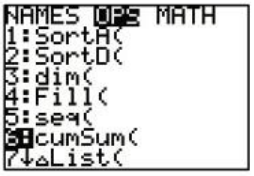
## Using the Ti graphic calculators *to process the height profile* and Graph data

Press **STAT** then select **1: Edit…** to open a spread sheet.



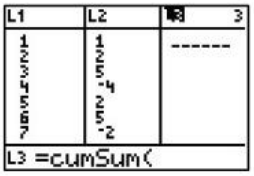
Just like MSExcel or other spread sheets you enter the data in columns or lists **L1**, **L2** etc. Type in your distance along the transect in **L1** and the height change for each metre along the transect in **L2**.

Use the cursor to get to the top of **L3**. Press **ENTER** then **2nd** and **LIST** and select **OPS** (use the cursor to move one to the right). This opens up a menu of functions rather like in Excel.

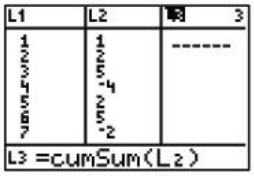


Select **6:cumSum(** This is the **cumulative sum function.**

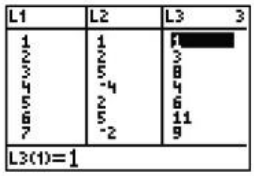
Press **ENTER** and the function will appear at the bottom of the screen
**L3 = cumSum(**



Press **2nd** and **L2** then **)**. Press **ENTER** and you will see the spread sheet calculate the cumulative sum of all your samples. This is your **height profile**



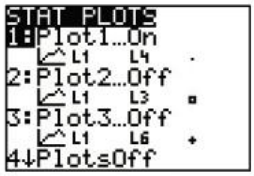
You can store other data for abiotic factors (e.g. light, temperature) along the transect in subsequent columns.



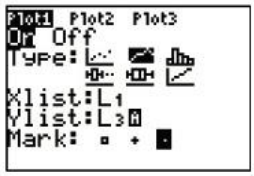
### Graphing the data

To show the height profile plot a graph using **STAT PLOT**

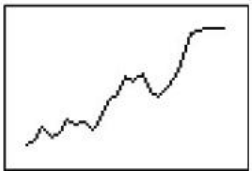
Press **2nd** and **STAT PLOT**. Open **Plot 1** by pressing **ENTER**



Using the cursors and **ENTER**
Select **ON**
Select the line graph plot
In **Xlist:** Press **2nd** and **L1**
In **Ylist:** Press **2nd** and **L3**



Then to see the graph Press **ZOOM** and select **9:ZoomStat**



You should be able to see a scaled graph showing the height profile of your transect.

# 14B USING INVERTEBRATE PITFALL TRAPS ON A LINE TRANSECT

Time: 1 hour setting up and 1 hour to identify

## Syllabus reference: Option C.1

**Skill**: Use of a transect to correlate the distribution of plant or animal species with an abiotic variable

## Materials

**Trap line:** String can be marked at five metre intervals with marker tape.

**Making pitfall traps:** To make the traps, collect plastic bottles such as *Evian* water bottles. Using a cutter, cut off the base of the bottle about 14cm high. Using scissors, make cuts 2cm deep at 4cm intervals around the cut edge and fold this inward.

*Warning: Sharp instruments. Take care to cut away from yourself.*


Cut off the coned top from what is left of the bottle and discard this. Cut out a panel, about 10cm wide from remaining sleeve and discard this also. The remaining section can be stretched over pegs (wooden barbecue skewers), placed around the trap, acting as a roof. Leave a 1cm space between the roof and the soil surface.

## Method

The line transect could be set up to look at relative abundance in a number of situations:

**For example**: Field-transition zone-wood.

Along a succession zone lake to woodland.

The traps could be left for various intervals of time: 24 hours, 12 hours, morning/afternoon.

### The Key

This will have to be relevant to your local habitat. Here, the following were used:

***Insects of Britain and Northern Europe:*** Michael Chinery; Collins ISBN 0 00 219216 0

***Grassland studies:*** Juliet Brodie; George Allen and Unwin ISBN 0 04 57 40208

***Animals under logs and stones:*** C. Philip Wheater & Helen J. Read; Naturalists Handbook #22 Richmond Publishing ISBN 0 85546 301 5

### Record sheet

In the record table, Order has been given as the lowest level of classification for most groups. However, a few groups have only been classified to the level of Class where further identification is difficult.

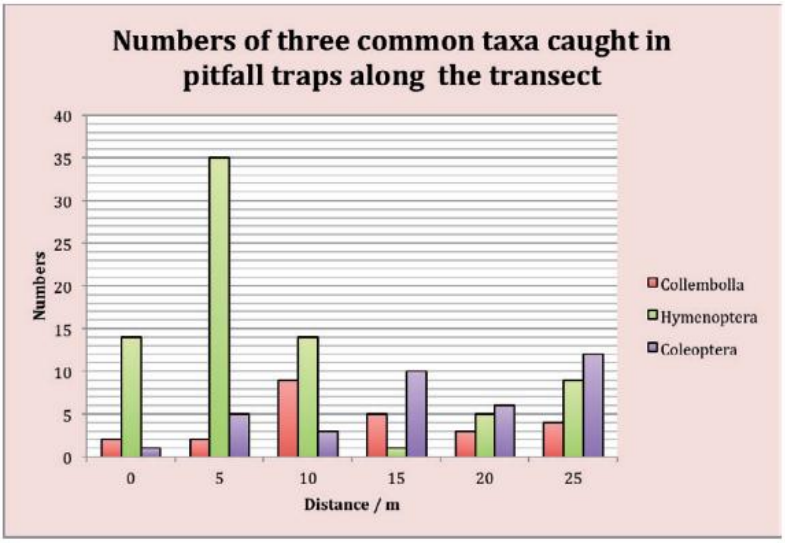
**Warnings**: *Work in the field has its risks:*
*The teacher should have visited the site first, obtained permission from and owners to work there and verified any safety risks.*
*The weather forecast for the day needs to be consulted.*
*Adequate protection from cold or wet conditions must be provided for.*
*Excessive sunlight and heat must be also considered.*

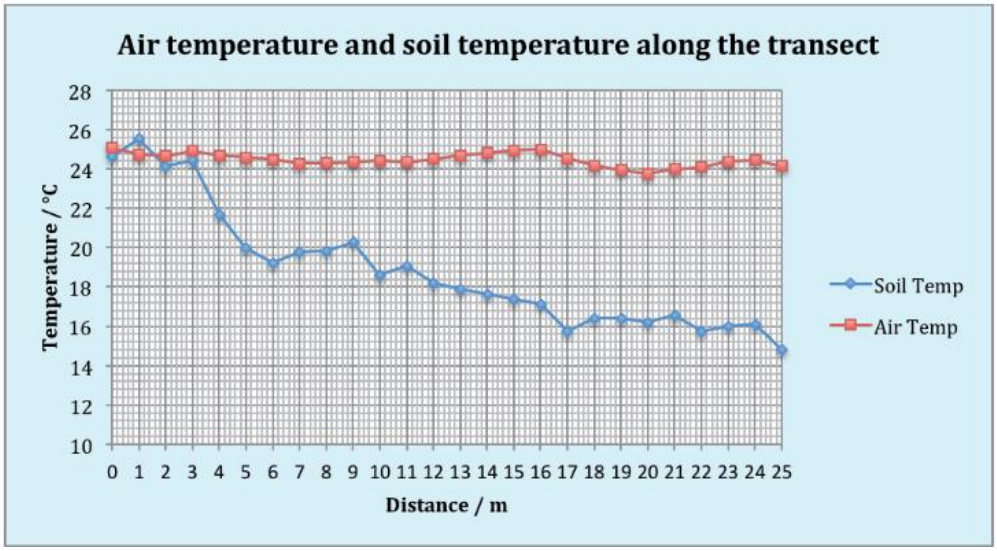
## Data

Data from five parallel transects, placed from a lawn to a woodland the transition being at 10-15m.

The data logging material used to obtain these results is from Vernier ®.

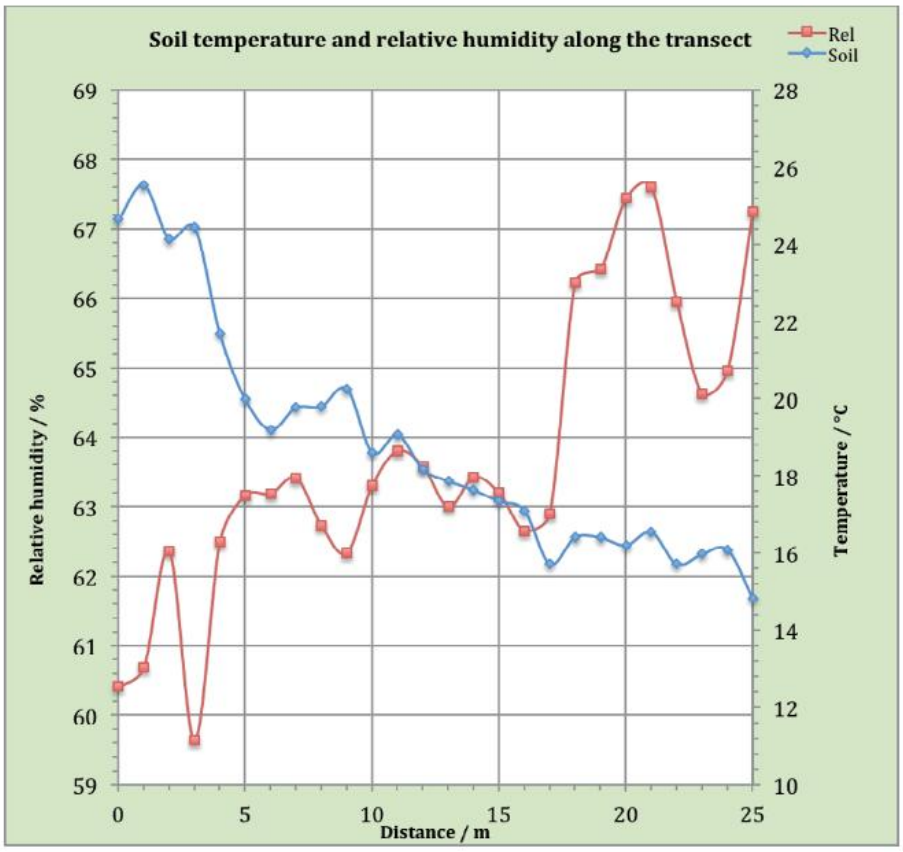
| Habitat | Distance / m | Acarina | Araneae | Isopoda | Diplura | Protura | Collembolla | Thysanura | Orthoptera | Dermaptera | Diptera | Hymenoptera | Coleoptera | Class Diplopoda | Class Chilopoda | Class insecta | Class Mollusca | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lawn | 0 | | 7 | 1 | | | 2 | 1 | | | | 14 | 1 | 3 | | | | 29 |
| | 5 | | 5 | | | | 2 | 1 | | 3 | | 35 | 5 | | 1 | 1 | | 53 |
| Transition | 10 | | 4 | | 1 | | 9 | | 2 | 1 | | 14 | 3 | | 1 | | | 35 |
| | 15 | 3 | 8 | | | | 5 | | | | | 1 | 10 | 1 | | | | 28 |
| Woods | 20 | 1 | 7 | 1 | | | 3 | | | | | 5 | 6 | 1 | 1 | | 1 | 26 |
| | 25 | 1 | 7 | | | | 4 | | | 1 | 23 | 9 | 12 | | | | | 57 |



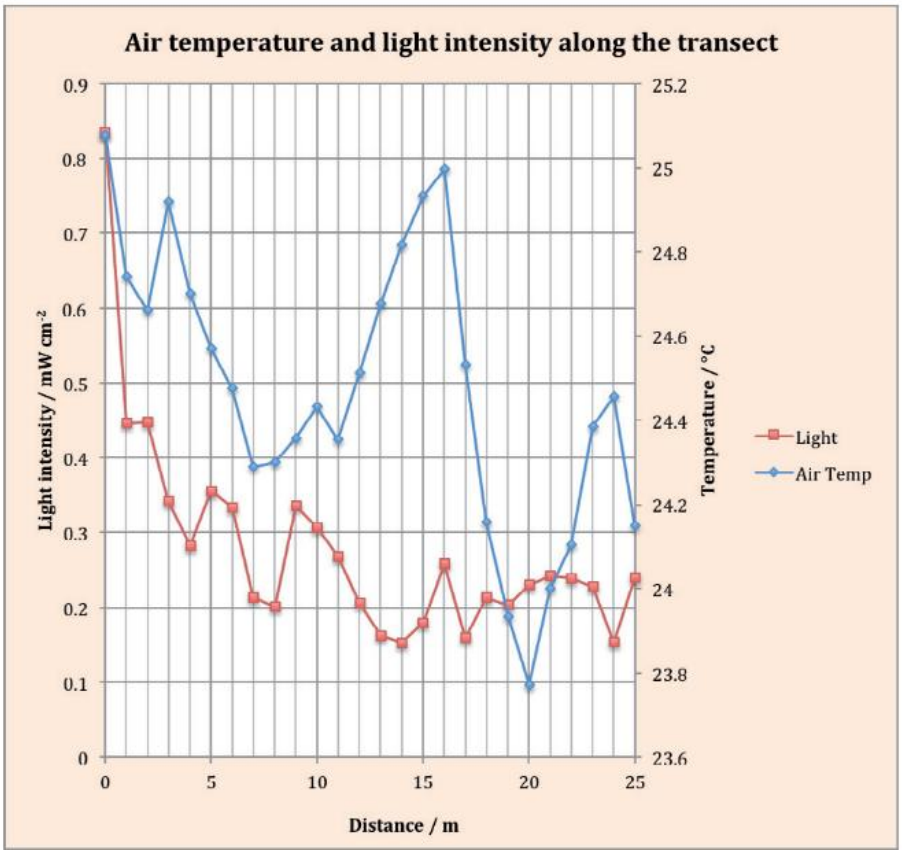




Profiles of various abiotic factors, measured at 1m intervals along the transect.

The air temperature can be seen to remain more stable across the transect than the soil temperature, which drops as the transect penetrates the woodland.




There is a clear inverse relationship between the soil temperature and the relative humidity.

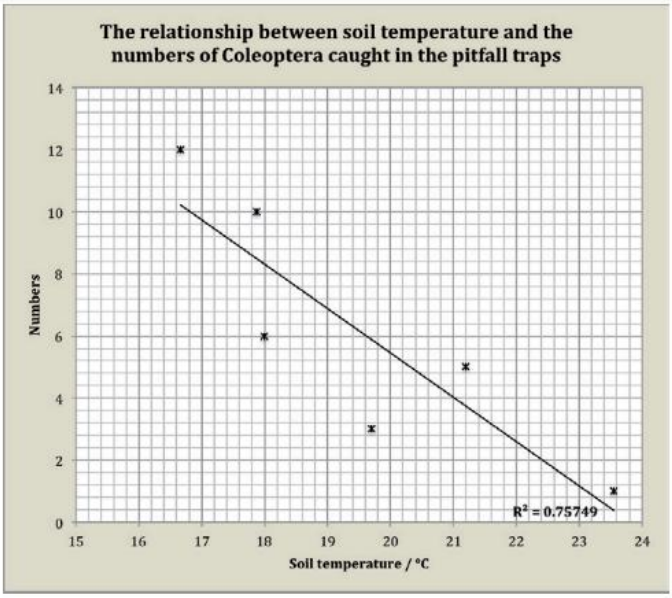



Here, the light intensity and the air temperature show a direct relationship.

Looking at these profiles and the distribution of the animal groups it may be possible to identify a relationship.

The Coleoptera numbers in the traps appear to rise as the soil temperature falls.

Plotting soil temperature values for the positions of the six traps gives a negative correlation, as expected. The coefficient of determination (R2) shows that the trend is quite a strong one.




| Soil temp / °C | Coleoptera numbers |
|---|---|
| 22.2 | 1 |
| 19.8 | 5 |
| 17.5 | 3 |
| 15.6 | 10 |
| 15.4 | 6 |
| 15.3 | 12 |
| r = | -0.796 |

This suggests a negative correlation, though the sample size is very small.

This does not mean that the soil temperature directly affects the numbers of Coleoptera. It could be some other factor that influences the beetles, such as their food, which is in turn influenced by soil temperature. Furthermore, Coleoptera is a very large Order of the insects, each species will have its own niche.

## To investigate further

- Measuring, for example, the number of Coleoptera per unit area could be carried out by sampling a given area or volume of soil or even by mark and recapture methods.
- Project work could be carried out on distribution and abundance at different time intervals, including seasonal influence.
- Investigating the other factors influencing the animals caught in the traps (e.g. the presence of bait in the traps, the position of the traps, other factors such as soil pH)

# 14C MEASUREMENT OF A DIVERSITY INDEX AND A BIOTIC INDEX

Time: 4 hours

## Syllabus reference: Option C.2

**Skill**: Investigation into the effect of an environmental disturbance on an ecosystem.

## Syllabus reference: Option C.4

**Skill**: Analysis of the biodiversity of two local communities using Simpson's reciprocal index of diversity.

**Required knowledge:** Biotic and abiotic factors. Agents bringing about the breakdown of organic material in water systems.

## Materials and Method

A heavy, robust, long-handled (1m to 1.5m long) pond net is good, as the currents can be quite strong.

Sorting trays, such as the one seen in the photos are ideal for sorting the animals into groups, these trays are also used for presenting geological specimens.

**White material**: bowl, sorting tray and plastic teaspoons for sorting, small organisms are easier to observe against a white background.

Plastic pipettes are more practical than glass for use in the field.

Rubber gloves are also useful for warmth and possible protection against waterborne pathogens.

Hand lens: ×10 magnification should be sufficient for most field observations.

Tools can be marked with bright insulating tape or paint to prevent loss.

### Keys

Much time is taken in working through the keys for precise identification of the organisms.

However, it is a good exercise to key out to species whenever this is possible.

Some observations for identifying specimens may require a binocular microscope, so a few specimen bottles would be useful in the field to collect difficult specimens for later identification in the laboratory.

*White bowls, white plastic spoons and white geological sorting trays are useful when searching for animals.*

*A flow meter is a very useful tool when studying river systems. It measures the flow rate nearer to where the animals are living.*

## Measuring abiotic factors

| Measurement | Method |
|---|---|
| Depth of the river (m) | Metre ruler |
| Width of the river (m) | Metre ruler or marked string |
| Water current (ms-1) | Flow meter or the time taken for a leaf or a small branch to float one metre distance |
| Temperature (° C) | Temperature probe or thermometer |
| Oxygen (mg $dm^{-3}$) | Oxygen probe or oxygen test |
| pH | pH probe |
| Light (Wm$^{-2}$ or lx) | Light meter at 1 metre above the surface |

## Oxygen

An oxygen probe is practical, however, they are expensive and they need to be calibrated before use if an absolute value is required. The percentage saturation of oxygen takes into consideration the water temperature. It can be calculated from the table below, that shows the oxygen concentration of fully saturated water:

### Dissolved $O_2$ (mg $dm^{-3}$)in saturated water at a given altitude / m

| °C | 0 | 200 | 400 | 600 | 800 | 1000 | 1500 | 2000 |
|---|---|---|---|---|---|---|---|---|
| 0 | 14.64 | 14.29 | 13.93 | 13.57 | 12.2 | 12.91 | 12.11 | 11.39 |
| 1 | 14.22 | 13.89 | 13.54 | 13.18 | 12.83 | 12.55 | 11.77 | 11.07 |
| 2 | 13.82 | 13.51 | 13.16 | 12.82 | 12.48 | 12.20 | 11.45 | 10.76 |
| 3 | 13.44 | 13.14 | 12.81 | 12.47 | 12.14 | 11.87 | 11.13 | 10.47 |
| 4 | 13.09 | 12.79 | 12.47 | 12.14 | 11.82 | 11.55 | 10.84 | 10.19 |
| 5 | 12.74 | 12.46 | 12.14 | 11.82 | 11.51 | 11.25 | 10.55 | 9.92 |
| 6 | 12.42 | 12.14 | 11.83 | 11.52 | 11.21 | 10.97 | 10.28 | 9.67 |
| 7 | 12.11 | 11.84 | 11.54 | 11.23 | 10.93 | 10.69 | 10.03 | 9.42 |
| 8 | 11.81 | 11.55 | 11.25 | 10.96 | 10.66 | 10.43 | 9.78 | 9.19 |
| 9 | 11.53 | 11.27 | 10.98 | 10.69 | 10.41 | 10.18 | 9.54 | 8.97 |
| 10 | 11.26 | 11.01 | 10.72 | 10.44 | 10.16 | 9.94 | 9.32 | 8.76 |
| 11 | 11.01 | 10.75 | 10.48 | 10.2 | 9.93 | 9.71 | 9.10 | 8.55 |
| 12 | 10.77 | 10.51 | 10.24 | 9.97 | 9.70 | 9.49 | 8.90 | 8.36 |
| 13 | 10.53 | 10.27 | 10.01 | 9.75 | 9.49 | 9.28 | 8.70 | 8.17 |
| 14 | 10.30 | 10.05 | 9.79 | 9.54 | 9.28 | 9.07 | 8.51 | 7.99 |
| 15 | 10.08 | 9.84 | 9.58 | 9.33 | 9.08 | 8.88 | 8.32 | 7.82 |
| 16 | 9.86 | 9.63 | 9.38 | 9.14 | 8.89 | 8.69 | 8.15 | 7.65 |
| 17 | 9.66 | 9.43 | 9.19 | 8.95 | 8.70 | 8.51 | 7.98 | 7.49 |
| 18 | 9.46 | 9.24 | 9.00 | 8.76 | 8.53 | 8.34 | 7.81 | 7.34 |
| 19 | 9.27 | 9.05 | 8.82 | 8.59 | 8.36 | 8.17 | 7.66 | 7.19 |
| 20 | 9.08 | 8.88 | 8.65 | 8.42 | 8.19 | 8.01 | 7.50 | 7.05 |
| 21 | 8.90 | 8.70 | 8.48 | 8.25 | 8.04 | 7.85 | 7.36 | 6.91 |
| 22 | 8.73 | 8.54 | 8.32 | 8.10 | 7.88 | 7.70 | 7.21 | 6.77 |
| 23 | 8.57 | 8.38 | 8.16 | 7.94 | 7.73 | 7.55 | 7.08 | 6.64 |
| 24 | 8.41 | 8.22 | 8.01 | 7.79 | 7.58 | 7.41 | 6.94 | 6.52 |
| 25 | 8.25 | 8.07 | 7.86 | 7.65 | 7.44 | 7.27 | 6.81 | 6.39 |
| 26 | 8.11 | 7.92 | 7.72 | 7.51 | 7.30 | 7.14 | 6.69 | 6.28 |
| 27 | 7.96 | 7.78 | 7.58 | 7.37 | 7.17 | 7.04 | 6.57 | 6.16 |
| 28 | 7.82 | 7.64 | 7.44 | 7.24 | 7.04 | 6.88 | 6.45 | 6.05 |
| 29 | 7.69 | 7.51 | 7.31 | 7.11 | 6.92 | 6.76 | 6.33 | 5.94 |
| 30 | 7.56 | 7.38 | 7.18 | 6.99 | 6.80 | 6.64 | 6.22 | 5.83 |

## The river

Two or three different stations could be chosen for example:

(a) above and/or below a dam

(b) in tributaries and above and in the main river

(c) at a shaded area and unshaded area of the river

(d) in fast, lotic, and slow, lentic, areas

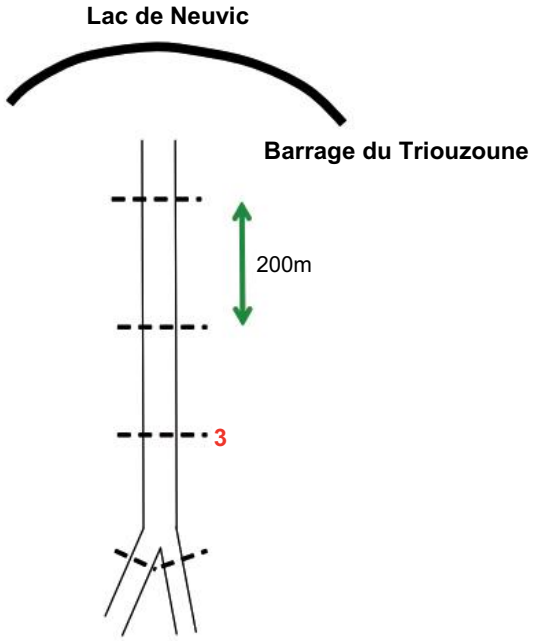
(e) in a stretch of the river running through a town and a stretch running through a country area

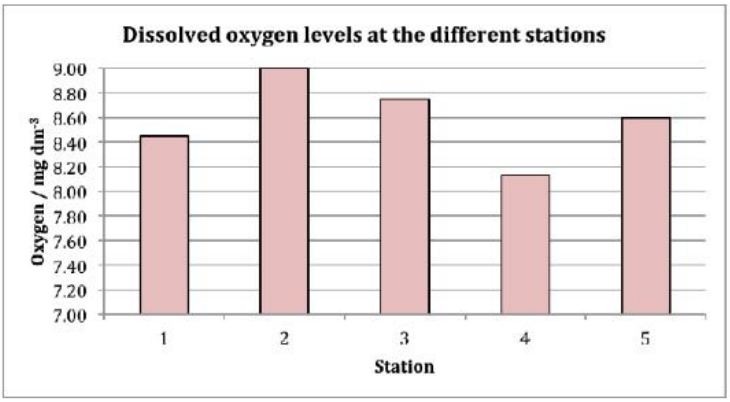
The water should not be too deep or too fast, it should be possible to cross the river (to measure the width). It should be fairly easy to clear an area of the large stones or small rocks for the kick sample. Check that you are allowed to work in this part of the river. Finally, ensure that there are no known sources of pollution in the vicinity, for health reasons.

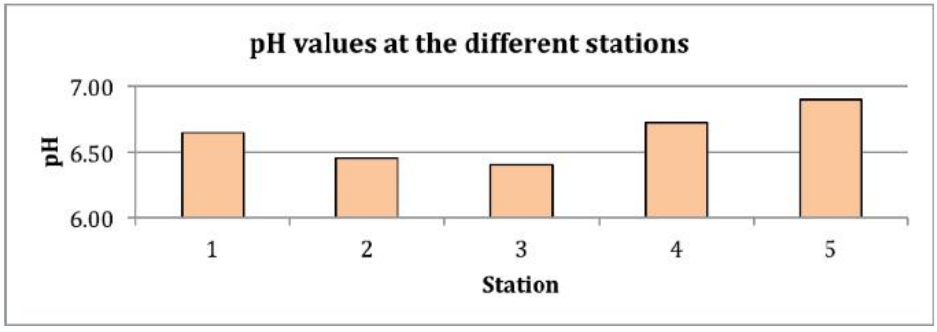
*Warning: Polluted areas must be avoided for health reasons*


## Data

The following data were taken from a river flowing below a dam. Authorisation from the electricity generating agency was obtained prior to the visit.



**Benthic animals captured by kick sampling in the River Triouzoune, Corrèze, France.**

| Organisms | STATION | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| Plecoptera | Leuctridae | | | | | |
| | Nemouridae | | | | | |
| | Perlidae | | 1 | | | |
| Ephemeroptera | Baetedae | | | | | |
| | Ecdyonuridae | | | | | |
| | Ephemerellidae | | | | | |
| | Ephemeridae | | | | | |
| | Leptophlebidae | | | | | |
| Trichoptera | | | | | | |
| With cases | Limnephilidae | 1 | | 2 | 4 | |
| | Glossosomatidae | | | | | |
| | Odontoceridae | | | | | |
| | Sericostomatidae | | | | | |
| Without cases | Hydropsychidae | 1 | | | | 1 |
| | Philopotomidae | | | | | |
| | Polycentropedie | | | | | |
| | Rhyacophilidae | | 5 | 12 | 1 | 4 |
| Odonata | Zygoptera | | | | 1 | |
| | Anisoptera | | | | | |
| Megaloptera | Sialidae | | | | | |
| Crustacea | Gammeridae | | | | | |
| | Asellidae | | 2 | | | |
| Gastropoda | Ancylidae | | | | | |
| Diptera | Simulidae | 2 | | | | |
| | Chironomidae | 2 | 1 | | | |
| Coleoptera | Dytiscidae | | | | | |
| | Hydrophilidae | | | | | |
| | Helodidae | | | | | |
| | Elminthidae | | | | | |
| Hirudinea | Glossiphonidae | | | | | |
| | Hirudinae | | 6 | 14 | 7 | 6 |
| Oligochaeta | Tubificidae | | | | | |
| Others | Worm | | | 5 | | |
| | SIMPSON'S INDEX | 7.5 | 4.0 | 3.1 | 2.9 | 2.6 |
| | BIOTIC INDEX | 5 | 6 | 5 | 5 | 5 |

## The Biotic index and the Diversity index

The Biotic index used here is based on that of Trent and Verneaux. For the purpose of this exercise, the above Biotic indices of Trent and Verneaux have been simplified, requiring identification to be carried out only as far as Family. It is considered that it will be fairly rare to find more than one species of the same Family in any one site. Also, considering the accuracy of the identification possible in the field in the time given, identification to Family is sufficient.

In the above-mentioned biotic indices, Trent excludes *Baetis rhodani* from the Ephemeroptera line, including it with the Trichoptera, Verneaux does not. Verneaux classes Ecdyonuridae with the Plecoptera line, Trent does not. A number of other differences arise between the biotic indices of Trent and Verneaux. In this exercise, for simplicity, all organisms will be classed within their major representative group.

Many biotic indices exist and all vary in detail. Ideally, each region should develop a biotic index to accommodate for local conditions and particularities of the organisms found. This simplified exercise does still give a good idea of the biotic index and compares well with results obtained using the indices of either Trent or Verneaux.

Biotic indices do not take into account abundance, so a unique appearance of 1 Plecoptera will considerably increase the index, even if a low diversity is seen. For a diversity index, the equitability between the species is important. One useful exercise is to compare the index measured with what it would be if all the species found were in equal numbers. This would give the theoretical maximum diversity index for a site ($D_{max}$).

The biotic index is an indication of organic pollution. Other forms of pollution, such as toxic pollution, may lead to a low diversity of organisms being present or high numbers of those groups tolerant to a particular pollutant. In the results table for this exercise only organisms more than 0.5cm are considered, so Hydrocarina and Platyhelminthes do not appear.

## Some points for consideration

- Organic pollution reduces the oxygen content of the water, so those organisms most sensitive to oxygen, such as Plecoptera will be absent in poorly oxygenated water.
- Stress will reduce the species diversity. Species sensitive to the stress will fall in numbers or even disappear. Species that tolerate the stress will increase in numbers. The environment will fit the fundamental niche of the tolerant species more and more and their will be less competition.
- One possible source of pollution could be agricultural run off (this could include animal waste, other fertilisers and pesticides). This leads to organic pollution and low biotic indices. To see if agricultural run off has an effect on the river organisms, sampling could be carried out by a farm and compared to an area where there is no run off.
- Even in areas where human impact is quite low, dead leaves falling in the river from surrounding woodland can have their impact. The organic mater added to the river during the autumn leaf fall may contribute significantly to the energy imput in the river and at the same time its decomposition will consume dissolved oxygen.
- The biotic index and diversity index of the river should only be indicative of that particular site. Many factors influence the biotic index found.

**Geographical region**: Different representative groups will be found in different regions. An absence of a particular group may just be because it does not live here. Repeating sampling and collecting data over many years would reveal this.

**Season**: Some organism may be absent at certain periods in the year. Sampling should be carried out at different times of the year.

**Current**: Some organisms are better adapted to fast flowing water than others.

Sampling could be carried out in areas of the river with different currents.

**Distance from a source of pollution:** The Biotic Index would appear low near the source of pollution. Sampling could be carried out at various distances from a known source of pollution.

**Other factors** that may influence the biotic index or the diversity index: light, and temperature of the water, introduced alien species, such a pond weed.

To see the evolution of a section of river over the course of time it is important to always sample at the same area at regular intervals of time.

# 15A ENERGY FROM FOOD

Time: 1.5 hours

## Syllabus reference: Topic D.1

**Skill**: Determination of the energy content of food by combustion.

## Materials

Dry food samples: Potato chips/crisps, cocktail snacks e.g. *Curlie Wurlies*, and dried fruits can be used.

*Warning: As nut allergies are quite common, foods containing nuts or products of nuts (e.g. peanut oil) should be avoided*


## Analysing the results

Some pieces of food appear to give more energy than other pieces of the same kind of food. There is a simple reason for this. What is it?

Not all pieces of food are the same size.

It would be simple to compare the results if everyone had used exactly the same amount of food. Is this a practical solution?

What must be done to each result in order to compare your results with the results of others?

Students may suggest cutting the food samples to be exactly the same mass, this is not realistic. Measuring the mass of the food samples, so the energy per unit mass of food is obtained, will permit a valid comparison. Nevertheless, the food samples need to be about the same size and not so large that the water reaches boiling point. If the water boils, the temperature will not rise any further.

## Evaluate this method and suggest improvements

When the food samples are burned, ideally, ash is all that should remain. All the organic matter should have been oxidised. In reality, the food samples are rarely completely burned.

When the food sample burns, all the energy released should be transmitted to the water in the test tube. This is not the case. Energy is lost when the Bunsen ignites the food sample. When the burning food is transported to the test tube and while the burning food is under the test tube, energy radiates out in all directions, not exclusively into the test tube.

Enclosing the test tube in a chamber would limit the radiation of the energy from the burning food sample.

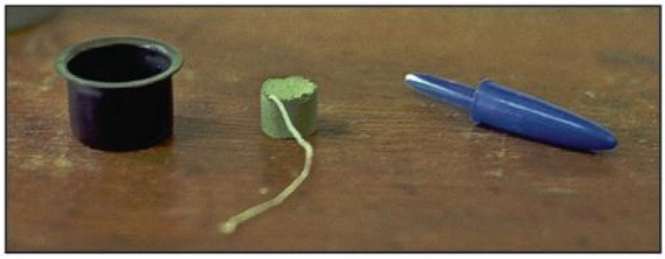
Instead of a test tube, a beaker with a larger flat bottom may give more realistic values.

## Research

Find out how a bomb calorimeter works and compare it to your method.

The bomb calorimeter works on the same principles as this experiment, however, the combustion is much shorter and the burning sample is enclosed in a vessel surrounded by an accurately measured volume of water.

The food sample is ground up and pressed into a pellet of given volume with a short cotton fuse.

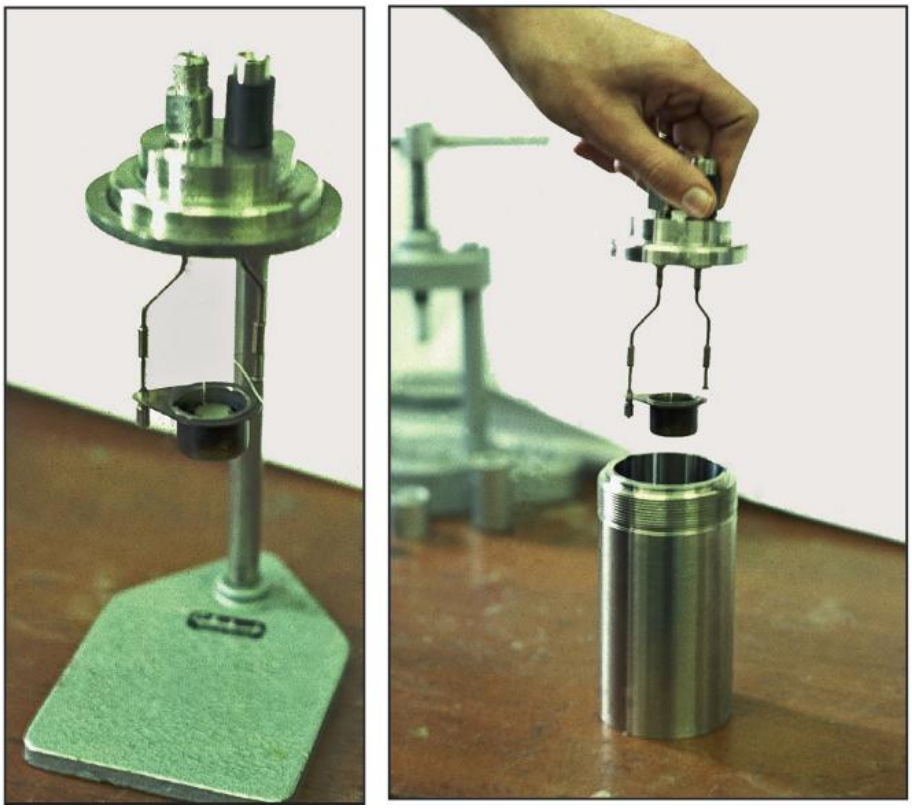


*Pellet of dried food with the cotton fuse (centre)*

*Bomb vessel (right) and the bucket that holds the water (left)*

It is massed and suspended in the bomb vessel. This vessel is made of a metal alloy that rapidly transmits the heat energy released. The bomb vessel is filled with pure oxygen and connected to an electricity supply. The whole vessel is immersed in an insulated bucket of water of known volume.

The cotton fuse lies across a wire connected to the electric terminals inside the bomb vessel. When the electricity is switched on, the wire heats, the fuse burns and the food sample burns very quickly in the pure oxygen (it explodes). The temperature of the water is measured before the explosion and during the ignition. Because the release of energy is so short, a more precise estimate of the energy content is obtained.



*The bomb vessel being loaded with a food sample.*

The bomb calorimeter needs to be calibrated to determine the specific heat capacity of the materials that is it made from.

# 15B HEART RATE AND BLOOD PRESSURE

Time: 1.5 hours

## Syllabus reference: Topic D.4

**Skill**: Measurement and interpretation of the heart rate under different conditions

**Skill**: Interpretation of systolic and diastolic blood pressure measurements

## Materials

**Stethoscope**: Make sure the ear pieces are properly cleaned before and after use. Certain models "switch" on and off by rotating the diaphragm head.

**Heart rate monitor:** Monitors that use a probe attached to the chest may need to be moistened or humidified on the surface facing the skin before they are attached.

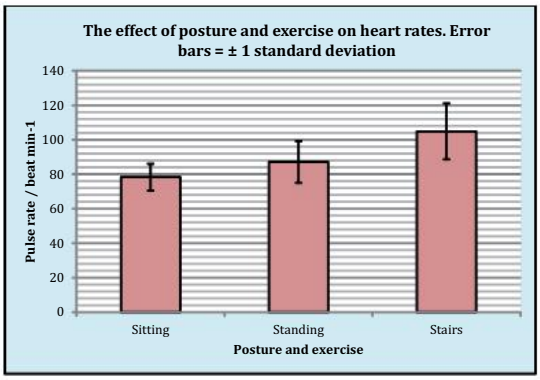
## Method

Investigations on heart rates and blood pressure can be combined with investigations on the ECG and ventilation rates.

## Data

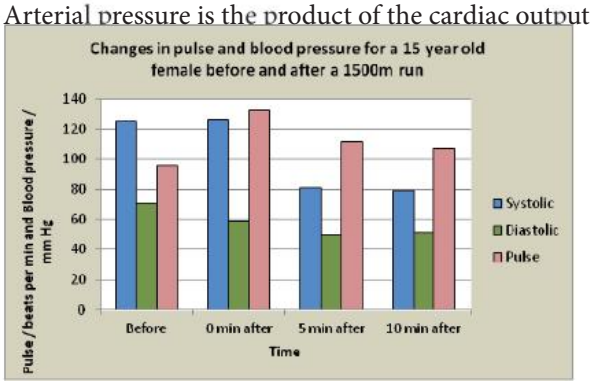
### Heart Rate and Posture

The pulse increases slightly between sitting and standing. It increases a little more with a bit of light exercise.




### Blood Pressure and Exercise

Arterial pressure is the product of the cardiac output and the peripheral resistance to blood flow.

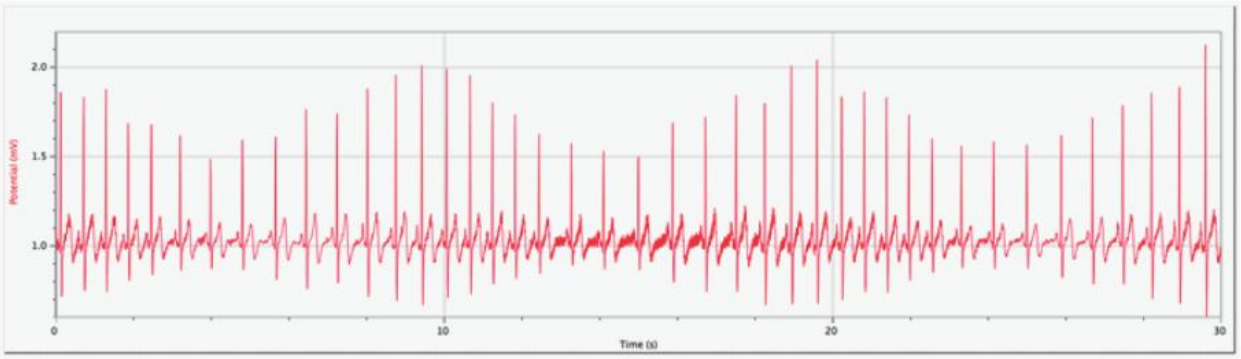


Increases in cardiac output will increase the systolic pressure, while an increase in peripheral resistance will increase the diastolic pressure. Vasoconstriction of blood vessels increases the resistance to blood flow and will increase the difference between systolic and diastolic pressure. After 1500m of running, the vasodilation of the arterioles in the muscles will reduce the resistance to blood flow so the diastolic pressure is lowered. The systolic pressure has not changed that much indicating this is probably a fit individual.

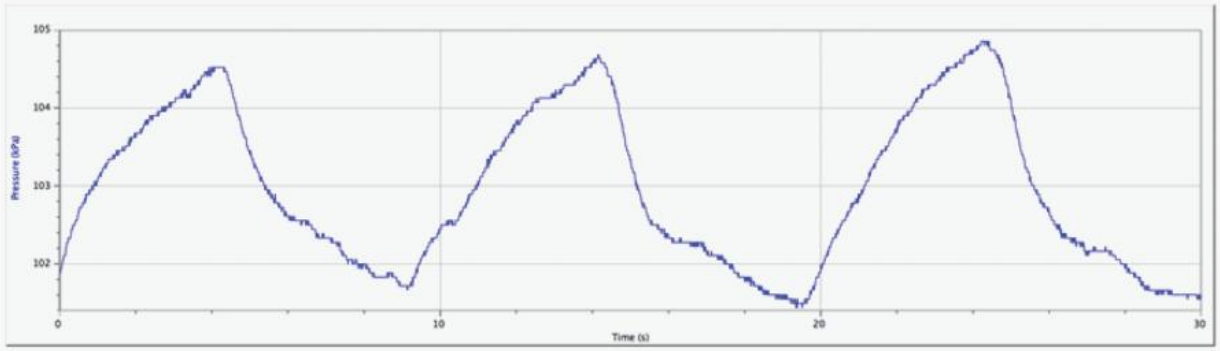
What is interesting is that the pulse pressure (the difference between the systolic and diastolic pressure) has increased. This is normal, the resistance to blood flow has dropped and the stroke volume of the heart (blood pumped per beat) has increased. This large difference in pulse pressure drops back within 5 minutes of resting.

The diastolic pressure may lower even further after running as the skin arterioles dilate further to radiate heat from the body. While running, the wind chills the skin by causing the evaporation of sweat. This effect stops when resting but there is still a lot of heat to be dissipated just after a run.

## Heart Rate and Ventilation




**ECG trace over 30s during 10s inspiration expiration cycle**




**Ventilation over 30s during 10s inspiration expiration cycle**

Using the ECG sensor can reveal some interesting relationships. The heart rate tends to increase as a person breathes in. Here the intervals between the ventricular contractions (the spikes) are seen to get smaller during inhaling and wider during exhaling. Stretch receptors in the lungs inhibit the cardio-inhibitory centre of the brain, thus the heart rate speeds up during inhaling and slows during exhaling. This is normal and it is called sinus arrhythmia.

The amplitude of the ECG trace may also change as the subject breaths due to the change in orientation of the heart during breathing.

# 15C TAKING AND READING AN ELECTROCARDIOGRAM

Time: 1 hour

## Syllabus reference: Topic D.4

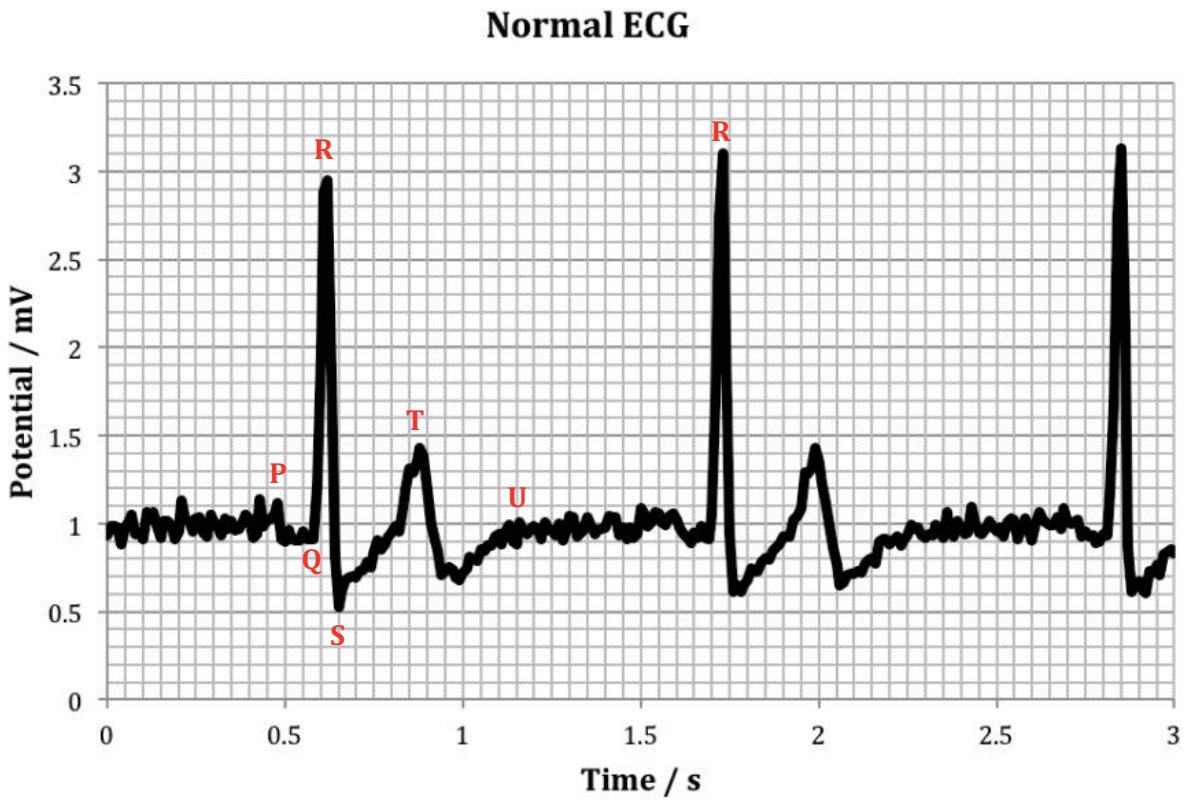
**Skill**: Mapping of the cardiac cycle to a normal electrocardiogram (ECG) trace

## Data

The ECGs taken by an apparatus in school are not of the same quality and precision as those used by medical personnel. Doctors use more electrodes placed around the heart. Thus, though the readings can be used to investigate the electrical activity of the heart, they should not be relied upon for medical diagnosis.

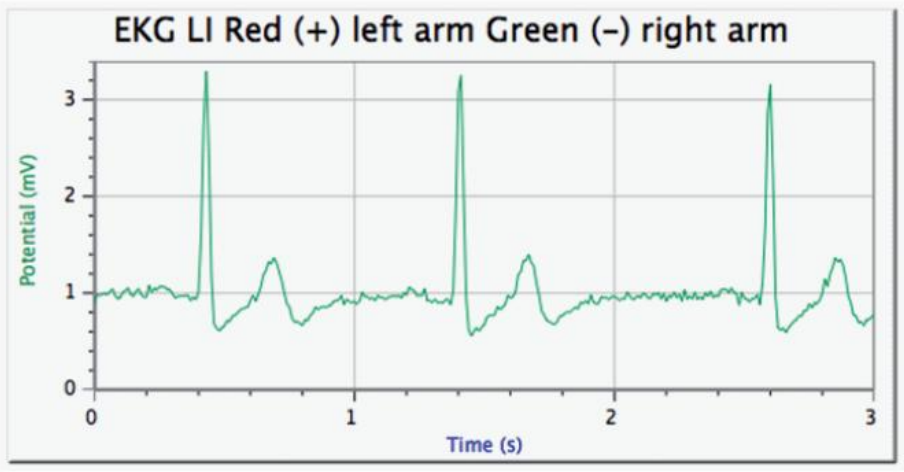
### Reading an ECG

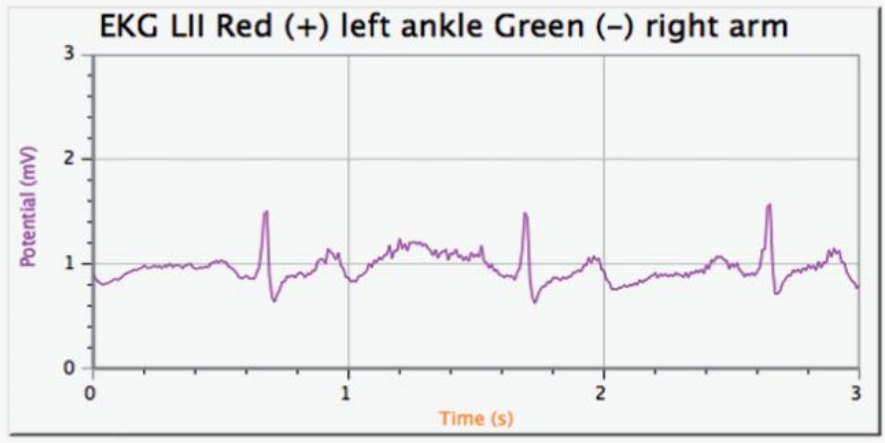
### Observations from the ECG

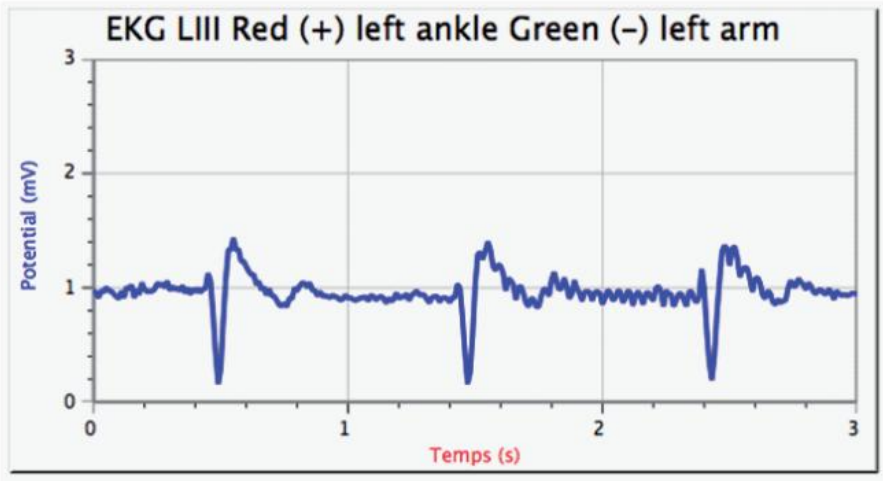



- The cardiac rhythm from R to R = 1.1s which gives a fairly slow heart rate.
- The conduction of the wave of contraction from the atria to the ventricles P to R = 0.15s
- The QRS complex = 0.075s
- The ventricular activity, Q-T = 0.3s R-R = 1.1s
- So, Q-T/R-R = 0.27 which is less than half of R-R, as it should be.

## Analysing the axis of the heart




The highest QRS amplitude is at position 1 for this subject. Therefore, the angle of the heart is about 0° to the norm.

# 7A THE NUCLEOSOME

**Time:** 1 hour

## Syllabus reference: Topic 7.1

Skill: Utilization of molecular visualization software to analyse the association between protein and DNA within a nucleosome.

## The nucleosome structure

- There are 4 pairs of histones in the core (an octomer).
- The histones are arranged as a disk with the DNA molecules wrapped around the edge.
- The DNA strand is wrapped twice around the core particle.
- The DNA is also held in place by another histone, H1.

## Basic amino acids

Histidine (H), Arginine (R) and Lysine (K) in the FASTA representation of the core histones.

## Proportion of basic amino acids

**H2B**

MPEPAKSAPAPKKGSKKAVTKAQKKDGKKRKRSRKESYSVYVYKVLKQVHPDTGISSKAM
GIMNSFVNDIFERIAGEASRLAHYNKRSTITSREIQTAVRLLLPGELAKHAVSEGTKAVT
KYTSAK

**H2a.a**

MSGRGKQGGKARAKAKTRSSRAGLQFPVGRVHRLLRKGNYSERVGAGAPVYLAAVLEYLT
AEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNKLLGRVTIAQGGVLPNIQAVLLPKK
TESHHKAKGK

**H3.1**

MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTE
LLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACEAYLVGLFEDTNLCAIHAKRVTI
MPKDIQLARRIRGERA

**H4**

MSGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGVLK
VFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQGRTLYGFGG

| Histone | H2B | H2a.a | H3.1 | H4 |
|---|---|---|---|---|
| **Total amino acids** | 127 | 131 | 137 | 104 |
| Basic amino acids | 31 | 30 | 33 | 27 |
| % | 24 | 23 | 24 | 26 |

Nearly a quarter of the amino acids in these proteins are basic. Their position in the structure of the protein will also be important. Being polar amino acids they will probably be present on the outside surface.

## Histones are conservative proteins

In the example shown 98% of the Japanese rice fish amino acids are the same as human amino acids in the H3.1 sub-unit. This is extremely conservative for two organisms that are quite distantly related. This makes sense as the histones have a vital role in packaging the DNA molecules. Packaging the DNA in the nucleus not only manages to store this large molecule efficiently but it also permits controlled access to genes that are being expressed in a cell. If the histones were subject to variation through mutation, gene expression would be affected. This would be rapidly eliminated by natural selection.

# 8A INHIBITORS OF UREASE

Time: 1 to 1.5 hours

## Syllabus reference: Topic 8.1

Skill: Distinguishing different types of inhibition from graphs at specified substrate concentration

Required knowledge: Enzyme inhibition, if this is to function as an illustrative investigation.

## Materials

**Urease:** The urease used in this investigation is an extract from Jack Beans and it had an activity of 11200 units $g^{-1}$. The suspension needs to be thoroughly mixed before samples are taken. If it is prepared the previous day its activity will increase.

Universal pH liquid indicator is added directly to the reaction mixture to provide a visual check. It does not seem to affect the enzyme's activity. Colour charts on the packets of universal indicator paper are useful for following the pH changes in the test tubes. However, a series of tubes of given pHs coloured with the indicator is better.

Pieces of indicator paper suspended from bungs inserted on top of the test tubes are an alternative to adding the liquid indicator to the tubes. The indicator paper should be moistened with distilled water.

**pH 4 Citrate buffer:** 38.55$cm^3$ of 0.2 mol $dm^{-3}$ $Na_2HPO_2$ mixed with 61.45$cm^3$ of 0.1 mol $dm^{-3}$ citric acid.

This is used to reduce the pH at the beginning of the experiment and it also serves to slow down the reaction. Without the buffer the same mixture would take less than four minutes to reach its end point. The differences between the flasks would be difficult to judge.

Urease has an optimum activity around pH 5.

Lead nitrate should be disposed of according to local regulations. Other heavy metals could be substituted e.g. zinc or copper salts can be used. The results shown below were obtained using apparatus from Vernier®. It was analysed using their program Loggerpro®.

## Method

**Alternative method: Urease tablets may be used instead.**

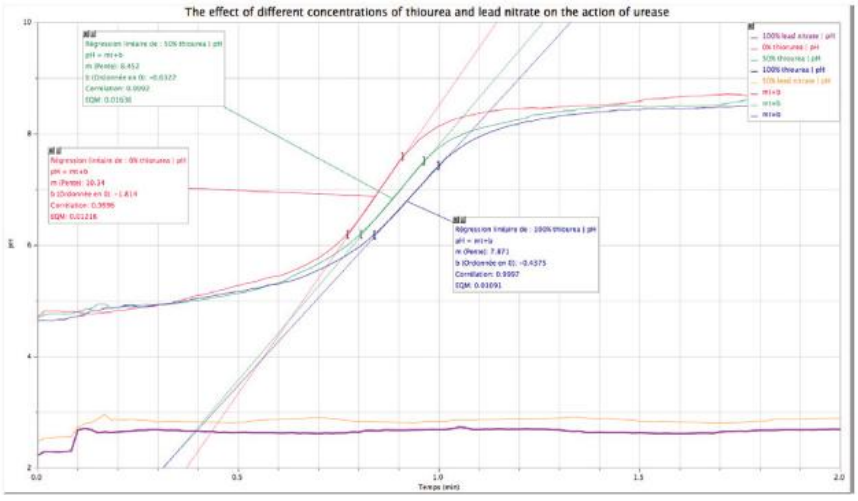
Add 2 $cm^3$ of buffer to each tube and 1 urease tablet. 10% Urea (1.67 mol $dm^{-3}$),

10% thiourea (0.61 mol $dm^{-3}$), 10% lead nitrate (0.3 mol $dm^{-3}$). Follow the method as indicated in the Student's guide.

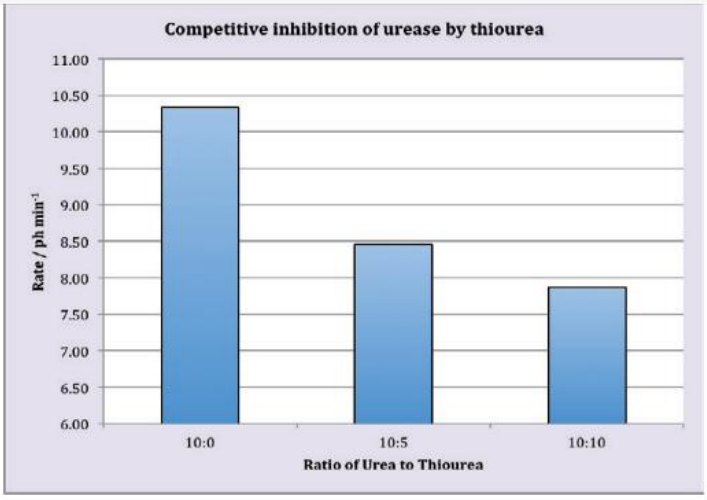
## Data

Using the solutions given, the reaction should give clear enough results in 30 minutes. Flask 1 turns basic first, followed by flask 2 and then flask 3. All these flasks ultimately arrive at the highest pH on the scale. Flasks 4 and 5 do not change at all. In fact flasks 4 and 5 become more acidic as lead nitrate is an acid salt.

Using the linear fit function on the graph it is possible to determine the rate of change in pH. The rate gets slower as the concentration of thiourea increases.



## Some points for consideration




- Thiourea is inhibiting urease activity but its effect is a function of its concentration. It behaves as a competitive inhibitor. The similar chemical structure or urea $CO(NH_2)_2$ and thiourea $CS(NH_2)_2$ can be used to suggest this hypothesis. Lead nitrate inhibits urease regardless of its concentration. Lead nitrate seems to be a non-competitive inhibitor or possibly irreversible inhibitor. Heavy metals, such as lead, tend to bind reversibly with the –SH group of the amino acid cysteine. Lead nitrate is an acidic salt, so students might propose that the acidic pH of the tubes 4 and 5 inhibit the enzyme activity.
- The real nature of the inhibition expressed here would require a more thorough investigation involving several substrate concentrations for the same inhibitor concentration. Competitive inhibitors do not reduce the maximum velocity of the enzyme ($V_{max}$) where as non-competitive inhibitors do.

## To investigate further

- This method could be extended further to investigate the effect of substrate concentration or temperature on enzyme activity.
- Other heavy metals can be substituted for lead. Copper and zinc have been suggested but iron and nickel can be tried too. Once again, local regulations on the disposal of toxic compounds must be adhered to.

# 9A TRANSPIRATION IN PLANTS

Time 1.5 hours

## Syllabus relevance: 9.1

Skill: Measurement of transpiration rates using potometers. (Practical 7)

Skill: Design of an experiment to test hypotheses about the effect of temperature or humidity on transpiration rates.

## Materials

Lime (Tilia) and poplar (Populus) branches work well. The branches should be freshly cut. They can be kept in water in a cool place for up to 24 hours.

The capillary tubing should be 40cm long.

The rubber tubing on the end of the capillary tubing must be soft and flexible.

To submerge the apparatus underwater a long window box is suitable.

Nail varnish or petroleum jelly (Vaseline ®) is suitable to close the wounds after removing the leaves.

## Method

Cut the branch just above a node and make sure the end of the branch is smooth before inserting it in the rubber tubing. Choose branches which are a bit bigger than the diameter of the rubber tubing.

The apparatus needs to be set up again if any air bubbles are in the tubing apart from the one introduced in the glass end. An alternative way of introducing an air bubble is by gently squeezing on the rubber tubing to push a drop of water out of the glass end, then place the end back in the water. Or dabbing the end of the tubing with a piece of absorbent paper.

## Data

The branches used here were approximately 50cm long.

| Plant | Branch with leaves | | | Branch with leaves removed | | |
|---|---|---|---|---|---|---|
| | Time for 100mm / min ± 0.1min | Rate / mm $min^{-1}$ | Volume / $mm^3$ $min^{-1}$ | Time for 100mm / min ± 0.1min | Rate / mm $min^{-1}$ | Volume / $mm^3$ $min^{-1}$ |
| **Lime** | 1.2 | 83 | 65 | 3.2 | 31 | 25 |
| **Poplar** | 1.0 | 100 | 79 | 4.5 | 22 | 17 |

## Some points for consideration

*How would the absence of leaves influence the rate of transpiration?*

The absence of leaves decreases the rate of transpiration through the plant supporting the idea that transpiration occurs through the leaves.

*What must be assumed when calculating the rate of transpiration through the branch in $cm^3$ $h^{-1}$ using the potometer?*

In calculating the rate of movement through the leaves it must be assumed that all the water taken up by the plant is lost via the leaves.

*What other unit of measurement could be found in order to make the calculation more valuable? Explain how you would carry out this measurement.*

It is interesting to calculate the surface area of the leaves, over which the water is lost, assuming that water is lost from the lower leaf surface.

This gives the units e.g. $cm^3$ $h^{-1}$ $m^{-2}$

The surface area can be found by drawing a leaf on graph paper and counting the squares. Then multiplying this by the number of leaves on the branch. Alternatively a photograph can be taken, imported into a video capture program to calculate the area using the Integral function.

## To investigate further

- Air movement and the influence of temperature by using a hair dryer on cold and hot air.
- Surface area and the rate of transpiration by using branches with different numbers of leaves.
- The rate of transpiration in different plants.
- Looking at the influence of wounds, by comparing the rate of transpiration in branches with the leaves removed applying petroleum jelly or leaving the wounds open.

# 9B STOMATA: THE SITE OF TRANSPIRATION IN PLANTS

Time: 2 hours

**Syllabus reference: 9.1**

Aim: Measurement of stomatal apertures and the distribution of stomata using leaf casts, including replicate measurements to enhance reliability are possible experiments.

## Part A Observation of the potted plant

### Materials

Choose plants with large leaves e.g. Dahlia, geranium and use transparent plastic bags such as freezer bags.

**Preparation of the cobalt chloride paper:** Leave filter paper in 5% aqueous solution of cobalt chloride solution for five minutes. Remove the papers from the solution and dry in an oven at 30°C. Keep in a dry place.

## Method

Either small bags can be placed over individual leaves or a larger bag can be used to cover the whole plant and attached to the stem to avoid evaporation from the soil surface. Once the plastic bags are attached, the potted plant should be kept in a light cupboard for 24 hours before the experiment or under a bright fluorescent lamp.

The students must not touch the cobalt chloride paper with their fingers, the squares could be placed in a closed Petri dish to transport the paper from the oven to the bench.

## Data

Condensation is seen inside the plastic bags.

The cobalt chloride paper on the lower surface turns pink faster.

## Hypotheses

Water is lost by the plant via the leaves.

More water is lost by the plant via the lower surface of the leaves.

# Part B The site of transpiration: Observation of stomata

## Materials

Choose leaves which are not hairy (e.g. ivy)

## Alternative methods

Cut the leaf into 1cm squares and glue them onto a drop of Superglue® using forceps at all times.


### *Warning Avoid skin contact with the Superglue®*

Allow the glue to dry thoroughly for five minutes. Peel away the leaf and observe the glue imprint.

The micrometer slide can be used to measure the diameter of the field view.

The surface area = $\pi r^2$

## Data

Examples of the distribution of the stomata:

| Leaf | Surface | Number of stomata Trial 1 | 2 | 3 | Average number of stomata | Class average | Diameter of field view / mm | Surface area / mm² | Density per mm² |
|---|---|---|---|---|---|---|---|---|---|
| Poplar | Upper | 4 | 4 | 4 | 4.0 | 3.0 | 0.5 | 0.2 | 15 |
| | Lower | 11 | 14 | 16 | 13.6 | 16.0 | 0.5 | 0.2 | 80 |
| Lime | Upper | 4 | 3 | 3 | 3.3 | 1.2 | 0.5 | 0.2 | 6 |
| | Lower | 11 | 5 | 8 | 8.0 | 9.0 | 0.5 | 0.2 | 45 |

Values of stomatal densities found in other species:

| Species | Stomata/mm² Upper epidermis | Stomata/mm² Lower epidermis |
|---|---|---|
| Ivy *Hedera helix* | 0 | 158 |
| Bean *Phaseolus vulgaris* | 40 | 281 |
| Tomato *Lycopersicon esculentum* | 12 | 131 |
| Geranium *Pelergonium sp.* | 19 | 59 |
| Maize *Zea mays* | 52 | 68 |

## Looking inside the leaf

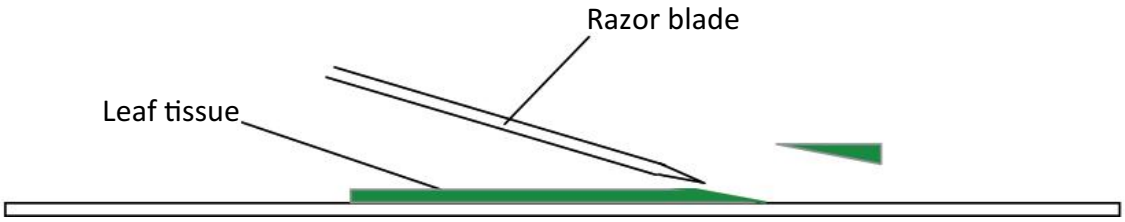
The internal structure of the leaf can be observed by making sections.

### To make oblique sections of a leaf

Cut strips of leaf tissue to 1cm × 3cm. Glue the strip down using Superglue® on a slide, lower side down.

*Warnings: Avoid skin contact with the Superglue. Sharp instrument.*


Using a razor blade cut a very oblique section towards one end of the leaf (see diagram below).




Trim the leaf down to a piece about 1cm long which includes the oblique section you have just cut. Stain the cut surfaces in iodine green stain (0.1% aqueous) for 1 to 2 minutes. Soak up the excess stain using filter paper and add a few drops of distilled water. Cover with a cover slip and observe under medium power.

This reveals the architecture of the spongy mesophyll quite clearly.

### To make a transverse leaf section

For example:

1. Cut a 3cm length of elderberry pith or carrot root longitudinally into two equal parts. Place one of the leaves between the two halves of the elderberry pith, with the midrib in the middle. Cut away the excess parts of the leaf that are not sandwiched between the elderberry pith cylinder.
2. Place the cylinder on the bench holding the two cut sections together between your thumb and forefinger and cut away one end to ensure a flat perpendicular section. At the cut end, cut the thinnest section possible throughout the elderberry pith and leaf. Cut the section by pushing the razor blade away from you.
3. Using forceps, transfer the leaf section to the watch glass containing the bleach. Cut the end of the cylinder again to ensure a flat and perpendicular section. Make sure the leaf has not moved. Carry on until you have five sections to make sure that at least one out of the five will be a good section

# 9C CELL TYPES AND TISSUES IN THE PETIOLE OF CELERY (Apium graveolens)

Time: 2 hours

## Syllabus reference: 9.1

Skill: Drawing the structure of primary xylem vessels in sections of stems based on microscope images.

## Syllabus reference: Topic 9.2

**Skill**: Identification of xylem and phloem in microscope images of stem and root.

Required knowledge: The organisation of plant tissue.

## Materials

**Ethanoic acid:** 10%

**Carmine stain:** 1g carmine + 4g potassium alum for 100cm$^3$ of distilled water. Boil this for

1 hour, allow to cool, filter and add 1cm$^3$ of formalin (methanal).

**Iodine green:** 0.5% aqueous.

Lignin can also be identified by using acidified phloroglucinol (careful, it is made with concentrated hydrochloric acid).

*Warning: Hydrochloric acid is caustic, wear gloves and eye protection when preparing it or staining with it.*


Phloroglucinol makes the lignified cell walls turn red.

**Phloroglucinol:** Dissolve 5g phloroglucinol (benzene 1,3,5-triol) in 100cm$^3$ of 95% ethanol. Then slowly add strong hydrochloric acid until it begins to precipitate.

Cellulose can also be identified using Schultz solution, it makes the cellulose turn violet. It may also react with starch and turn it black. Starch, however, is not found in cell walls.

**Schultz solution:** Dissolve 21g of zinc in 60cm$^3$ of 2mol dm$^{-3}$ hydrochloric acid. Boil to reduce the volume of the liquid to 30cm$^3$. Add more zinc if necessary to provide a neutral solution. Add 2.4g potassium iodide and 0.03g of iodine crystals. Mix and filter.

If these stains are available try preparing a few slides of celery petiole and other tissues with them. These stains can be placed directly on to freshly cut sections (no need to bleach) but you cannot add the two stains to the same slide. The stains will take a few minutes to penetrate the cell walls and react with the chemicals.

Other plant tissue: Ivy (Hedra helix) stem sections, reed (Phragmites sp.) sections, asparagus (Asparagus officinalis) stem.

Trial runs should be carried out on any new tissue tried and the time left in the various liquids and the concentration of the liquids adapted accordingly.

Prepared slides of root and stem sections should be available to observe the distribution of the tissues in these organs.

## Method

Avoid using tissues from the bases of the petioles or in the older parts of the petioles. The tissue is not always intact here.

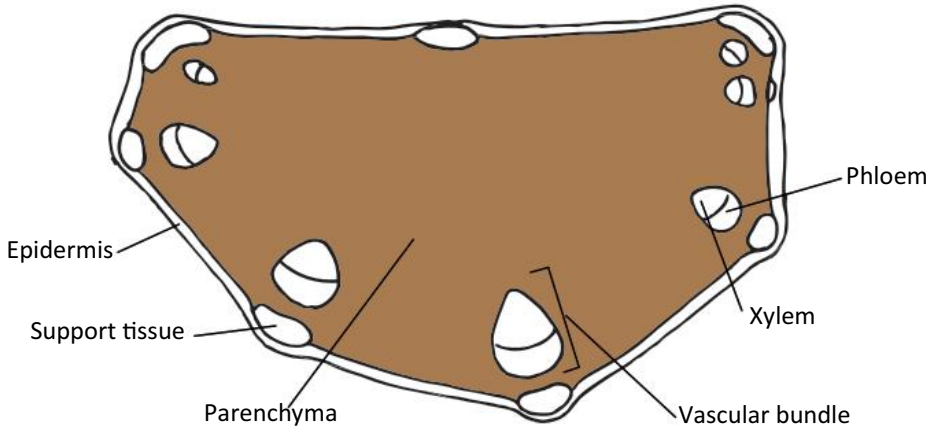
The plant tissue should be left in the bleach until the tissue decolourises, if this is less than 5 minutes the tissue should be removed from the bleach and transferred to the distilled water. 5 to 10 minutes is usually sufficient in the carmine stain.

The time left in the iodine green should be long enough so that the stain is taken up, but not so long that the entire section turns green. After passing the section in the iodine green, washing in water will remove the excess stain leaving a tissue mostly pink with prominent xylem stained green. The final washing in distilled water should not be too long as the stains can be washed out if they have not been well fixed in the tissue.

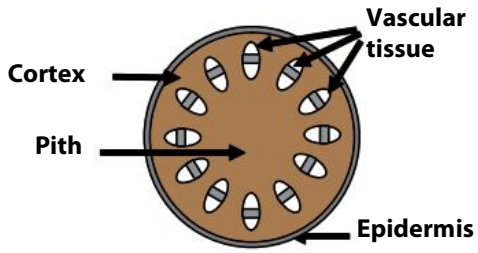
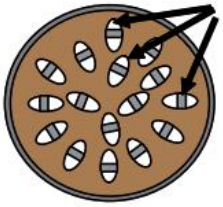
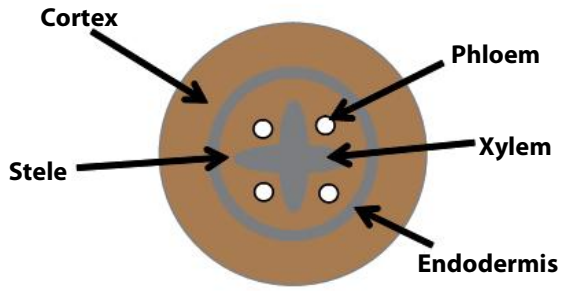
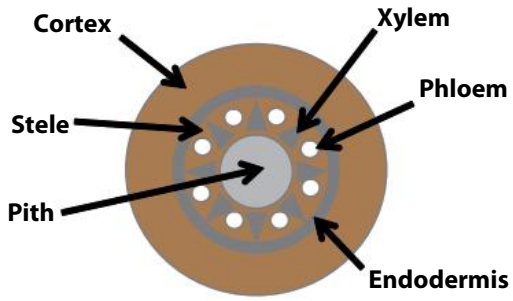
For other plant tissues **trial runs are necessary** and the times left in the bleach and the stains should be adapted accordingly. Not all tissue types are necessarily present in all the tissues observed.

## Results

### Transverse section of celery




Distribution of tissues in stems and roots

| Dicotyledons | Monocotyledons |
|---|---|
| Vascular tissue in a ring round the stem   | Vascular tissue scattered throughout stem  |
| The vascular tissue in the root forms a 3 to 5 pointed star   | The vascular bundle in the root is a many pointed star and the centre is filled with pith tissue   |

## Research

In the celery tissue:

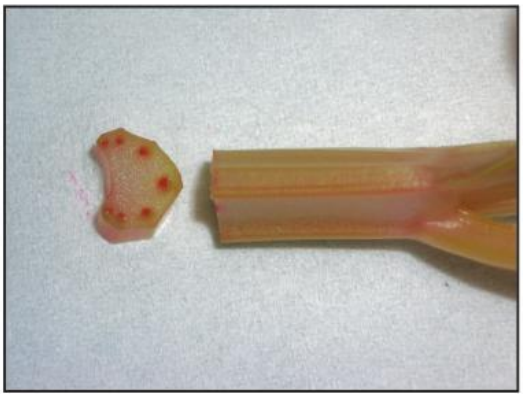
The bulk of the petiole is made up of regularly packed parenchyma cells, with large vacuoles, rounded cells, that maintain the shape of the tissue. The xylem is prominent, showing rounded, pentagonal or hexagonal cells that are large and hollow for water transport. The phloem, for transport of organic solutes. The sieve structures are usually not seen as they are, perhaps, removed by the bleach. Prominent groups of cells with thickened walls appear around the edge of the petiole to support it.

The petiole also supports the leaf and will orientate it towards the sun.

### Tracing sap flow in vascular bundles

The vessels used for the transport of sap up the stem can be identified by using dyes that travel in the fluid flow. Once again celery is good material for this especially the yellow leaves that grow in the centre of the celery. The stain can be observed to rise up the stem. Eosine (1% aqueous disodium eosine) or methylene green (1% aqueous) work well. Methylene blue, however, tends to get stuck at the nodes.

The xylem tissue stains up clearly so long as the tissue is not left for too long (1 hour is enough). Beyond this, the stain tends to spread into neighbouring tissues.



*Celery showing eosin transport.*

*Tracing the translocation of methylene green in celery*

# 9D THE STRUCTURE AND ADAPTATIONS OF FLOWERS

Time: 2 hours

## Syllabus reference: 9.4

**Skill**: Drawing of half-views of animal-pollinated flowers.

**Required knowledge:** General structures of wind and insect pollinated flowers, cross and self pollination.

Comparing the flowers of a wind pollinated and an insect pollinated plant.

## Materials

**Season:** Early spring to mid-summer

Hand lenses or, better still, binocular microscopes will be needed if wild flowers are used. Wild flowers, in general, are smaller than domesticated varieties.

## Some points for consideration

- *What features of these two flowers permitted you to identify them as being insect pollinated or wind pollinated. Explain how these features adapt the flower to insect or wind pollination*

**Features:** The insect pollinated flower can be distinguished by large colourful petals enclosing the reproductive organs, a scent that may be detected and nectaries may be observed at the base of the petals. The pollen tends to be sticky and it is not easily blown away. Wind pollinated flowers do not have large colourful petals, their reproductive organs tend to be exposed. The stigma may be feathery to catch pollen floating on air currents. The pollen is easily blown away from the anthers which hang out of the flower.

**Adaptations:** The insect pollinated flowers are adapted to their method of pollination by being able to attract insect pollinators using colour and scent. They provide a reward in the form of nectar. The pollen grains, being sticky, are less likely to fall off the insect's body. Scents will vary depending upon the pollinator. Some smell sweet but others that attract flies may smell foetid.

- *How may these plants avoid self pollination?*

Self pollination can be avoided by ripening the male and female parts at different times.

The male and female parts of the same flower may be positioned at different levels.

- *The floral formula does not include the colour of the flower. In what way is the colour important for the flower's function? Is the colour important in identifying the flower?*

The colour of insect pollinated flowers provides contrast. The colours may appear differently to the eyes of insects as they have different colour vision. Plants that use birds or bats as pollinators tend to use different colours to those adapted to insects. Though colours of flowers may vary from plant to plant it is usually genetically determined and can be used for identification purposes. Domesticated varieties of plants have more variety in their colouration due to artificial selection. The flower structure of domesticated plants may also make them poorly adapted for effective pollination (e.g. floribunda roses).

# 9E SEED STRUCTURE AND GERMINATION

Time: 1 hour initially, followed by regular observations over two weeks.

## Syllabus reference: 9.4

Skill: Drawing internal structure of seeds.

Skill: Design of experiments to test hypotheses about factors affecting germination.

## PART A Using tetrazolium indicator to determine seed viability

### Materials

Tetrazolium indicator: 1% aqueous

This is a redox indicator, it is reduced by active dehydrogenase enzymes in living cells. From a colourless compound it turns bright red.

For easy cutting soak the seed for a few hours first.

Large seeds are the easiest to work with (e.g. maize, wheat, barley, and peas). Beans give different results to the cereals as the cotyledons stain up as well as the embryo.

Reduced seed viability can be simulated by heating the seeds in an oven for a few minutes.

## PART B Germinating a sample of seeds to determine seed viability

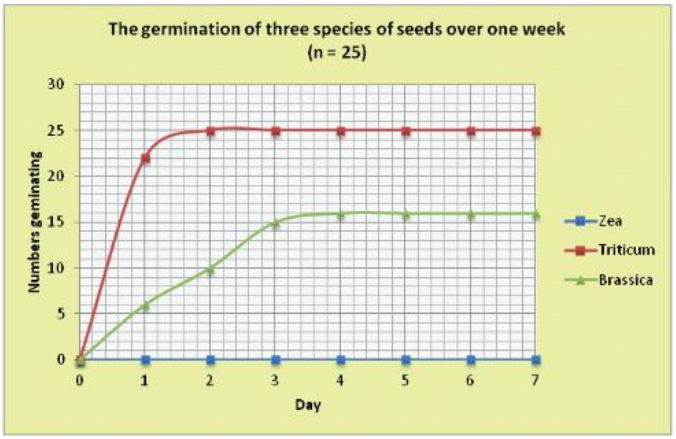
### Method

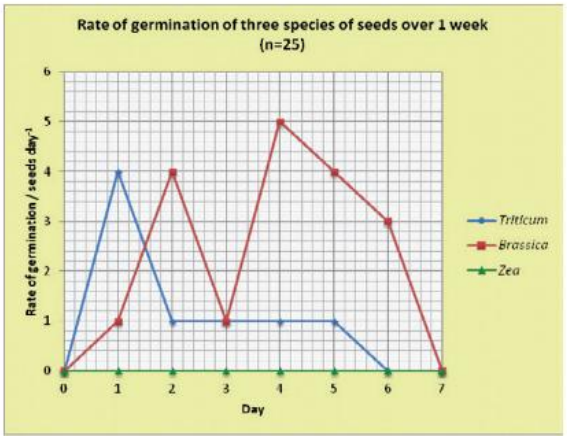
Surface sterilisation: Some seeds (e.g. peas, beans and maize) seem to be more open to attack by mould growth than others. Surface sterilisation can reduce this problem.

Seeds can be surface sterilised by soaking in 1% bleach (hypochlorite) for 1 minute. Then they should be washed 4 or 5 times in sterile water (bottled mineral water will be adequate for most purposes).

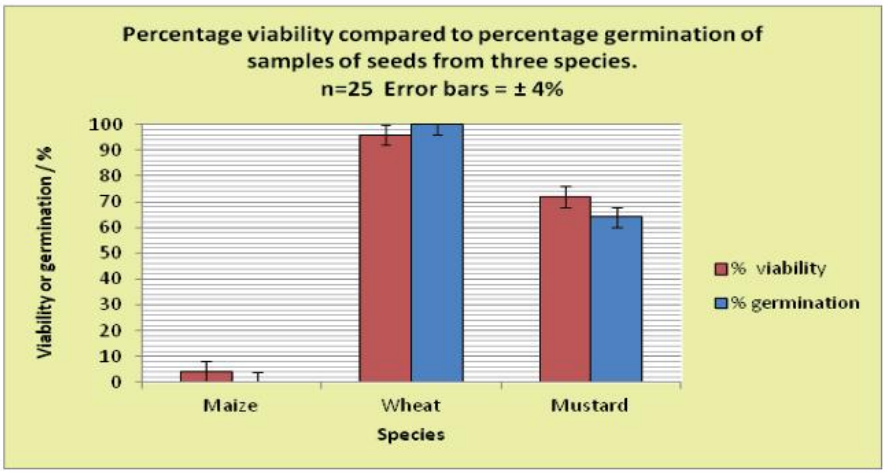
### Data

Results will depend upon the species, the storage conditions and the age of the seeds. In this example kept under optimal conditions for a week, the maize seeds (*Zea*) seem to have lost their viability. The initial rate of germination can be determined from the initial slope of the graph. The wheat seeds (*Triticum*) germinate faster than the mustard seeds (*Brassica*).




Plotting the rates of germination can reveal changes in the rate of germination. In this sample the mustard (*Brassica*) seems to pause at day 3 before resuming germination.




The results will vary for the reasons given above if they are from different species or different batches. Results, which vary between groups using the same batch of seeds, could be due to chance or experimental error.

They could be treated statistically to see if they are significantly different from one another using the $\chi 2$ test of association.

The percentage germination test may give different results than the tetrazolium test. The tetrazolium test only tests for the viability of the seed (i.e. to see if it is living). Even if they are alive, the seed will only germinate if certain optimal conditions are met (e.g. humidity, temperature, and the absence of inhibitors or the presence of promoters).

## To investigate further

- Compare the viability of seeds of different species of plants.
- Compare the viability of seeds of the same species but of different ages.
- Compare the viability of seeds kept under different conditions (e.g. heating, freezing and exposure time to these conditions).
- Compare the percentage germination of seeds under different conditions.

# Structure of seeds

## Data

Classification of the seeds suggested:

| Seed | Dicotyledon | Monocotyledon | Endospermic | Non-endospermic |
|---|---|---|---|---|
| Vicia fabia | • | | | • |
| Phaseolus vulgaris | • | | | • |
| Helianthus annuus | • | | | • |
| Triticum sp. | | • | • | |
| Allium cepa | | • | • | |

## To investigate further

Dissect a large seeds to remove more or less of their food store, followed by observations on their growth and development.

# 10A THE GENETICS OF MAIZE (Zea mais)

Time:: Approximately 1 hour

## Syllabus reference

Topic 10.2

Skill: Use of a chi-squared test on data from dihybrid crosses

## Part A Monohybrid cross

### Data

| Colour | Numbers | % |
|---|---|---|
| Black kernels | 142 | 70.3 |
| Yellow kernels | 60 | 29.7 |
| TOTAL | 202 | |

If black is dominant to yellow then this cob must be the result of a cross between two heterozygous black plants.

If **B** = the black allele and **b** = the yellow allele:

| | | | | |
|---|---|---|---|---|
| **Parents** | **Phenotypes** | **Black kernels** | × | **Yellow kernels** |
| | Genotypes | Bb | | Bb |
| | Gametes | B and b | | B and b |

**Offspring**

| | B | b |
|---|---|---|
| B | BB | Bb |
| b | Bb | bb |

| | Black | Yellow |
|---|---|---|
| Phenotypes | | |
| Expected Ratio | 3 | 1 |
| | 75% | 25% |

### $\chi^2$ test

| Phenotypes | Observed (O) | Expected (E) | O-E | $(O-E)^2/E$ |
|---|---|---|---|---|
| Black kernel | 142 | 151.5 | -9.5 | 0.596 |
| Yellow kernel | 60 | 50.5 | 9.5 | 1.787 |
| Totals | 202 | 202 | | 2.383 |

The number of degrees of freedom (n) = 2 - 1 = 1

The calculated value for $\chi^2 = 2.383$ so $p>0.1$. This is below the critical value of 3.84 ($p=0.05$). The null hypothesis is retained. There is no significant difference between the observed and the expected results.

# Part B Dihybrid cross

## Data

| Phenotypes | Numbers | % |
|---|---|---|
| Black Smooth | 78 | 56.5 |
| Black Wrinkled | 26 | 18.8 |
| Yellow Smooth | 27 | 19.6 |
| Yellow Wrinkled | 7 | 5.1 |
| TOTAL | 138 | |

If smooth allele (**S**) is dominant to wrinkled allele (**s**) and black allele (**B**) is dominant to yellow allele (**b**) we would expect a 9:3:3:1 ratio in the $F_2$ generation if seed colour and seed shape genes are not linked. This can be shown be the following genetic diagram:

| | | | | |
|---|---|---|---|---|
| **Parents** | **Phenotypes** | **Black Smooth** | × | **Yellow Wrinkled** |
| | Genotypes | BBSS | | bbss |
| | Gametes | BS | | bs |
| $F_1$ | Phenotype | | Black Smooth | (Selfed) |
| | Genotype | | BbSs | |
| | Gametes | | BS, Bs, bS, bs | |

$F_2$ Genotypes

| | BS | Bs | bS | bs |
|---|---|---|---|---|
| BS | BBSS | BBSs | BbSS | BbSs |
| Bs | BBSs | BBss | BbSs | Bbss |
| bS | BbSS | BbSs | bbSS | bbSs |
| bs | BbSs | Bbss | bbSs | bbss |

| **Phenotypes** | Black Smooth | Black Wrinkled | Yellow Smooth | Yellow Wrinkled |
|---|---|---|---|---|
| **Expected ratio** | 9 | 3 | 3 | 1 |
| | 56.25% | 18.75% | 18.75% | 6.25% |

## $\chi^2$ test

| Phenotypes | Observed | Expected | O-E | $(O-E)^2/E$ |
|---|---|---|---|---|
| Black Smooth | 78 | 77.6 | 0.37 | 0.0018 |
| Black Wrinkled | 26 | 25.9 | 0.12 | 0.0001 |
| Yellow Smooth | 27 | 25.9 | 1.12 | 0.0485 |
| Yellow Wrinkled | 7 | 8.6 | -1.63 | 0.3079 |
| Totals | 138 | 138 | | 0.3583 |

The number of degrees of freedom (n) = 4 - 1 = 3

The calculated value for $\chi 2$ is 0.3583 so $p>0.9$. This is below the critical value of 7.81 ($p=0.05$): the Null hypothesis is retained. There is no significant difference between the observed and expected results. Therefore, we can say that these results show that the genes for seed shape and seed colour in maize are not linked. A test cross between the F1 and a yellow smooth kernel plant should produce a 1:1:1:1 ratio for the four different phenotypes.

# 11A HOMEOSTASIS AND RED BLOOD CELLS

Time:: 2 hours.

## Syllabus reference

Topic 11.3

Application: Consequences of dehydration and over hydration.

## Materials

The animal blood, such as pig's blood must be as fresh as possible. A trial run should be done to verify the state of the blood. Pipette 1cm$^3$ of the blood into 9cm$^3$ of physiological saline. Then spin in a centrifuge. The sample is acceptable if the supernatant has a slightly orange tint. If it is cloudy red, the red cells have already hemolysed.

## Method

The animal blood is diluted, otherwise, the form of the cells is not clearly seen, as there are too many cells. Alternatively, 10cm$^3$ of blood can be diluted in 90 cm$^3$ of physiological solution and 1cm$^3$ of this dilution added to each of the salt dilutions. Crenation of the cells in the physiological saline may also be observed if the blood is not fresh enough. Before beginning the practical session, it is best to check the number of cells seen in the physiological saline dilution and adjust the volume of fresh blood added accordingly. When pipetting the diluted blood onto the microscope slides it is best to start with the most dilute solutions.

## Example of data

| NaCl / % | Microscope cells form | After 1 hour | Sediment after centrifugation | Supernatant after centrifugation |
|---|---|---|---|---|
| Physiological saline | Rounded<br>bi-concave discs | Cloudy.<br>Clear fluid at the top. | Thick | Clear |
| 0 | No cells | Clear red | No sediment | Clear red |
| 0.1 | Rare cells | Clear red | No sediment | Clear red |
| 0.3 | Fewer rounded cells | Clearer<br>No fluid at the top | Little | Clear red |
| 0.5 | Ro□unded cells | Cloudy. Less clear fluid | Less sediment | Clear red |
| 0.7 | Biconcave discs | Cloudy. Less clear fluid | Less sediment | Clear |
| 0.9 | Biconcave discs | Cloudy. Less clear fluid | Thick | Clear |
| 1.0 | Slight crenation | Cloudy. Less clear fluid | Thick | Clear |
| 2.0 | Crenation | Cloudy. Less clear fluid | Thick | Clear |

**N.B.** If the blood is not fresh some crenation will be observed in the 0.9% solutions and the supernatant will have a slight orange colour.

## Some points for consideration

- *What is the shape of the cells observed in the physiological saline solution?*

In the physiological saline the blood cells appear as biconcave discs.

- *Explain your observations for the shape and the numbers of the red blood cells in the different salt concentrations.*
  In the lower saline concentrations, fewer cells are observed and their aspect is rounded. Here, the outside concentration is lower than the internal concentration of the red blood cells. The external environment is hypotonic (less negative water potential) to the cells. The cells are relatively hypertonic (more negative water potential). Water enters the cells and some of the cells hemolyse. In the higher concentrations the cells appear crenated. The external environment is higher then the internal concentration of the red blood cells. It is hypertonic (more negative water potential) to the cells. The cells are relatively hypotonic (less negative water potential). Water leaves the cells and the cells crenated.

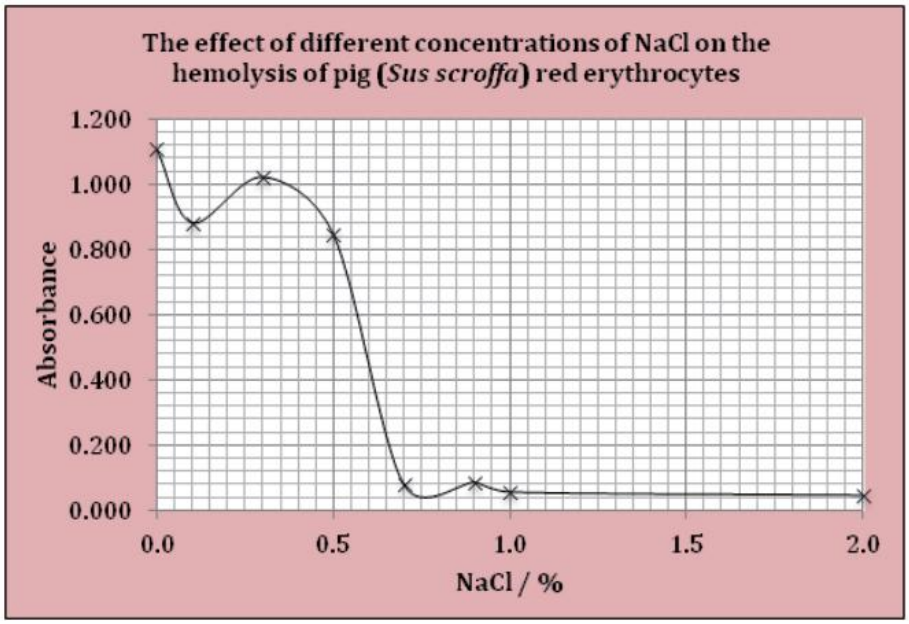
- *In which salt concentrations was little or no change seen? Explain why.*
  No change should be seen in the 0.9% solution, this being the same as the physiological saline solution. The surrounding solution is isotonic (the external and internal water potentials are the same) with the internal solution of the red blood cells. There is no net water movement.

- *In the tubes that were left for 1 hour explain why some tubes became clear whilst others became cloudy.*
  In the higher concentrations, the tubes became cloudy as the cells begin to crenate and start to sediment out. In the lower concentrations the tubes become homogeneously clear red as the cells begin to hemolyse.

- *At the top of some of the tubes, there is a clear layer of liquid. What is this liquid? Explain the appearance of this liquid in these particular tubes.*
  As the cells sediment towards the bottom of the tube in the higher concentrations a clear layer appears at the top of the tubes. The clear liquid appearing is the blood plasma diluted in the saline solution.

- *Explain your results from the observations made after centrifugation.*
  The use of the centrifuge illustrates that the cells remain intact in the higher salt dilutions, leaving a clear solution above. As the solutions become more dilute the number of intact cells decreases, hence, the thickness of the sediment decreases. As the cells hemolyse, thus releasing their contents into the solution, the supernatant becomes clear red.

## Data




### Interpretation

The results from the colorimeter confirm the visual observations on the test tubes. At low sodium chloride concentrations up to 0.5 % the blood cells hemolyse and the hemoglobin is present in the supernatant. Above 0.6% the cells are intact to absorbance is almost zero. Absorbance at these levels is probably due to damaged blood cells in the original sample of pig's blood.

# 12C STUDYING INVERTEBRATE BEHAVIOUR

Time 2 hours

## Syllabus reference: Option A.4

Skill: Analysis of data from invertebrate behaviour experiments in terms of the effect on chances of survival and reproduction.

Required knowledge: Behavioural responses: Taxis and kinesis

## Materials

**Choice chamber:** Usually available from biological suppliers.

Home made ones are easily made using large (10mm diameter) Petri dishes. Holes in the lid can be made by drilling or melting holes in the top cover. The partition inside can be constructed using modelling clay and the fine mesh netting can be made from stockings.

## Animals

Small organisms such as mealworms (*Tenebrio molitor*) or woodlice (e.g. *Oniscus asellus*) are suitable.

Use different animals for each test. If the same mealworms are to be used a second time, leave them for at least 30 minutes beforehand in the usual container in which they are kept.

*Ethics: The conditions used should not extend outside the natural limits experienced by the organism.*


## Method

The response by the animals may be complicated by other behaviours. Low lighting is recommended when light is not the independent variable. Woodlice are known to huddle in groups and many invertebrates will "hide" in corners. In some cases using individual organisms in a choice chamber may be necessary.

### Suggested investigations and their materials

- Light/dark: Lamp + black paper
- Warm/cold: Ice
- Combination of variables e.g. dark and humid. Dry and light.
- Graph paper can be used to plot individual animal movement or a video camera can be used for video capture.

# 12D TAKING AN ELECTROMYOGRAM

Time: 1 hour

## Syllabus reference: Option A.4

Aim: Data logging using an ECG sensor to analyse neuromuscular reflexes.

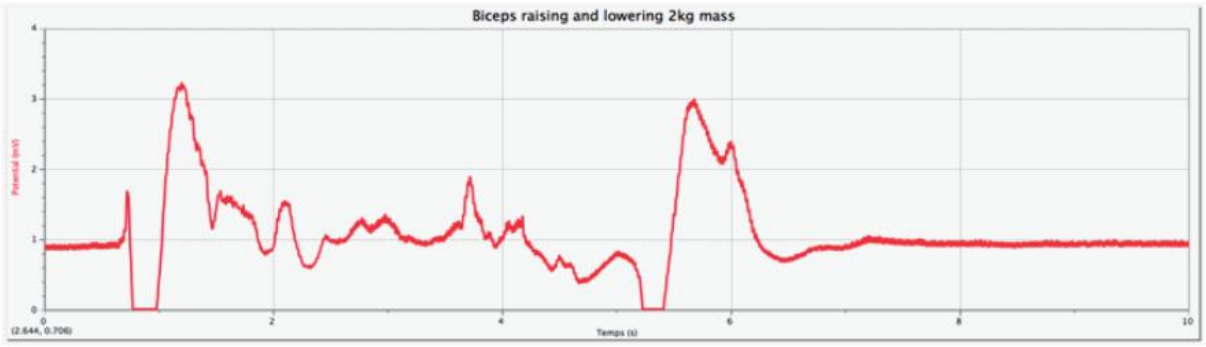
## Materials

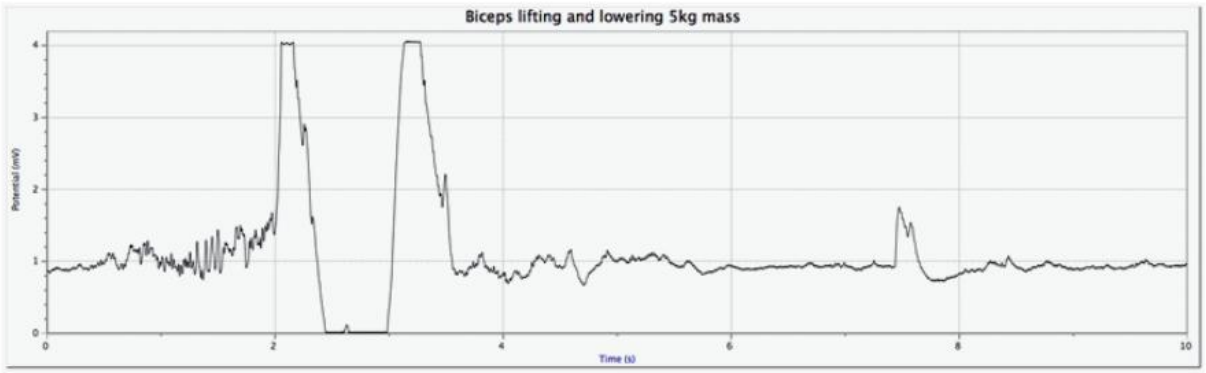
2kg, 5kg and 10kg masses: buckets of water or bags of sand

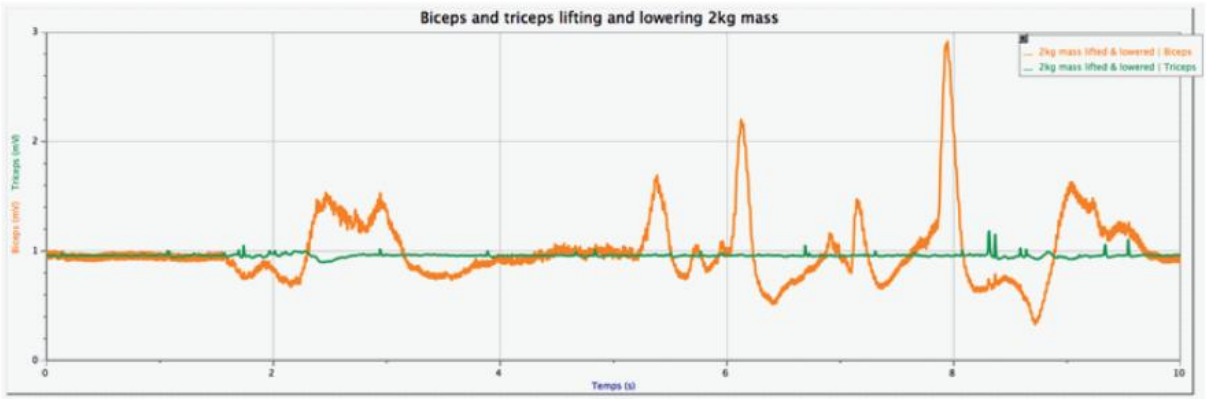
The equipment used to obtain these data was a Vernier ® EKG sensor and readout is displayed in Vernier Loggerpro®.

## Data

## Analysing a reflex




If the reflex hammer is fitted with an accelerometer and the ECG recording is triggered when the hammer's acceleration drops below $1 ms^{-2}$, the ECG will record the muscle activity between the stimulus and the response, in other words, the time of the reflex. The foot jerk reflex using the Achilles tendon is a convenient reflex to analyse. The electrodes are stuck to the gastrocnemius (the calf muscle).

# 13C COMPARING PROTEINS FROM DIFFERENT SPECIES

Time: 1 hour

## Syllabus reference: Option B.5

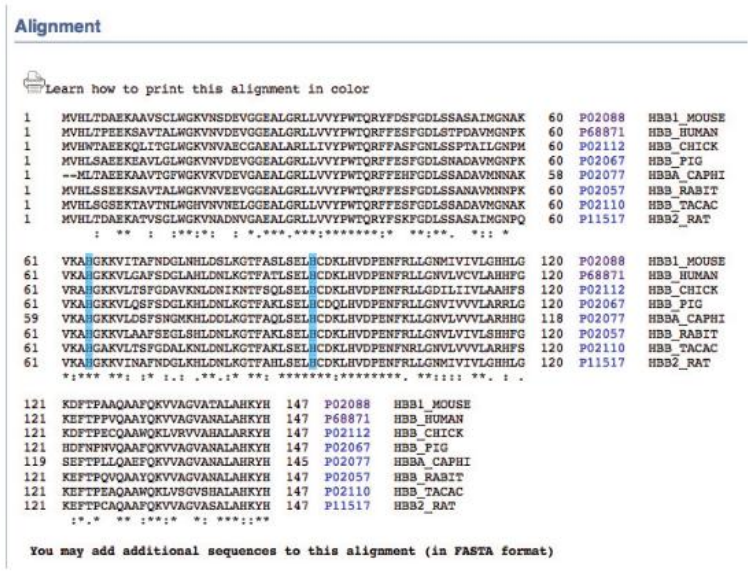
Skill: Use of software to align two proteins.

## Materials

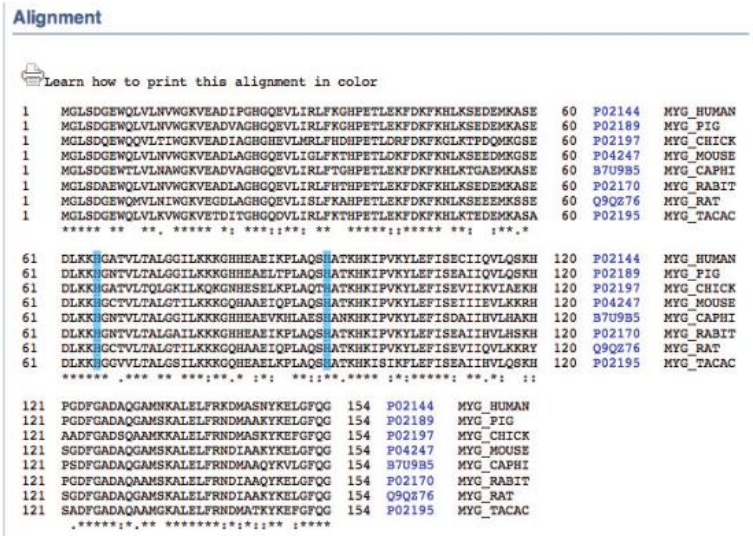
'Align' uses Clustal Omega program to align the sequences of the amino acids written in FASTA format. This should work on most computers.

## Data

The Metal binding application used to highlight where the iron containing heme group is bound to the beta hemoglobin molecule gives the following result:




Myoglobin showing the same detail for the same species.




The myoglobin molecule is a bit shorter than beta hemoglobin (154 amino acid residues as opposed to about 147 for the beta haemoglobin) but the metal binding site is almost in the same position with the same residues, histidine.

Other comparisons can be made between different proteins in the same species e.g. myoglobin, alpha- and beta-hemoglobins expressed after birth, or gamma-, epsilon- or zeta-hemoglobin expressed before birth.

# 13D MAKING A CLADOGRAM FROM AMINO ACID SEQUENCES

Time 1 hour

## Syllabus reference: Option B.5

**Skill**: Use of software to construct simple cladograms and phylograms of related organisms using DNA sequences.

## Data

The alignment of the beta hemoglobin molecules for the eight species gives the following FASTA format:

**Alignment**

Learn how to print this alignment in color

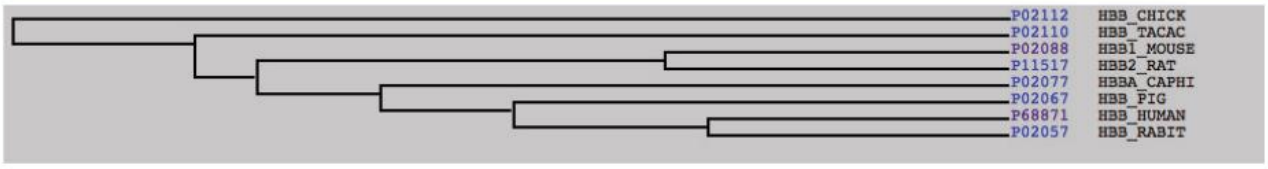
```
1    MVHLTDAEKAAVSCLWGKVNSDEVGGEALGRLLVVYPWTQRYFDSFGDLSSASAIMGNAK  60  P02088  HBB1_MOUSE
1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60  P68871  HBB_HUMAN
1    MVHWTAEEKQLITGLWGKVNVAECGAEALARLLIVYPWTQRFFASFGNLSSPTAILGNPM  60  P02112  HBB_CHICK
1    MVHLSAEEKEAVLGLWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSNADAVMGNPK  60  P02067  HBB_PIG
1    --MLTAEEKAAVTGFWGKVKVDEVGAEALGRLLVVYPWTQRFFEHFGDLSSADAVMNNAK  58  P02077  HBBA_CAPHI
1    MVHLSSEEKSAVTALWGKVNVEEVGGEALGRLLVVYPWTQRFFESFGDLSSANAVMNNPK  60  P02057  HBB_RABIT
1    MVHLSGSEKTAVTNLWGHVNVNELGGEALGRLLVVYPWTQRFFESFGDLSSADAVMGNAK  60  P02110  HBB_TACAC
1    MVHLTDAEKATVSGLWGKVNADNVGAEALGRLLVVYPWTQRYFSKFGDLSSASAIMGNPQ  60  P11517  HBB2_RAT
         :  **  :  :**:*:  : *.***.***:*******:*  **:**.  *:: *

61   VKAHGKKVITAFNDGLNHLDSLKGTFASLSELHCDKLHVDPENFRLLGNMIVIVLGHHLG  120  P02088  HBB1_MOUSE
61   VKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG  120  P68871  HBB_HUMAN
61   VRAHGKKVLTSFGDAVKNLDNIKNTFSQLSELHCDKLHVDPENFRLLGDILIIVLAAHFS  120  P02112  HBB_CHICK
61   VKAHGKKVLQSFSDGLKHLDNLKGTFAKLSELHCDQLHVDPENFRLLGNVIVVVLARRLG  120  P02067  HBB_PIG
59   VKAHGKKVLDSFSNGMKHLDDLKGTFAQLSELHCDKLHVDPENFKLLGNVLVVVLARHHG  118  P02077  HBBA_CAPHI
61   VKAHGKKVLAAFSEGLSHLDNLKGTFAKLSELHCDKLHVDPENFRLLGNVLVIVLSHHFG  120  P02057  HBB_RABIT
61   VKAHGAKVLTSFGDALKNLDNLKGTFAKLSELHCDKLHVDPENFNRLGNVLVVVLARHFS  120  P02110  HBB_TACAC
61   VKAHGKKVINAFNDGLKHLDNLKGTFAHLSELHCDKLHVDPENFRLLGNMIVIVLGHHLG  120  P11517  HBB2_RAT
     *:*** **: :* :.: .**.:* **: *******:********. **:::: **. : .

121  KDFTPAAQAAFQKVVAGVATALAHKYH  147  P02088  HBB1_MOUSE
121  KEFTPPVQAAYQKVVAGVANALAHKYH  147  P68871  HBB_HUMAN
121  KDFTPECQAAWQKLVRVVAHALARKYH  147  P02112  HBB_CHICK
121  HDFNPNVQAAFQKVVAGVANALAHKYH  147  P02067  HBB_PIG
119  SEFTPLLQAEFQKVVAGVANALAHRYH  145  P02077  HBBA_CAPHI
121  KEFTPQVQAAYQKVVAGVANALAHKYH  147  P02057  HBB_RABIT
121  KEFTPEAQAAWQKLVSGVSHALAHKYH  147  P02110  HBB_TACAC
121  KEFTPCAQAAFQKVVAGVASALAHKYH  147  P11517  HBB2_RAT
      :*.*  ** :**:*  *: ***::**
```

**You may add additional sequences to this alignment (in FASTA format)**

## Cladograms

The following cladogram derived from the alignment is visible under **Guide tree:**

**Guide tree**




This shows the relatedness of these species for their beta (β) hemoglobin protein.

## Analysing the cladogram

- *Which animal has the most similar beta hemoglobin to us?*

The rabbit.

- *Which is the most different protein from humans?*

The chicken is most different from humans. It is most closely related to the echidna (P02110) which is an egg laying mammal that diverged from the mammals at an early stage in their evolution. Given that mammals and birds share a common ancestor amongst the reptiles this is not surprizing.

- *Are there any surprising relationships?*

The human and the rabbit are the most closely related are the  most closely related on this cladogram, more closely related than the pig that resembles us physiologically.

- *Do you think this cladogram represents the real relatedness?*

All cladograms are hypothesis based on the principle of parsimony. That is the least number of changes needed to explain the differences observed. It is possible that the real pattern was more complicated.

In addition, because the genetic code is degenerate and this analysis is based on the amino acid sequence there are some mutations that are silent. A cladogram based on the nucleotide sequence would be more accurate.

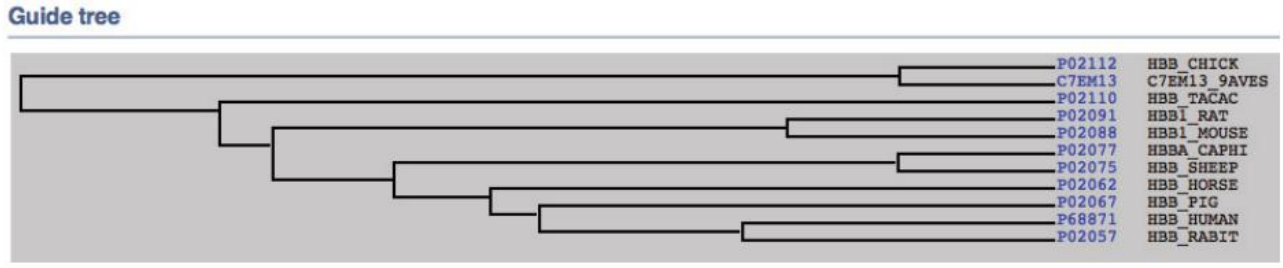
Hemoglobin is an important molecule and the survival of an animal would depend upon it. Some mutations may have arisen that were eliminated by natural selection. These would not be observed.

- *What would need to be done to produce a phylogram showing the period of time since different species diverged?*

The rate of mutation would need to be calibrated from known divergences in the fossil record. Or the molecular clock hypothesis could be applied assuming that mutations that result in changes in the protein occur at a regular periodicity.

## Making predictions

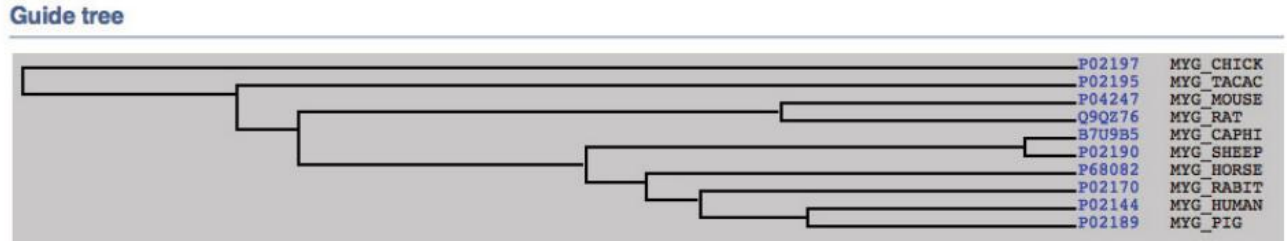
Adding in three more species (sheep P02075, horse P02062 and goose C7EM13):




Interestingly, the sheep is not in the same immediate clade as the goat in this analysis. However, the goose and the chicken are in the same clade, which is reasonable.

## Trying a different protein

The  myoglobin cladogram for the 10 of the same species (there is no data for the goose):

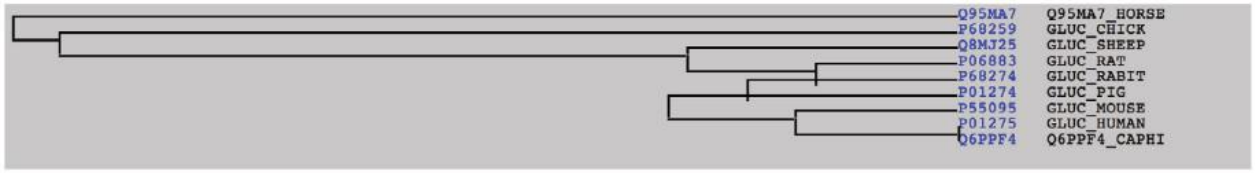



Here we see that the sheep and the goat are in the same clade which is more reassuring, as are the human and the pig.

The fact that the results are different is not surprising since these cladograms are only established from the amino acid sequences of single proteins and these proteins are subject to natural selection.

The cladogram for glucagon is completely different and it does not succeed in giving realistic phylogeny:

**Guide tree**




This protein is very short in most of these species and it shows a lot of difference between them as the alignment data below shows:

**Alignment**

Learn how to print this alignment in color

```
1    MALWTRLLPLLALLALWAPAPAQAFVNQHLCGSHLVEALYLVCGERGF-----FYTPKAR   55  P01315  INS_PIG
1    MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGF-----FYTPKTR   55  P01308  INS_HUMAN
1    MALWMRFLPLLALLVLWEPKPAQAFVKQHLCGPHLVEALYLVCGERGF-----FYTPKSR   55  P01322  INS1_RAT
1    ------------------------------------MKSIYFVAGLFVMLVQGSWQRSLQD   25  P01275  GLUC_HUMAN
1    ------------------------------------MKTIYFVAGLLIMLVQGSWQHALQD   25  P55095  GLUC_MOUSE
1    ----------------------------------MKMKSIYFIAGLLLMIVQGSWQNPLQD   27  P68259  GLUC_CHICK
1    ------------------------------------MKTIYFVAGLFVMLVQGSWQRSLQN   25  P01274  GLUC_PIG
1    ------------------------------------------------------------    0  Q6PPF4  Q6PPF4_CAPHI
1    ------------------------------------------------------------    0  P68274  GLUC_RABIT
1    ------------------------------------MKTVYIVAGLFVMLVQGSWQHAPQD   25  P06883  GLUC_RAT

56   REAENPQAGAVELGGGL--GGLQALALEGPPQKRGIVEQ---CCTSICSLYQL----EN-   105  P01315  INS_PIG
56   REAEDLQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQ---CCTSICSLYQL----EN-   107  P01308  INS_HUMAN
56   REVEDPQVPQLELGGGPEAGDLQTLALEVARQKRGIVDQ---CCTSICSLYQL----EN-   107  P01322  INS1_RAT
26   TEEKSRSFS-----------ASQADPLSDPDQMNEDKRHSQGTFTSDYSKYLDSRRAQDF   74  P01275  GLUC_HUMAN
26   TEENPRSFP-----------ASQTEAHEDPDEMNEDKRHSQGTFTSDYSKYLDSRRAQDF   74  P55095  GLUC_MOUSE
28   TEEKSRSFK-----------ASQSEPLDESRQLNEVKRHSQGTFTSDYSKYLDSRRAQDF   76  P68259  GLUC_CHICK
26   TEEKSRSFP-----------APQTDPLDDPDQMTEDKRHSQGTFTSDYSKYLDSRRAQDF   74  P01274  GLUC_PIG
1    ------------------------------------------------------------    0  Q6PPF4  Q6PPF4_CAPHI
1    ----------------------------------------HSQGTFTSDYSKYLDSRRAQDF   22  P68274  GLUC_RABIT
26   TEENARSFP-----------ASQTEPLEDPDQINEDKRHSQGTFTSDYSKYLDSRRAQDF   74  P06883  GLUC_RAT

106  ------------------------------------------------------------   105  P01315  INS_PIG
108  ------------------------------------------------------------   107  P01308  INS_HUMAN
108  ------------------------------------------------------------   107  P01322  INS1_RAT
75   VQWLMNTKRNR-------------------NNIAKRHDEFERHAEGTFTSDVSSYLEGQAA   116  P01275  GLUC_HUMAN
75   VQWLMNTKRNR-------------------NNIAKRHDEFERHAEGTFTSDVSSYLEGQAA   116  P55095  GLUC_MOUSE
77   VQWLMSTKRNGQQGQEDKENDKFPDQLSSNAISKRHSEFERHAEGTYTSDITSYLEGQAA   136  P68259  GLUC_CHICK
75   VQWLMNTKRNK-------------------NNIAKRHDEFERHAEGTFTSDVSSYLEGQAA   116  P01274  GLUC_PIG
1    ----------------------------NNIAKRHDEFERHAEGTFTSDVSSYLEGQAA    31  Q6PPF4  Q6PPF4_CAPHI
23   VQWLMNT------------------------------------------------------    29  P68274  GLUC_RABIT
75   VQWLMNTKRNR-------------------NNIAKRHDEFERHAEGTFTSDVSSYLEGQAA   116  P06883  GLUC_RAT

106  ------------------------------------------------YCN-------   108  P01315  INS_PIG
108  ------------------------------------------------YCN-------   110  P01308  INS_HUMAN
108  ------------------------------------------------YCN-------   110  P01322  INS1_RAT
117  KEFIAWLVKGRGRRDFPEEVAIVEELGRRHADGSFSDEMNTILDNLAARDFINWLIQTKI   176  P01275  GLUC_HUMAN
117  KEFIAWLVKGRGRRDFPEEVAIAEELGRRHADGSFSDEMSTILDNLATRDFINWLIQTKI   176  P55095  GLUC_MOUSE
137  KEFIAWLVNGRGRRDFPEKALMAEEMGRRHADGTFTSDINKILDDMAAKEFLKWLINTKV   196  P68259  GLUC_CHICK
117  KEFIAWLVKGRGRRDFPEEVTIVEELRRRHADGSFSDEMNTVLDNLATRDFINWLLHTKI   176  P01274  GLUC_PIG
32   KEFIAWLVKGRGRR-----------------------------------------------    45  Q6PPF4  Q6PPF4_CAPHI
30   ------------------------------------------------------------    29  P68274  GLUC_RABIT
117  KEFIAWLVKGRGRRDFPEEVAIAEELGRRHADGSFSDEMNTILDNLATRDFINWLIQTKI   176  P06883  GLUC_RAT

109  ----------  108  P01315  INS_PIG
111  ----------  110  P01308  INS_HUMAN
111  ----------  110  P01322  INS1_RAT
177  TDRK------  180  P01275  GLUC_HUMAN
177  TDKK------  180  P55095  GLUC_MOUSE
197  TQRDLLGEYQ  206  P68259  GLUC_CHICK
177  TDSL------  180  P01274  GLUC_PIG
46   ----------   45  Q6PPF4  Q6PPF4_CAPHI
30   ----------   29  P68274  GLUC_RABIT
177  TDKK------  180  P06883  GLUC_RAT
```

# 14C POPULATION GROWTH IN YEAST

**Time:** 1 hour to set up and 15 minute counting periods.

## Syllabus reference: Option C.5

**Skill**: Modelling the growth curve using a simple organism such as yeast or species of *Lemna*.

## Materials

### Stock solution of yeast

Dried yeast is practical to use although other kinds, for example, fresh bakers yeast, could also be used.

Physiological saline may be available in the pharmacy or a 0.9% aqueous solution of sodium chloride.

The counting grid referred to here is the **Neubauer** (improved). Other counting grids commonly used are the Malassez or Thoma. Many types exist.

**Malassez:** The distance between the central bar and the cover slip is 0.2mm. The surface area of the smallest squares seen is 0.05mm × 0.05mm giving a volume of 0.0005mm$^3$.

**Thoma** (new): Similar dimensions apply in the "new" grid: the distance between the central bar and the cover slip is 0.1mm. The smallest square seen is 0.05mm × 0.05mm giving a volume of 0.00025mm$^3$.

## Method

Finding time available to carry out the counting may be difficult depending on the facilities available.The counting period should be as many days as possible. In trial runs good results were obtained during a counting period of 12-18 days. This varies depending on the type and state of the yeast cells and the handling of the yeast culture. It is important to maintain a steady temperature, as yeast cells are sensitive to temperature shock. The amount of glucose, oxygen or contamination by other microbes will also influence results.

Sterile flasks and sterile Pasteur pipettes are used at the beginning to minimise initial contamination of the culture. Sterile cotton wool from a packet can be used. After this, sterile techniques could be used, although, in the testing of this experiment good results were obtained and contamination was only observed after 18 days. If sterile techniques are to be used, the cotton wool should be passed through Bunsen flame each time it is removed and also the neck of the flask before replacing the cotton wool and sterile pipettes should be used each time. The workbench should also be cleaned with bleach for example and all manipulation carried out in an area close to a Bunsen Burner flame.

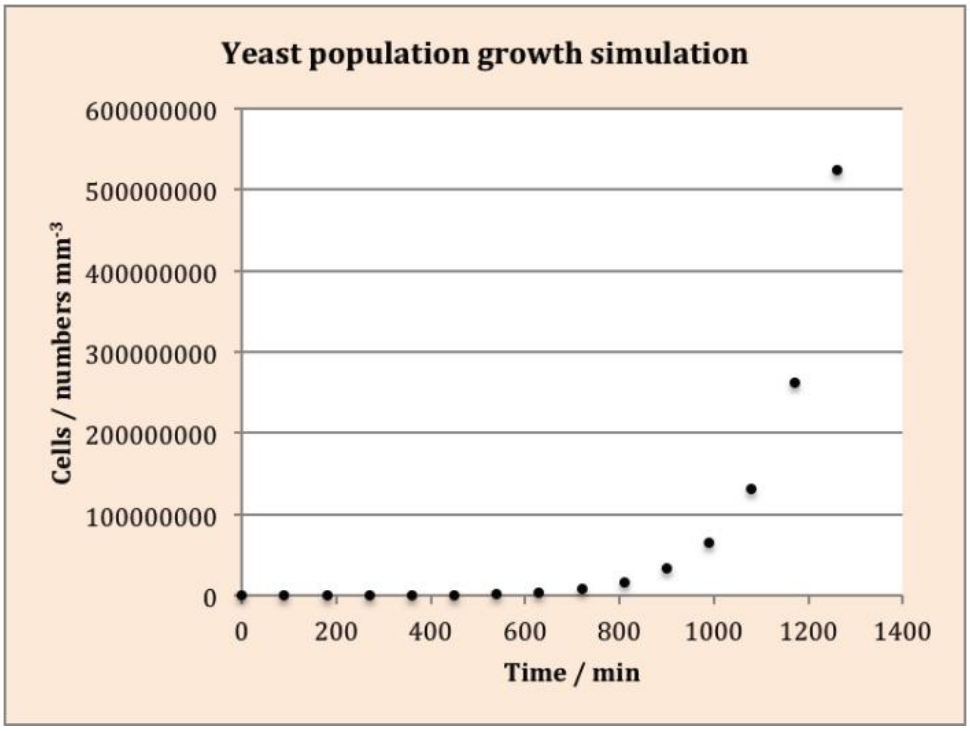
Students may need help in locating the correct squares in which to count.

### Simulating exponential growth in a spread sheet

The estimates suggested for yeast cell cycle duration were taken from:

<http://mpf.biol.vt.edu/research/budding_yeast_model/pp/wt_glucose.php>

The results below show the exponential growth curve for 21h (14 generations at 90 min per generation).




## Some points for consideration

- Various factors influencing the rate of growth of yeast cells: temperature, oxygen or absence of oxygen, substrate concentration, other minerals present and space.
- Plotting the numbers of yeast cells in $mm^3$ instead of numbers per $0.00025mm^3$ is a more practical unit to use. If either units of volume are used, this is better than a relative density i.e. 'numbers per counting area'.
- Rate of growth could be determined by plotting, for example: growth increments (the number by which the colony increased per day) **v** time unit.

## To investigate further

Many variables could be investigated:

- The concentration of the glucose
- The substrate used (other sugars could be tried: fructose, lactose, sucrose, etc.)
- Incubation temperature
- The size or shape of the culture containers
- The volume of culture solution used
- Aerobic and anaerobic conditions

# 15D MYOGLOBIN AND HEMOGLOBIN

Time:: 30 minutes to analyse data and unlimited time for further investigation

## Syllabus reference: Option D.6

Application: Consequences of high altitude for gas exchange.

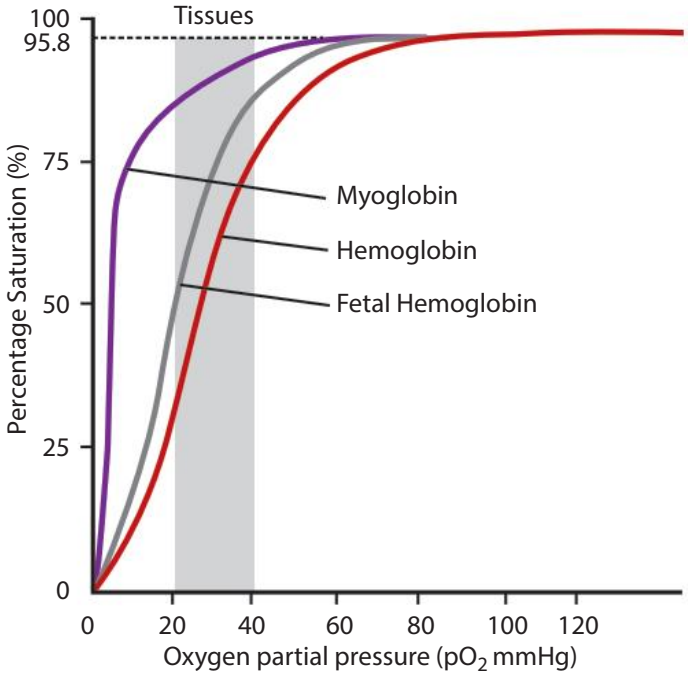
Skill: Analysis of dissociation curves for hemoglobin and myoglobin.

## Data

*Most reputable Biology Textbooks* and websites will include information and show dissociation graphs similar to this:*

Hemoglobin carries oxygen around the body, but when it reaches the muscles, the oxygen is taken over and stored by myoglobin. To be able to do this, myoglobin must have a higher affinity for oxygen than hemoglobin (at equal $pO_2$), but still be able to release the oxygen when the muscles need it at very low $pO_2$. Human fetal hemoglobin has a slight difference in primary structure which allows greater affinity with oxygen in utero. *See Figure below.*

Hemoglobin is made of four polypeptide chains and each chain has an iron-containing heme group which can bind a molecule of oxygen. Myoglobin is only made of one polypeptide chain, so only has one heme group and binds only one molecule of oxygen. As a result, there is no cooperative binding. Therefore, the oxygen dissociation curve of myoglobin is not sigmoidal. *See Figure below.*




Hemocyanins are a group respiratory pigments that are found in arthropods and molluscs. They contain the element copper rather than iron and change colour from colourless to blue with oxygenation. There are other less common respiratory pigments, including hemoerythrin and chlorocruorin.

## For further investigation

- Comparing the molecular structures of myoglobin and the various forms of fetal and adult hemoglobin.
- The effects or altitude training are mainly quantitative with the production of a greater amount of hemoglobin and myoglobin. However, there is evidence that there can be adverse effects as well (refer also to IB Guide Aim 8): refer to <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1332514/>

* see <www.ibid.com.au> for information about our complementary IB Biology Textbook

# Appendix 1 Informed Consent Form

Read this form and listen carefully to the description of the tasks that you are asked to carry out. Feel free to ask as many questions as you wish that are relevant to the investigation.

You are free to ask questions at any time during the course of the experiment.

You may stop your participation in the experiment when you wish.

The data obtained from each volunteer will be made anonymous in all subsequent documents.

You may ask for your data to be deleted.

**Investigation title:** _____

**Name of experimenter:** _____

**Location:** _____

**Date:** _____

## Description of the investigation and the role of the volunteer

## Possible risks or discomforts

I have read and understand these instructions.

I have no known health problem that may be incompatible with this investigation.

I understand that I may withdraw from the experiment at any time

**Signature** _____

**Date** _____

# Appendix 2 Table of random numbers

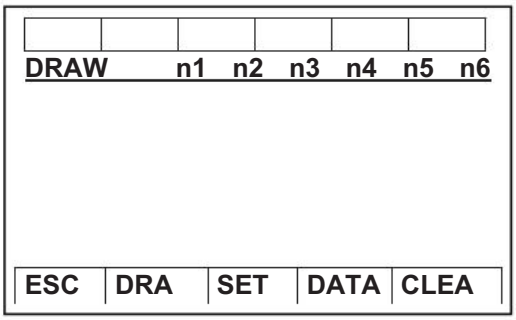
The numbers have been arranged in pairs of columns. These may be used as random co-ordinates (X and Y) or individual columns may be used as strings of random numbers.

| X | Y | X | Y | X | Y | X | Y |
|---|---|---|---|---|---|---|---|
| 71 | 5 | 52 | 83 | 11 | 40 | 36 | 51 |
| 53 | 41 | 77 | 82 | 100 | 46 | 49 | 39 |
| 58 | 86 | 5 | 59 | 68 | 49 | 16 | 11 |
| 29 | 79 | 59 | 99 | 2 | 21 | 47 | 78 |
| 30 | 37 | 47 | 91 | 58 | 33 | 26 | 46 |
| 77 | 96 | 30 | 23 | 10 | 10 | 63 | 75 |
| 1 | 87 | 62 | 70 | 10 | 59 | 54 | 60 |
| 76 | 6 | 65 | 98 | 80 | 17 | 16 | 83 |
| 81 | 95 | 26 | 24 | 28 | 93 | 94 | 2 |
| 71 | 36 | 28 | 53 | 5 | 10 | 65 | 21 |
| 30 | 41 | 91 | 56 | 51 | 55 | 44 | 79 |
| 38 | 41 | 26 | 69 | 46 | 92 | 27 | 30 |
| 30 | 71 | 79 | 91 | 35 | 54 | 87 | 24 |
| 95 | 33 | 38 | 83 | 40 | 41 | 75 | 48 |
| 98 | 63 | 29 | 2 | 27 | 85 | 27 | 25 |
| 40 | 21 | 92 | 54 | 6 | 83 | 67 | 34 |
| 28 | 19 | 63 | 92 | 24 | 67 | 26 | 4 |
| 16 | 58 | 63 | 43 | 98 | 72 | 9 | 48 |
| 16 | 8 | 43 | 68 | 6 | 100 | 3 | 21 |
| 65 | 46 | 10 | 50 | 39 | 34 | 32 | 86 |
| 36 | 51 | 7 | 19 | 65 | 21 | 8 | 76 |
| 49 | 39 | 11 | 68 | 44 | 79 | 59 | 92 |
| 16 | 11 | 33 | 45 | 27 | 30 | 75 | 62 |
| 47 | 78 | 13 | 36 | 87 | 24 | 93 | 35 |
| 26 | 46 | 0 | 15 | 75 | 48 | 33 | 15 |
| 63 | 75 | 54 | 70 | 27 | 25 | 54 | 48 |
| 54 | 60 | 66 | 93 | 67 | 34 | 8 | 22 |
| 16 | 83 | 54 | 53 | 26 | 4 | 63 | 99 |
| 94 | 2 | 83 | 9 | 9 | 48 | 41 | 13 |
| 65 | 21 | 8 | 76 | 3 | 21 | 96 | 3 |

# Appendix 3 Using a TI calculator to generate random numbers

Press **APPS** and select **Prob Sim**. This opens the 'Probability Simulation' program.
Press any key to open the menu.
(The **Probsim** program can be downloaded to the calculator from the Texas Instruments ® site.)

Select **6. Random Numbers**

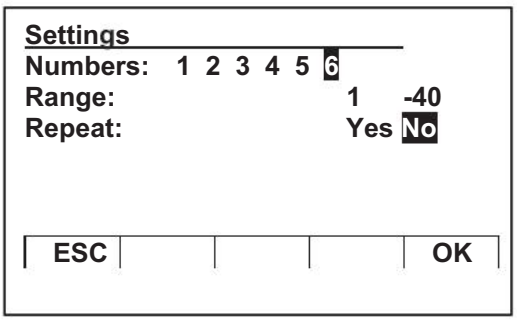



Select **SET** (press the **ZOOM** key).

**Numbers** determines the set of random numbers drawn. Up to six can be drawn at one time.

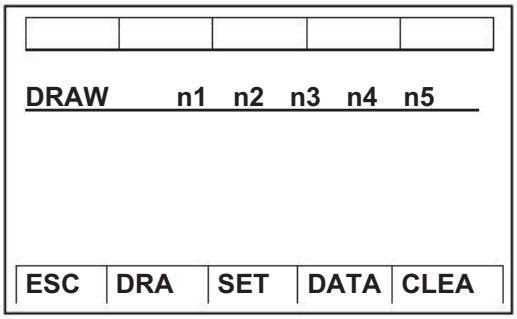
**Range** sets the range of the numbers that can be chosen (limited between 1 and 99)

**Repeat** determines whether you want a number to appear more than once or not.



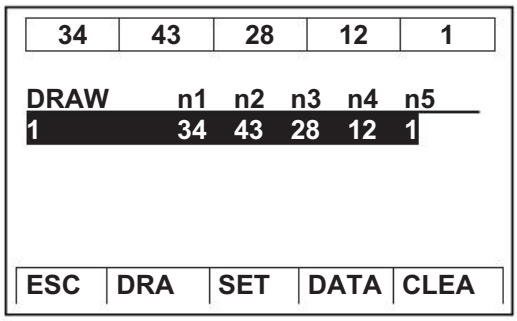

Make your choices and select **OK** (press the **GRAPH** key)

***Example:***
**Settings**
**Numbers: 5**
**Range: 1-50**
**Repeat: No**



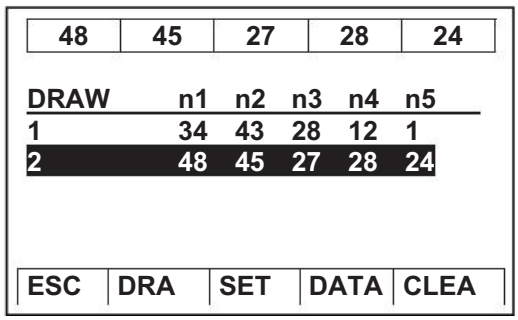

Select **OK** (press the **GRAPH** key)

Select **DRAW** (press the **WINDOW** key)




If you want more random numbers with the same settings, select **DRAW** again.

To start again using the same settings select **CLEAR** (press the **GRAPH** key).
To change the settings select **SET**.




To quit **Random Numbers** select **ESC** (press the **Y=** key).