BIOLOGY GCSE
REQUIRED PRACTICALS

Introduction

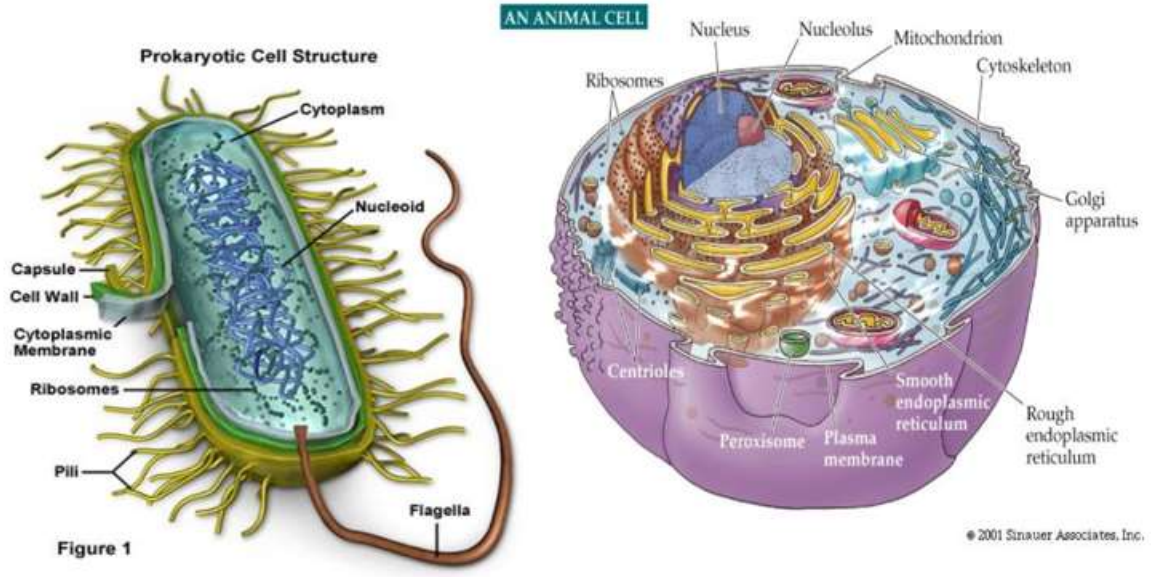
Biology is broken down into many subjects such as cytology, genetics, neurology and ecology, each of which are covered in the assignment.

Biology serves as a basis to solving real world problems we face, such as new drug discoveries and mitigating the risks associated with globalisation to the worlds biodiversity.

These issues can only be resolved through understanding, knowledge and delivering innovative ideas on a local and global scale. The more that is learned and understood, the better (and healthier) our world can strive to become.

Cell Biology

Prokaryotic vs Eukaryotic Cells



https://biochemanics.files.wordpress.com/2013/03/125817436.png (accessed: 1st June 2017)

RECAP: Cells are the basic unit of living organisms.

They contain DNA (the Cell blueprint), Cytoplasm and Cell membrane.

Understanding Cell Biology

Eukaryotic cells can be seen (as dots) through a good magnifying lens (x10 or x15). A good budget non-zooming stereo microscope costs £90.00 and is a useful piece of equipment for the biologist.

An Optical (light) microscope can produce an image than will enable the researcher to identify some organelles, such as the nucleus and vacuoles. The low cost of optical microscopes makes them useful in a wide range of different areas, even for fun.

A scanning electron microscope can clearly identify all organelles in detail (extremely important for understanding DNA and chromosomes).

Using techniques, apparatus and materials, both plant and animal cells will be studied by observation through a light microscope.

This very first practical experiment sets the baseline for the entire scope of required practical sessions at GCSE.

Fundamental Parts of Cells – Plant Cells (onion epidermis)

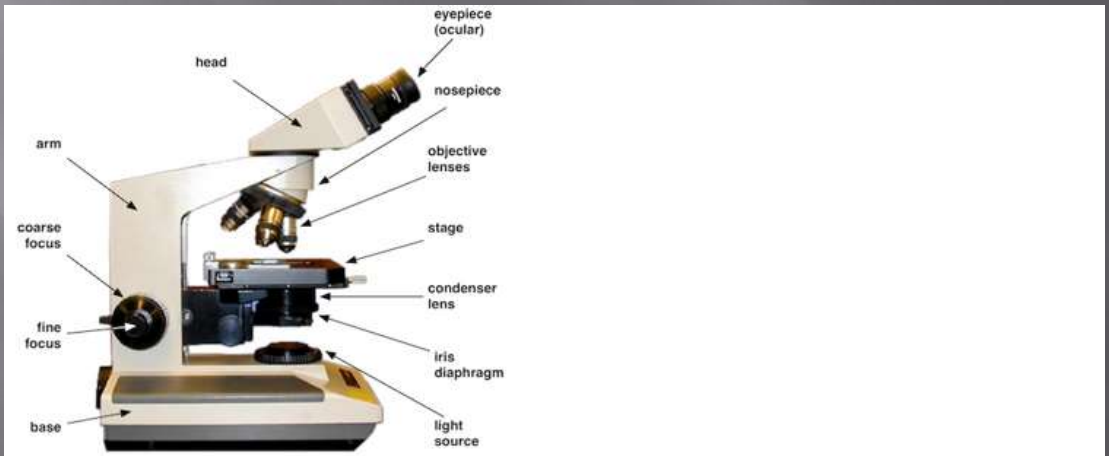
Microscopy – Method:

- Squeeze a drop of water from a pipette onto a microscope slide.
- With a knife, remove the outer layer of the onion and cut a small section.
- Expose and peel one of the epithelial layers from the inside of the onion with forceps.
- Place this thin layer flat on the drop of water on the microscope slide.
- Add two drops of iodine solution onto the onion tissue.
- Lower a coverslip onto the slide – do not touch the flat surface, hold on edge and lower on the onion tissue using forceps.
- Soak up any access liquid (water / iodine) which may have been displaced by the coverslip.

Microscopy

Using the microscope:

- Place the slide on the stage
- Use the lowest magnification lens (moving the nosepiece) first
- Use the course adjustment knob to clarify the image
- Rotate the nose piece again to obtain a higher magnification
- Use the fine adjustment knob to clarify the image
- Make a clear and labelled drawing of the onion cells highlighting the stained areas – drawing should include: Cell wall, Nucleus, Vacuole and Cytoplasm



http://www.doctortee.com/dsu/tiftickjian/cse-img/biology/cell/micro/microscope-diagram.jpg (accessed 3rd July 2017

Observing and Noting Cells

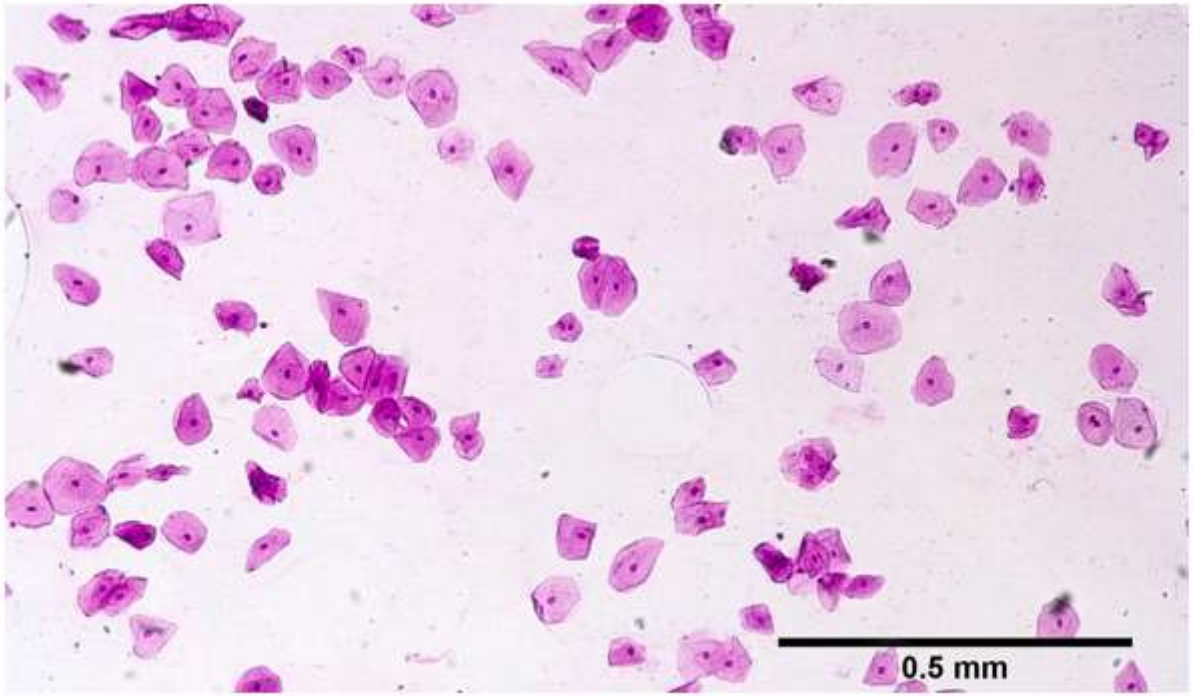
http://creatiwittyblog.com/wp-content/uploads/2013/01/world-microscopic-wonders-epidermal-cell-onion.jpg
(Accessed 3rd Jul 2017)

Animal Cells (cheek cells)

Microscopy – Method:

- Using cotton swabs, swipe the inside of our mouths.
- Then transfer the cells onto a clean glass slide.
- Place the swab immediately into disinfectant afterwards.
- Add one drop of concentrated Methylene blue to the cells on the slide.
- Lower a coverslip onto the slide, holding the edge ensuring that there are no air bubbles in between the slide and the cover slip.
- Soak up any excess chemical which may have been displaced by the coverslip.
- Make two labelled drawings (low (10x) and high magnification (100x)) of the cheek cells highlighting the stained areas. Drawings should include: Cell wall, Nucleus, Vacuole and Cytoplasm.
- Disinfect all glassware and equipment after use.

Observing and Noting cells



https://classconnection.s3.amazonaws.com/934/flashcards/2507934/jpg/cheek_cell1363146212875.jpg
(accessed 3rd July 2017)

Risk Assessment:

- Iodine solution may irritate the eyes so safety goggles should be worn. Refer to Hazcards 54A and 54B
- Methylene blue is an irritant and toxic in high concentrates. Refer to Hazcard 70 (dyes and indicators)
- Wash off any spillages on the skin immediately.
- Wipe up solution spills with a damp cloth and rinse it well.

Transport through cells

RECAP: Three types of movement into / out of Cells. These are;

Osmosis – The movement of water concentration across the cell membrane.

Active Transport –The transport substances via channel proteins against their concentration gradient. This requires Energy [ATP].

Diffusion – The simple movement down a concentration gradient through gaps in the phospholipid bilayer.

The next experiment involves calculating the effect of surface area and size on diffusion and considers the problems faced by large animals.

Diffusion

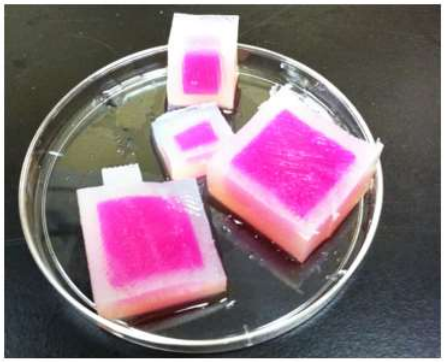
Method:

- Using the tools provided, cut three cubes of agar into the following sizes: 0.5cm x 0.5cm, 1cm x 1cm, 2cm x 2cm.
- Place 20cm^3 HCL into a beaker and place over a white sheet.
- Using forceps, place the smallest cube into the beaker and time the colour change.
- Wash out the beaker and repeat this for the second cube.
- Wash out the beaker and repeat this for the largest cube.
- Record the timed data in a table.

Diffusion

Results:

- As length doubles, surface area multiplies.
- As length doubles, volume multiplies.
- As length doubles, the surface area to volume ratio halves.
- The rate of diffusion is around same for each cube due to the standardisation of the agar used – other than its size, it is the same for each test.



http://2.bp.blogspot.com/-9hfM2Q3CN6E/UlGdMWVtGhI/s1600/photo+2.JPG

(accessed 4th July)

Osmosis

This experiment uses potato to investigate the movement of water through a semi-permeable membrane.

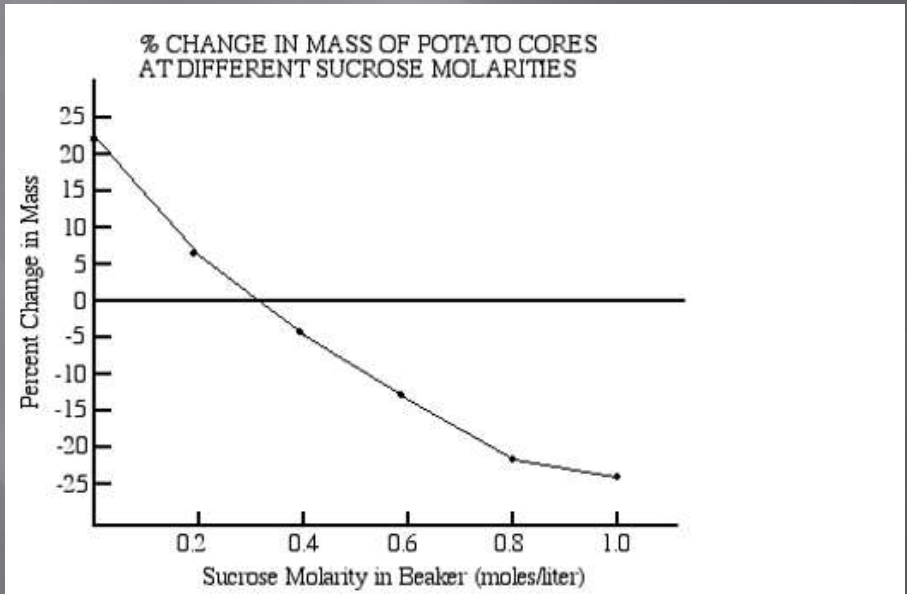
Method:

- Make up 10cm^3 samples of 1.0, 0.8, 0.4, 0.2 and 0.1 mol/dm^3 sucrose solutions. Place distilled H_2O into the beaker labelled 0.0 mol/dm^3.
- Use a cork borer to cut six potato cylinders of the same diameter and cut to the same length (3cm).
- Accurately measure and record the length and mass of each potato cylinder.
- Dry the samples with kitchen paper to remove any moisture and weigh all six – record this as initial weight.
- Add one potato cylinder to each boiling tube of the solutions above (labeled and on test tube rack). Top up with the solutions until it completely covers the potato core.
- Leave for 15 minutes occasionally agitating.
- Remove all samples one by one, dry with a paper towel and record the second weight.
- Calculate the change in mass and then % change (plot on a graph).

Osmosis

Results:

- The before and after sizes vary depending upon the solutions they are placed into. Shrinkage (where cells have plasmolysed) can be found in samples of high sucrose concentrations and swelling (where cells have become turgid and burst) in the hypotonic concentrations.



http://www2.sluh.org/bioweb/apbio/labs/apl01masspercentchangegraph.png

(accessed 4th July)

Risk Assessment

- Take care with the solutions (Hydrochloric acid) used: wear eye protection and rinse splashes off the skin.
- Take care when using electrical balances near water.

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Sharps

including **scalpels, knives, syringe needles, seekers, etc** (2013)

Source	Hazard	Comment
Scalpels, knives and other blades	⚠ DANGER	Cuts and puncture wounds can lead to infection, especially if the blade or point is contaminated by contact with living or once-living material. Careless use and handling of scalpels, syringes with needles, seekers and other sharps can lead to cuts and puncture wounds. Sharp scalpels are safer to use than blunt ones because there is less risk of them slipping as less force needs to be used. Carrying scalpels, syringes with needles, seekers and other sharps especially in crowded rooms, can present a hazard to the user and others. Carelessly-disposed sharps can present a hazard to waste handlers and others.
Syringe needles	⚠ DANGER	
Seekers and other sharps	⚠ DANGER	

Typical control measures to reduce risk

- Follow your teacher's guidance on safe practice in relation to the material being dissected.
- Cut in a direction away from yourself and where possible cut using a cutting board, dissection tray or pad or similar.
- Wear eye protection when changing scalpel blades or cutting material likely to "flick" (eg, cartilage or bone).
- Count sharps at the beginning and end of the lesson.
- Carry sharps with the blade or point protected, eg in a shallow tray, and do not carry them at all if you are likely to be jostled..
- Dispose of used sharps in a proper, safe container, eg a sturdy box, clearly labelled, and sealed and wrapped before disposal.

Food and Nutrition

Background:

- Starch can be found in a variety of different foods, mainly potatoes, bread, rice, pasta and cereals and should make up just over a third of the food we eat.
- Protein can be found in eggs, milk. yogurt, fish and meat etc.
- Glucose is the building block of most carbohydrates, simple sugars, but in large amounts can be unhealthy.
- Most fats and oils contain both saturated and unsaturated fats in different proportions and can be found in cheese, milk, lard, meat products, vegetable oils etc.

Simple tests are used by the food industry to identify what types of food contain how much of starch, glucose, protein etc. for packaging information (consumers).

Food and Nutrition

Testing for starch.

Method:

- Label test tubes 1 – 4
- In tube 1, add 20ml 10% glucose
- In tube 2, add 20ml 1% starch
- In tube 3, add 20ml 1% albumin
- In tube 4, add 20ml d H_2O (blank)
- Add 3 drops of iodine to each tube and agitate
- In a table write down observations made (colour changes)

Positive tests colour turns black / blue

Food and Nutrition

Testing for glucose.

Method:

- Half fill a beaker with water and place on a tripod and gauze, heat with a Bunsen burner
- Label test tubes 1 – 4
- In tube 1, add 20ml 1% starch
- In tube 2, add 20ml 10% glucose
- In tube 3, add 20ml 1% albumin
- In tube 4, add 20ml d H_2O (blank)
- To each tube, add 10ml of benedict's solution
- Place the tubes in the beaker of hot water over the Bunsen burner, keeping the water just boiling
- After 5 minutes, turn out the flame, allowing the tubes to cool slightly, place them on the rack and observe colours.

Red / orange – positive for glucose (maltose / fructose)

Cloudy green is sometimes seen with 1% starch

Food and Nutrition

Testing for protein

Method:

- Label test tubes 1 – 4
- In tube 1, add 20ml 10% glucose
- In tube 2, add 20ml 1% starch
- In tube 3, add 20ml 1% albumin
- In tube 4, add 20ml d H_2O (blank)
- To each tube, add 5ml of sodium hydroxide solution
- To each tube, add 5ml of dilute copper sulphate solution
- Agitate the contents
- Place them on the rack and observe colours.

Purple – positive for protein

Food and Nutrition

Testing for fats

Method:

- Label test tubes 1 – 4
- In tubes 1 and 2, add 20ml ethanol
- In tube 1, add one drop of vegetable oil
- Agitate and allow the oil to dissolve in the alcohol
- In tube 3 and 4, add 20ml H_2O
- Tip the contents of tube 1 into tube 3
- Tip the contents of tube 2 into tube 4
- Agitate the contents
- Place them on the rack and observe colours.

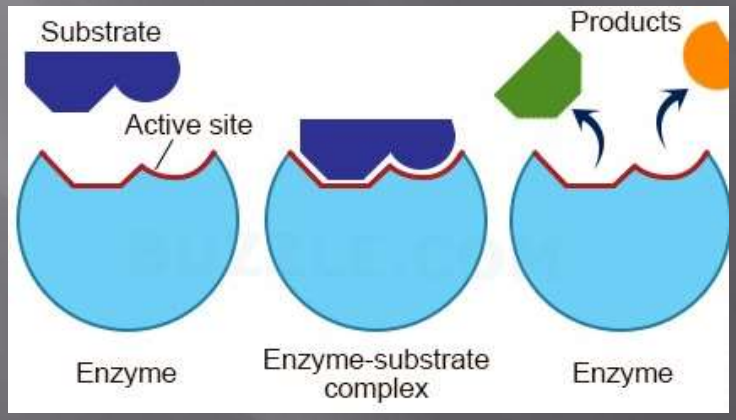
Ethanol and fat (tube 1) should become a cloudy emulsion

Enzymes

RECAP: Biological catalysts made up of amino acids / proteins designed to speed up reactions inside our bodies.

Many Enzymes are involved in this process:

- In saliva, there is an enzyme which catalyses the breakdown of starch into smaller molecules (Maltose). This active enzyme is called amylase and has an active site to which starch fits exactly. Other enzymes in the body carry out this function in the same way.
- Lipase (Fat into Glycerol and Fatty acids)
- Protease (Protein into Amino Acids and Sugars)



Effect of amylase on starch

Method:

- Prepare a water bath
- In four labelled test tubes (1-4), add 5cm^3 (2%) starch using a syringe
- In number 2 and 3 tubes, add 2cm^3 amylase solution
- Agitate the tubes and leave for 5 minutes
- In tubes 1 and 2, add 3 drops of iodine
- Add 3cm^3 benedict's solution to tubes 3 and 4
- Place tubes 3 and 4 inside a water batch for 5 minutes

Record findings:

Effect of amylase on starch

Results:

- Test tube 1 + iodine = blue / black (positive starch)
- Test tube 2 (amylase) + iodine = brown
- Test tube 3 (amylase) + benedict's = Red / orange (positive glucose (starch broken down)
- Test tube 4 + benedict's = blue

Effect of Temperature on Enzyme activity

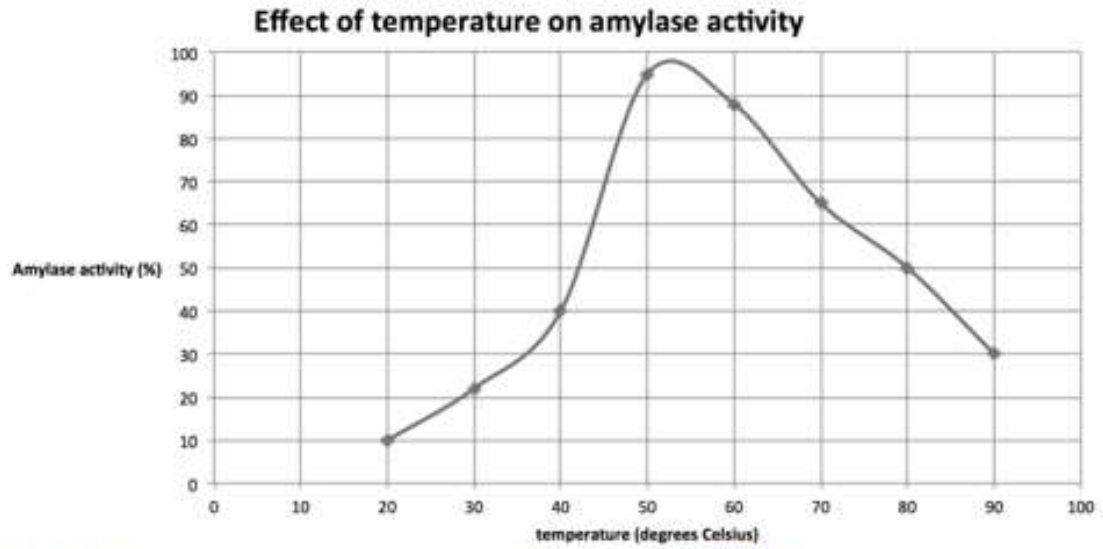
Method:

- Label 6 test tubes (1–6)
- In tubes 1, 2 and 3 add 5cm^3 (1%) starch solution
- Add 6 drops of iodine in each tube
- Add 1cm^3 amylase in tubes 4, 5 and 6
- Have three different temperature baths at ice cold, room temperature and 35^{o}C
- Place tubes 1 and 4 in ice water
- Place tubes 2 and 5 in room temperature water
- Place tubes 3 and 6 in warm water and leave all tubes in baths for 5 minutes
- Make a note of the time and pour the amylase solution from 4, 5 and 6 into their corresponding tubes, 1, 2 and 3 respectively.
- Shake and place back in to the baths

Effect on Temperature on Enzyme activity

Note the reaction times – express them on a graph
(i.e. when blue colour disappears / starch is broken down)

Results:



http://cdn.intoscience.com/csa/amylase-temp.018-001.png
(accessed 3rd July 2017)

Effect of pH on Enzymes (amylase)

Method:

- Test tubes labelled 1 – 5
- Place 5cm^3 of 1% starch in each tube
- Put 3 drops of iodine in each circle on a spotting tile
- In tube No. 1 place 1cm^3 of sodium carbonate solution (alkali)
- In tube No. 2 place 0.5cm^3 sodium carbonate
- Tube No. 3 (leave blank)
- In tube No. 4 place 2cm^3 ethanoic acid (acid)
- In tube No. 5 place 4cm^3 ethanoic acid
- Note the time.
- To each tube add 1cm^3 amylase and shake
- Remove a sample from each tube and drop into each iodine containing circle on the dropping tile
- Take the same samples at 30 second intervals and place into new circles
- Stop testing after 10 minutes

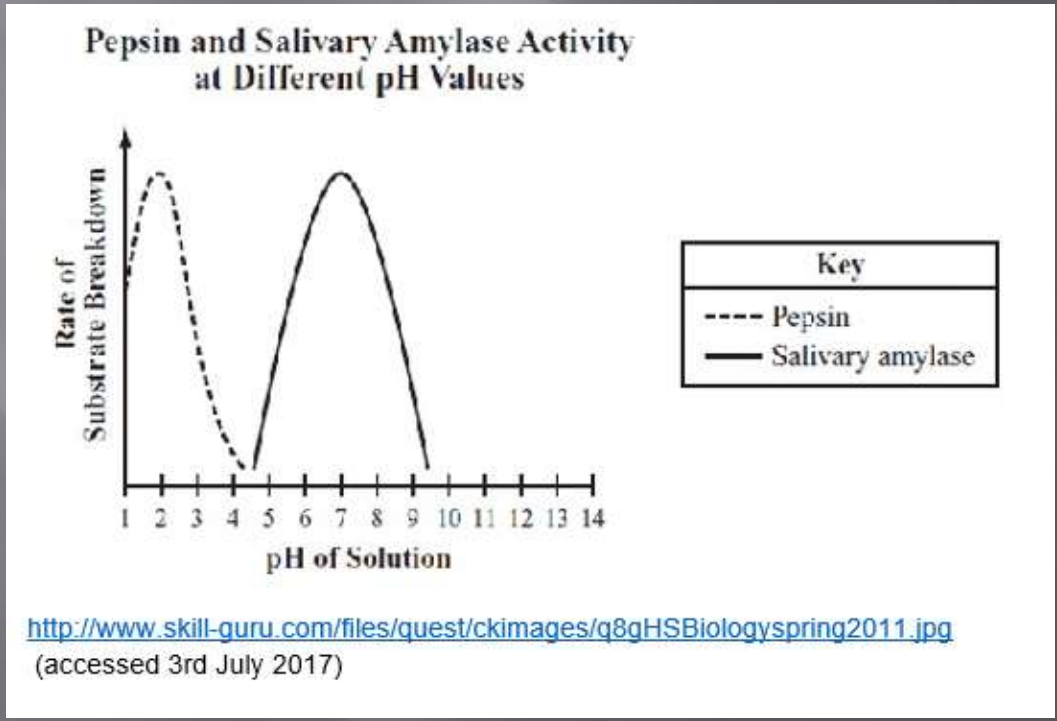
Effect of pH on Enzymes (amylase)

Records results:

Test the pH of each tube - Tube No. 3 should see best results (neutral).

Saliva is between 6.5 and 7.5 pH

Further representation may include:



http://www.skill-guru.com/files/quest/ckimages/q8gHSBiologyspring2011.jpg
(accessed 3rd July 2017)

The effect of pH on Enzymes (Pepsin and Egg White)

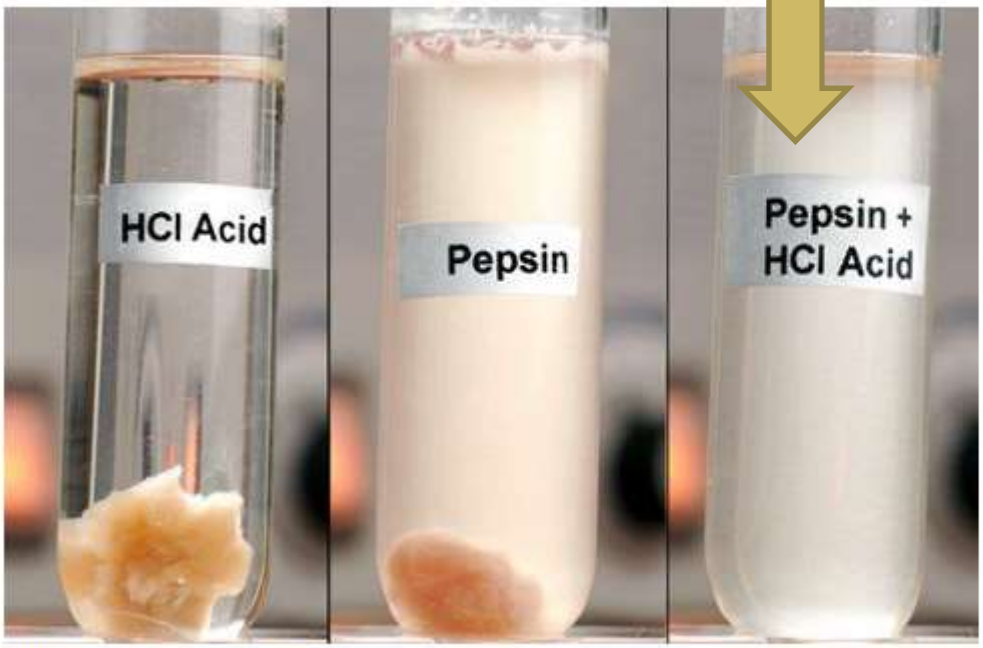
Method:

- Label tubes 1–5
- Add 5cm^3 of egg white to each
- In tube 1, add 2 cm^3 sodium carbonate
- In tube 2, add 0.5cm^3 sodium carbonate
- Leave tube 3 blank
- In tube 4, add 1cm^3 HCl
- In tube 5, add 2cm^3 HCl
- Add 1cm^3 (1%) pepsin to all tubes
- Place all tubes in a water bath (40^oC)
- After 15 minutes, take a pH reading and compare results.

The effect of pH (Pepsin and Egg White)

Results:

- The egg white solution goes from cloudy to clear indicating that pepsin works better in acidic conditions (range from 2-5 pH)



http://www.functionalfitmag.com/blog/wp-content/uploads/2011/12/A5000818-Digestion_experiment-SPL.jpg
(accessed 3rd July 2017)

Effect of Enzyme concentration on rate of reaction

Method:

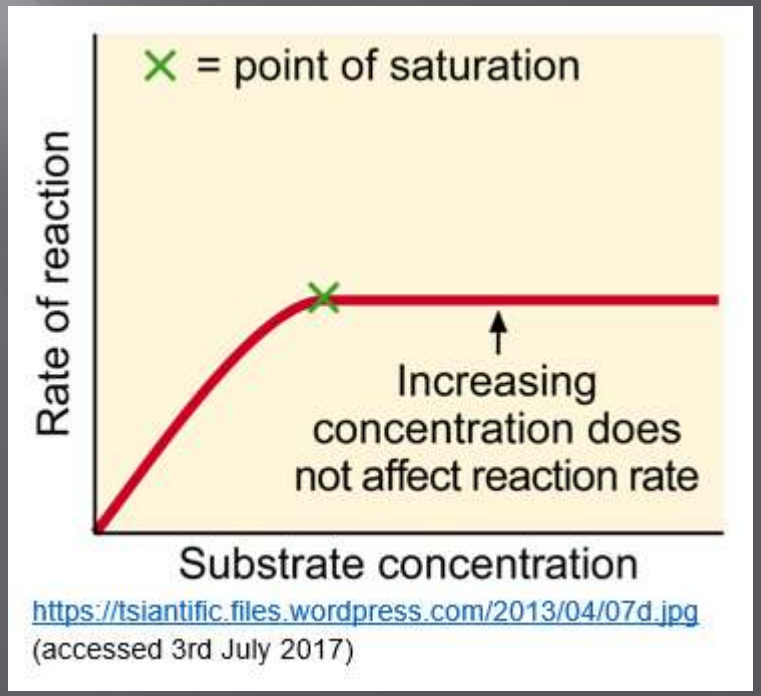
- Label test tubes 1–4
- Add 5cm^3 (1%) urea to each tube
- Add 2cm^3 to the dilute ethanoic acid to each tube
- Add ten drops universal indicator to each tube
- In tube 4, add 3cm^3 **boiled urease** solution
- In tube 3, add 4cm^3 urease
- In tube 2, add 3cm^3 urease
- In tube 1, add 2cm^3 urease
- Agitate all tubes and at 30 second intervals record the colour of the indicator

Effect of Enzyme concentration on rate of reaction

Results:

As the enzyme hydrolyses urea, the pH will become alkaline. Ethanoic acid and universal indicator solution are added to the reaction mixture.

The universal indicator solution is red at the beginning of the reaction and changes through to blue as the reaction proceeds, finally reaching end of reaction when colour change stops.



https://tsiantific.files.wordpress.com/2013/04/07d.jpg
(accessed 3rd July 2017)

Risk Assessment:

- Safety goggles should be worn throughout all experiments.
- Take care with boiling water.
- All solutions, once made up, are low hazard.
- Refer to Hazcard 2 for urease and other Enzymes

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Enzymes

includes **Amylase, Catalase, Cellulase, Diastase, Lipase, Proteases (eg, pepsin, trypsin), Urease**

Substance	Hazard	Comment
Enzymes Powders 'Biological' detergents contain enzymes.	HARMFUL	All enzymes may be sensitisers (see below) and may produce allergic reactions. They can cause asthma and irritate the eyes, nose and skin.
Enzymes Concentrated solutions	IRRITANT	All enzymes may be sensitisers (see below) and may produce allergic reactions. They can cause asthma and irritate the eyes, nose and skin.
Enzymes Dilute solutions Biological systems are, of course, rich sources of enzymes, eg, liver (catalase), saliva (amylase).	LOW HAZARD	The enzymes at these concentrations are unlikely to offer any significant risk.

Health and Disease

RECAP: Bacteria and fungi can be taken and cultured on agar to identify the pathogen and find a cure or antibiotic sensitivity.

The action of antibiotics, antiseptics and disinfectants can be investigated using cultures of microorganisms. Useful agents with antimicrobial properties, sometimes derived from plants are used to treat or prevent infection.

This type of experiment provides a good basis for identifying useful agents against bacterial infections and disease. However, sterile conditions (aseptic techniques) are needed to prevent contamination:

- The Petri dishes, nutrient agar jelly and other culture media must be sterilised
- The inoculating loops used to transfer microorganisms must be sterilised by hot flame
- The lid of the Petri dish is sealed to stop airborne microbes contaminating the culture.

Culturing microorganisms

Method: Preparing a lawn plate

- Wash hands before and after handling items and equipment
- Divide the base of the agar (petri dish) into 4 sections (A, B, C, D) and place a dot in the middle of each section
- Write initial and date along the edge of the plate lid
- With a lit Bunsen (blue flame), flame the neck of the bottle containing the culture (killing bacteria on the outside) and with a pipette remove 1ml of culture
- Flame the neck of the bottle, replace the lid and put to one side
- Pipette the culture onto the agar plate and close the lid
- Place the empty pipette straight into disinfectant
- Turn the Bunsen to yellow flame, dip the glass spreader into ethanol, tap excess, pass through the flame and wait for it to go out (20 seconds)
- Lift the lid of the agar dish and use the spreader to disperse the culture
- Place the used spreader straight into disinfectant
- The culture has now been successfully transferred onto the agar

Culturing microorganisms

Preparing the Discs

- Flame forceps over the Bunsen
- Lift the lid at an angle and using sterile forceps, place a dried paper disk over sections A, B, C on the plate over the dot, each time flaming the forceps
- Over dot D (control zone), pipette a drop of distilled H_2O only
- Place all used instruments into disinfectant
- Tape the lid (biohazard tape) to the base at each end and place into incubation for 2-3 days (20^{o}C – 25^{o}C)
- Observe the inhibition zone over each section, measuring the area with a ruler so that the diameter can be calculated.

There will be some clear difference (bacterial sensitivity to antiseptics, plant extracts or chemicals) in sections A – C.

The purpose of leaving section D as a control area – isolated colonies may be extracted from this.

Using a Bunsen burner – types of flame

Type of flame	Gas tap	Air hole	Appearance	Hazards	When used
Yellow flame	Fully (or partly) open.	Closed		This flame is still hot if you put your hand into it.	To light the burner and when it is not heating anything. It is easy to see and will not readily set fire to clothing etc. This flame is unsuitable for heating as it coats surfaces with soot (carbon).
Gentle and medium flames Yellow just disappears; pale lilac, almost invisible.	The gentle flame has the gas tap half open and the medium flame has the gas tap fully open.	Partly closed		It is difficult to see the flame in bright sunlight. Some risk of blowing out.	For general heating. Start with the gentle flame and then open the gas tap fully open to give the medium flame.
Roaring flame Inner cone blue, outer cone lilac.	Fully open.	Fully open		It is difficult to see the flame in bright sunlight. Some risk of blowing out. Do not partly close the gas tap if the air hole is fully open as this will extinguish the flame.	For very strong heating (which is not very often). The hottest section of the flame is just above the blue cone of unburnt gas.

Risk assessment

- It would be dangerous to incubate cultures at temperatures close to body temperature (37°C) because it may allow the growth of pathogens harmful to health. To control the Risk associated, the maximum temperature used is 25°C.
- Do not culture microbes other than those advised in the experiment.
- When using ethanol, ensure that it doesn't meet a naked flame. This is extremely flammable.
- Be careful when using Bunsen burners – Danger of hot surface or burns!

CLEAPSS STUDENT SAFETY SHEETS 1

Microorganisms

Source	Hazard	Comment
Samples from the environment	BIOHAZARD	Air, water and soil samples could be used, but not samples from high-risk areas, eg, toilets or the floors of changing rooms. All environmental samples could be contaminated with **pathogens** (organisms which cause disease).
Samples from humans	BIOHAZARD	'Finger dabs' could be used or hair from clean areas, eg, the scalp. Samples could, however, be contaminated with pathogens (see above).
Foods	BIOHAZARD	Any uncooked animal product (eggs, meat, cheese etc) may be contaminated with bacteria, especially *Salmonella* and *Escherichia coli* (*E. coli*) from the gut, which can cause food poisoning. Take care to prevent cross contamination between cooked and uncooked foods. Thorough cooking will destroy bacteria.
Purchased cultures (ie, samples of microorganisms bought from suppliers)	BIOHAZARD	Cultures bought from reputable suppliers (but not those from hospitals, etc) should be safe but may have become contaminated. *E. coli* is often studied in schools, but this is **not** the same strain of bacterium that causes food poisoning.

Ensure sterile conditions are achieved AT ALL TIMES.

Wash hands and maintain personal Hygiene!

Bacterial Decay

Microbes are responsible for Decay and spoiling of food.

This experiment sees the effect of bacterial decay on a range of milk types (UHT, pasteurised and fresh).

The experiment is widely used in the milk production industry to identify quality and standards in the range of milks.

Carbon dioxide and lactic acid are released as biproducts of activity.

An indicator will be used to detect oxygen release (resazurin).

Method:

- Place 6 tubes on a rack, adding $2cm^3$ of resazurin indicator to each
- Add $10cm^3$ of each milk sample to each tube, bung with cotton wool
- Agitate and place tubes in water bath (40^oC) and record the colour change of each tube every 5 minutes

Bacterial Decay

Results:

Resazurin gives milk a characteristic blue colour and the test is based on the ability of bacteria in the milk to reduce the blue dye.

The quality of the milk is noted by the colour change - from blue through mauve and purple and pink and finally white to indicate bad condition milk (high bacterial counts).

http://faculty.lacitycollege.edu/hicksdr/litmusmilk.jpg (accessed 4th July)

Color of Sample: Quality of Milk

1. Blue (no color change): Excellent
2. Blue to deep mauve: Good
3. Deep mauve to deep pink: Fair
4. Deep pink to whitish pink: Poor
5. White: Bad

Risk Assessment

- Resazurin solution should be prepared fresh for accurate readings.
- Do not open the tubes once resazurin is added.

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Dyes & Indicators

Note: Many dyes and indicators are used in school science, in the home and in industry. This *Sheet* applies to those listed below, which include those likely to be found in schools. Be warned that some dyes are toxic and may cause cancer, some may contain hazardous impurities or are made up in hazardous solvents, many have not been well researched because they are not much used in industry and suppliers often vary in the hazard classifications they use.

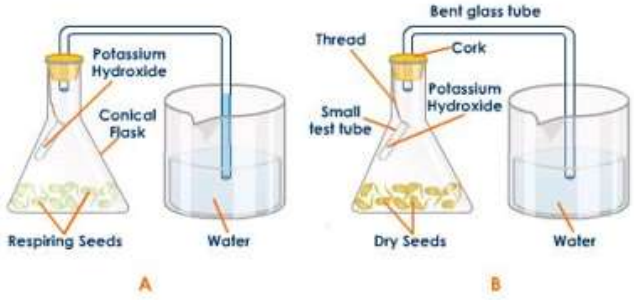
Substance	Hazard	Comment
Congo red, Methyl orange	 TOXIC	Congo red is a carcinogen. Methyl orange is toxic if swallowed.
Dyes and indicators, solid, including: **Acridine orange, Alizarin red S, Aluminon, Aniline blue, Cresol red, Crystal violet (Methyl/Gentian violet), Direct red 23, Disperse yellow 3/7, Dithizone, Eosin, Eriochrome black (Solochrome black), Indigo, Magenta (Basic fuchsin), Malachite green, May-Grunwald stain, Methyl green, Methyl red, Methylene blue, Procion dyes, Resazurin, Rosaniline, Sudan I, II and IV, Xylene cyanol FF.**	 HARMFUL & / OR IRRITANT	Skin contamination will be very obvious. This should be avoided and dusts of most dyes can irritate the eyes and lungs while some may act as sensitisers. Dyes for use in the home (eg, *Dylon*) may be classed as IRRITANT. Dust from Procion dyes may be a sensitiser, but the M-X dyes present a greater risk than the H-E dyes.
Dyes and indicators, solid, including: **Acid blue 40, Alizarin, Blue dextran, Brilliant yellow, Bromocresol green, Bromophenol blue, Bromothymol blue, Carmine, DCPIP (PIDCP), Diazine green (Janus green B), Fluorescein (Dichlorofluorescein), Indigo carmine, Litmus, Methyl blue, Murexide, Neutral red, Nigrosin, Orcein, Phenol red, Phenolphthalein, Rhodizonic acid, Sudan black, Sudan III, Thymol blue, Thymolphthalein, Toluidine blue, Xylenol orange.**	LOW HAZARD	The substances listed here are not usually classified as hazardous but note the comments in the box at the top of the sheet. They should therefore be used with caution. Skin contamination should be avoided.
Dyes and **indicators** Dilute *aqueous* solutions of the above (except Congo red) which include **Full-range pH indicator, Screened methyl orange** and **Universal indicator**.	LOW HAZARD	Dyes & indicators in dilute solutions are unlikely to offer significant risk because most are less than 1% by mass. However, some are made up in solvents other than water and the hazards may be greater, eg, ethanol [HIGHLY FLAMMABLE if more than 25% (v/v)] or ethanoic acid which may be CORROSIVE.

Respiration

Respirometers are used to measure respiration rates in plants and animals over a given period. In this practical a very simple respirometer will be used to detect the respiration rate in germinating seeds.

Method:

- Place 5g of germinating seeds into a conical flask (A) and 5g boiled seeds into flask (B)
- Set up the glass tubes into the bungs and suspend a tube of potassium hydroxide in each flask
- Place the other end of the tube in a beaker of water and record the level in each case
- Leave for 30 minutes and record the change in the level of water



http://images.tutorvista.com/content/respiration/aerobic-respiration-demonstration.jpeg (accessed 4th July)

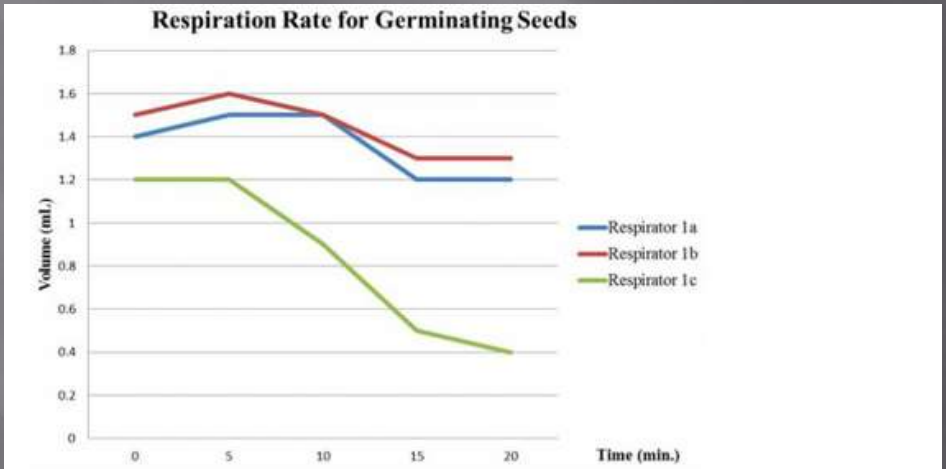
Respiration

Results:

The level of water in flask (A) will have increased as the rate of respiration and volume of air increases in the flasks, confirming that respiration is taking place in the first 10 minutes.

Flask (B) is a control and shows that no change has been recorded.

Sample graph below illustrates a typical graph that can be produced to show the results.



http://roughgarden-apbio.weebly.com/uploads/1/7/3/7/17371933/1362784672.jpg

(accessed 4th July)

Control Systems - Reflex Actions

RECAP: Reflex reactions in humans (and all animals) are controlled by the **reflex arc**. This experiment will identify the variation of reflex actions in individuals (on consumption of caffeine and no caffeine – blind test) .

It must be undertaken accurately and with no bias.

Method:

- Person 1 holds a hand out at 1m from the floor
- Person 2 holds a ruler until it is above person 1's hand (at 0cm) and lets its go (without telling them)
- The ruler is read at the point of person 1 catching it – repeat 3 times
- Swap over to allow person 2 do the test
- Each person should drink (A or B – caffeine or no caffeine)
- The reaction test is then repeated for each pair and results recorded

Reflex action

Results:

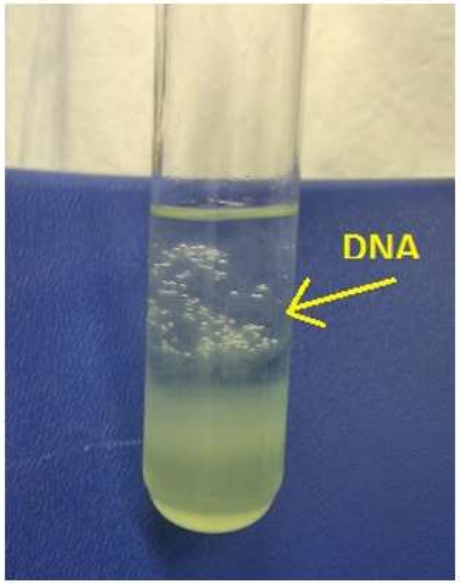
The reaction time should be quicker with consumption of caffeinated drinks following the first test. The results for the person (1 or 2) consuming the caffeinated drink (stimulant) should be faster reaction time.

Risk assessment

- Care should be taken to avoid injury from the falling ruler.
- Care from hot drinks (coffee)
- Take care not to consume drinks in the laboratory where chemicals and biohazards are present

Deoxyribonucleic Acid

RECAP: Inside the nucleus of a eukaryotic cell, there is DNA tightly looped around itself. There are up to 3 billion base pairs, storing its individual blueprint (info, amino acid sequence etc.).



http://study.com/cimages/multimages/16/DNA_from_kiwi_from_www.stagesofsuccession.com.jpg

(accessed 4th July)

In this experiment, DNA can be isolated from the plant cell and visually observed.

(Using a kiwi, banana, strawberry or thawed frozen peas).

Deoxyribonucleic Acid

Method:

- Peel the fruit and chop into small chunks, grind them into puree in a pestle and mortar
- Add 20ml of extraction fluid and mix it into the puree
- Salt and water help break the cell wall apart, increase surface area and allow the DNA to precipitate later on
- Place the mixture into a beaker and incubate in a water bath (60^{o}C) for 15+ minutes
- The mixture should then be filtered through a funnel into a test tube
- Tilt the tube and pour in a similar amount of ice cold ethanol over the filtered puree until it forms a layer. The ethanol will sit on the surface.
- Leave for 5 minutes until the DNA precipitates (forming white clumps)
- Use a glass rod or device to tease out (wind out) the DNA.

Risk Assessment

CLEAPSS

STUDENT SAFETY SHEETS 60

Ethanol

also applies to **Denatured alcohol and Methylated spirit**

Substance	Hazard	Comment
Ethanol Pure	 HIGHLY FLAMMABLE	There is a serious risk of liquid catching fire; its vapour may catch fire above 13 °C. The vapour / air mixture is explosive (from 3.3 to 19% ethanol). Breathing vapour may result in sleepiness: the concentration in the air should not exceed 5,760 mg m^{-3}.
Industrial denatured alcohol (IDA) [formerly **Industrial methylated spirit (IMS)**]	 HIGHLY FLAMMABLE	It is more hazardous than pure ethanol because of the presence of 5% (v/v) methanol which is TOXIC. It is often used as a solvent, eg, for chlorophyll and for indicators, eg, Universal indicator, phenolphthalein and in chromatography.
Completely denatured alcohol (CDA)	 HARMFUL	It contains methanol, pyridine and a purple dye. CDA has a bad odour and is not suitable for use indoors.
Surgical spirit *(It contains small amounts of castor oil, methyl salicylate and diethyl phthalate)*	 HIGHLY FLAMMABLE	It is suitable for demonstrating the cooling effect of evaporation. It can be applied to the skin on the back of the hand. It is used for medical purposes, eg, foot infections, cleaning the skin. It must not be swallowed.
Ethanol Dilute solution in water	LOW HAZARD	Alcoholic drinks contain ethanol, typically 3 to 7% (beers), 11 to 14% (wines), 30 to 40% (spirits). Although chemical hazards are low, there may be considerable effects on the body leading to a loss of judgement, slower reaction times, etc. Consumption is dangerous if driving a vehicle or operating machinery.

Typical control measures to reduce risk

- Use the smallest volume possible; wear eye protection.
- Make sure the room is well ventilated.
- Check that equipment for extinguishing fires is nearby, eg, damp cloth, bench mat, fire blanket.
- Do *not* use near naked flames; if heating is necessary, use an electrically-heated water bath or hot water from a kettle.

Growth responses - Tropisms

RECAP: A **tropism** is a growth response to an external stimulus.

Phototropism is the growth response of a plant to light and is exhibited by sun-loving species. An example of phototropism is when a plant is placed indoors, near a window. Stem curvature takes place as the plant grows towards the light.

Using seedlings grown at the same time, we can investigate the effect of light on the growth during germination. Various seeds can be used. Cress, white mustard and brassica variety are preferred due to quick germination for turnover of results.

The effect on Light

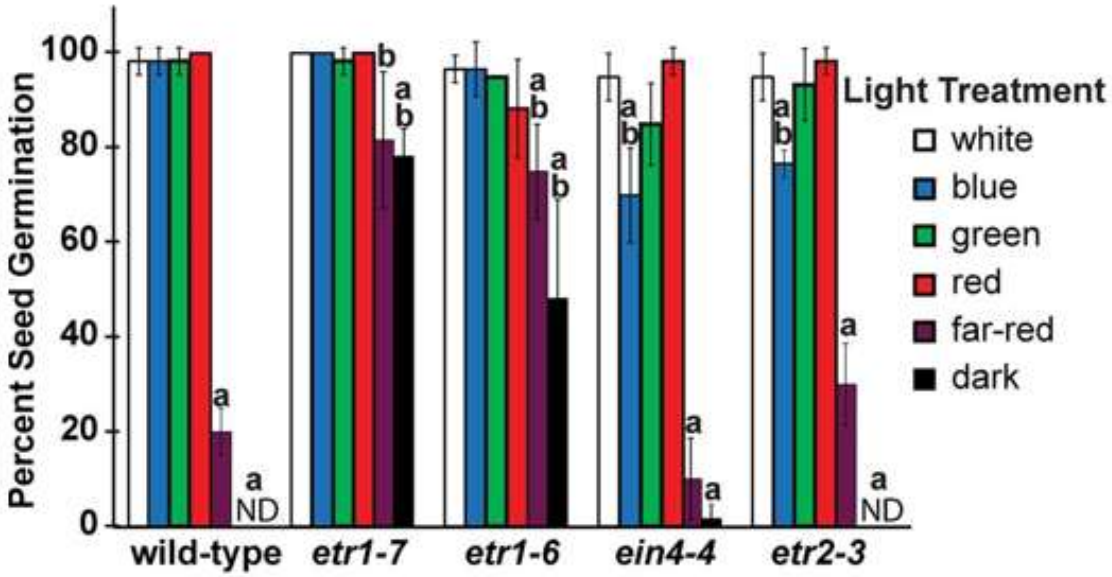
Method

- Using film cases with dark lids, make a hole about 10mm below the rim on each case
- Cut a 1cm square of each colour (red, green and blue) and black paper
- Cover the (1cm) hole of four cases with each one of the above
- With the fifth (control), leave blank, although tape over with clear tape / clear filter
- At the bottom of the cases, place a decent layer of shredded paper towel (this will be the growing medium) and dampen
- Place three seeds in each case, label (name and date) and cover with the different lids under a light source
- After two days, lift the lid and take a photograph (Arial view), remove the plantlets to allow a photograph to be taken from the front / side.
- Replace and leave for one week. Take further photographs

The effect on Light

Results:

Previous experimentations on various seeds:

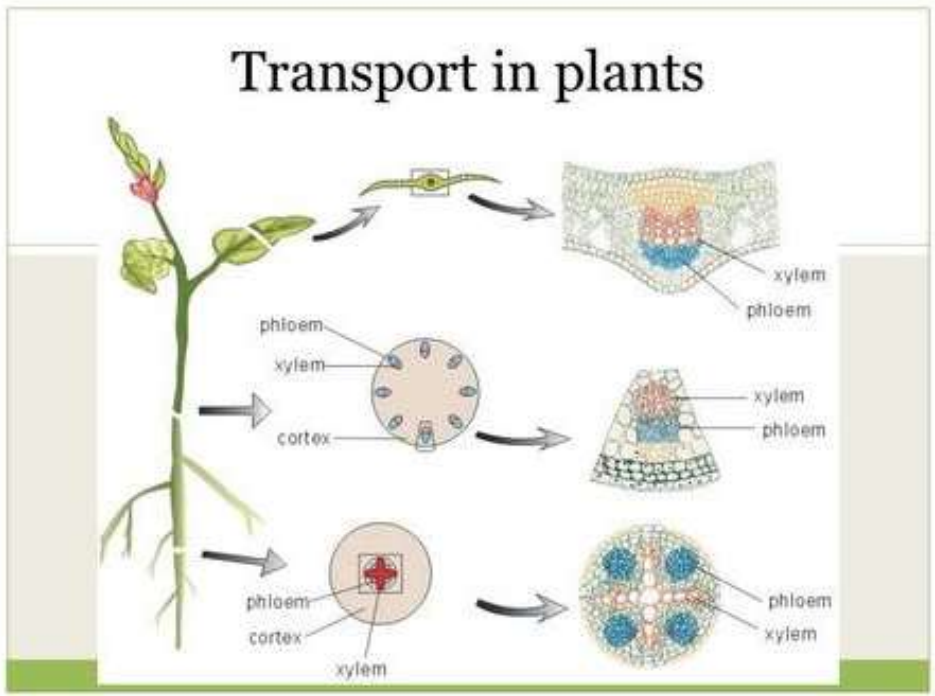


http://www.frontiersin.org/files/Articles/103099/fpls-05-00433-HTML/image_m/fpls-05-00433-g001.jpg

(accessed 4th June 2017)

Transport in Plants

RECAP: There are two types of vessels in plants: xylem (columns of dead hollow cells that enable water to be moved upwards through the plant by evaporation) and phloem (column of living cells that enables sugars and amino acids to be translocated from source to sink or storage region.

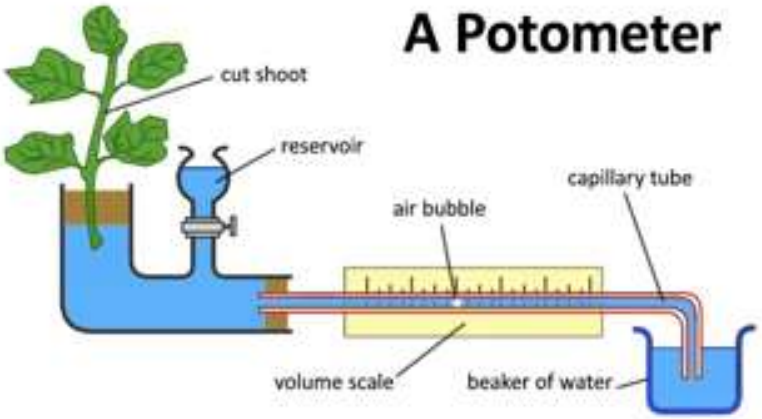


http://slideplayer.com/16/4900546/big_thumb.jpg

(accessed 5th July 2017)

Transport in Plants

In the next experiment, we are going to determine the effect of environmental conditions (light, air movement and temperature) on the uptake of water by plants. A potometer will be used to measure water uptake by timing how long a bubble takes to move a set distance in the capillary tube.



http://www.passmyexams.co.uk/GCSE/biology/measuring-transpiration.html

(accessed 5th July 2017)

Transport in Plants

Standard test – normal conditions

Method:

- Make eight marks on a capillary tube at 1cm intervals starting at 2cm from the free end
- Introduce a bubble to the capillary tube by lifting the potometer upwards (starting bubble)
- Place the capillary tube back into the water now that a bubble is present and retighten the screw on the boss
- Start the stop clock when the bubble touches the first marked line and stop the clock when the bubble travels 2cm to a marked line
- Calculate the rate of transpiration using:

$$\frac{\text{Distance (moved by air bubble in cm)}}{\text{Time taken (s)}}$$

Transport in Plants

- Changing environmental factors:
- Place a lamp 20cm from the light and measure the light intensity (lux)
- Repeat the bubble experiment
- Now calculate the rate of transpiration using:

$$\frac{\text{Distance (moved by air bubble in cm)}}{\text{Time taken (s)}}$$

- Remove the lamp and leave for five minutes for the plant to re-establish normal conditions
- Place a fan 50cm form the plant and measure air movement using the anemometer
- Repeat the bubble experiment
- Now calculate the rate of transpiration using:

$$\frac{\text{Distance (moved by air bubble in cm)}}{\text{Time taken (s)}}$$

Transport in Plants

- Remove the fan and leave for five minutes
- Place a radiant heater near and measure the temperature
- Now calculate the rate of transpiration using:

$$\frac{\text{Distance (moved by air bubble in cm)}}{\text{Time taken (s)}}$$

Results:

- The rate of transpiration increases with increased air movement
- The rate of transpiration increases with increased temperature
- The rate of transpiration increases with brighter light

Risk assessment:

Plant sap can be irritant!!

Glass and associated equipment can break if not handled correctly.

Photosynthesis

RECAP: Photosynthesis is the chemical change which happens in the leaves of green plants in the presence of light (oxygen and water).

Method:

- Cut 5cm pond weed plant (Elodea preferred)
- Place it in a test tube filling the tube with sodium hydrogencarbonate solution
- Place the tube in a beaker of water (measure and record temperature) – ensure it remains at room temperature throughout the experiment (topping up with cooler water when it gets too warm from heat generated)
- Secure gas collecting syringe

to clamp (apparatus set up as diagram)

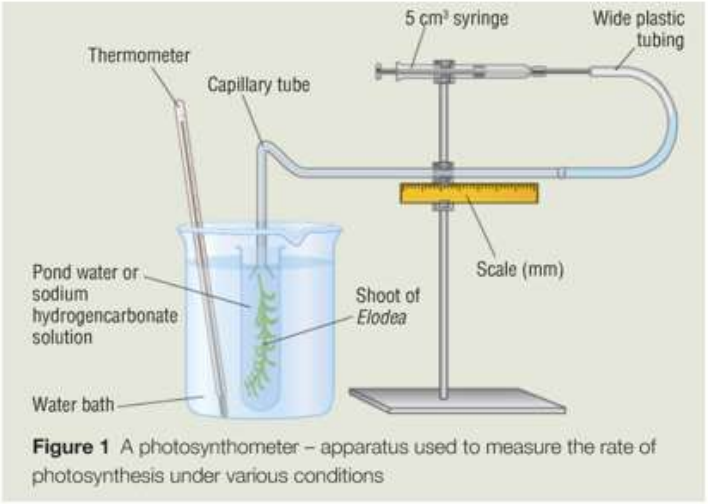


Figure 1 A photosynthometer – apparatus used to measure the rate of photosynthesis under various conditions

http://12knights.pbworks.com/f/1300188422/photsynthometer.png

(accessed 5th July 2017)

Photosynthesis

- Darken the surroundings, placing the lamp 10cm from the beaker and allow the plant to adjust
- Use the light meter (lux) to record light intensity falling towards the plant
- As the air bubbles increase, place the flared end of the tubing over the plant and measure the volume over five minutes (repeat twice and calculate the average of the results)
- At 20cm, 30cm, 40cm and then 50cm repeat and record the light intensity falling towards the plant
- As the air bubbles increase, place the flared end of the tubing over the plant and measure the volume over five minutes (repeat twice and calculate the average of the results)

Photosynthesis

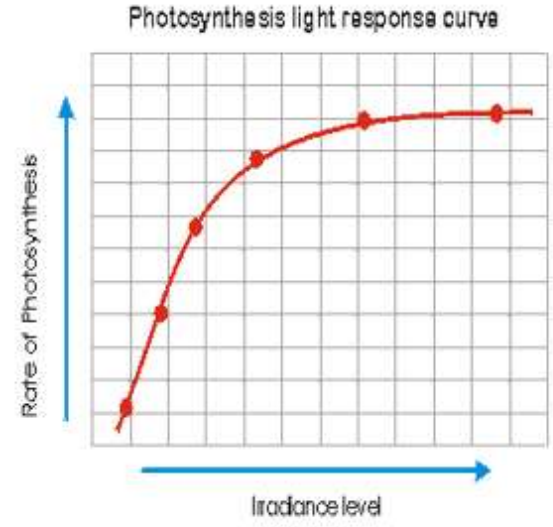
Results:

Oxygen is given off by plants during photosynthesis and is visible as bubbles in the experiment

The increasing light intensity increases the rate of photosynthesis, but not at very high intensities

As the distance from the plant increases, the light intensity directed onto the plant reduces.

Typical results graph generated:



https://13jhavra.files.wordpress.com/2012/01/rspcrve.gif

(accessed 5th July 2017)

Risk assessment

- Be careful when handling glassware
- Risk of lamps getting hot during use
- Care should be taken when using mercury-containing light bulbs (e.g. fluorescent tubes).
- Use light sources that absorb any UV light given off by the bulb/tube.
- Care should be taken with the presence of water and the electrical apparatus
- Sodium hydrogencarbonate solution (CLEAPSS sheet 33)

Ecology

What is Biodiversity? Its the variety of plant life (and animal life) in a habitat. A high level of diversity is ecologically very important e.f. to support food chains (we've all heard about the pyramid of biomass?)

How can this be measured? We can Identify and measure the Frequency, Distribution and Abundance: How much of something (i.e. plant life or animal life) is in that area (Habitat).

One Method is to use a Quadrat. The size of the quadrat is usually related to the size of the plants being studied.

- 10cm x 10cm quadrats - for very small plants, such as algae or bryophytes on tree trunks or walls
- 25cm x 25cm quadrats - for short grassland and other low-growing vegetation
- 50cm x 50cm quadrats / 1m x 1m for larger areas of long grass or heathland
- Larger areas - Tree and shrub species percentage cover recorded using a 50m^2 quadrat . . . And so on . . .

Ecology - Sampling

Flowering species that may be present – plantain, dandelions, white and red clover, speedwell, chickweed . . .

Q - What other type of grassland flowers could we look at?

Placing a good sample size of 20 quadrats and doing it randomly should eliminate sampling bias.

- Divide the area into a grid
- Choose the quadrat samples at random (numbers picked from bags)
- Place the quadrat where the coordinates meet

It obtains a good representation of the entire area – sparse or dense areas may be common (i.e. none in some quadrats, in others there may be 1, 2 or 3 flowers counted) depending on light and shade, wet or dry conditions . . .

Ecology - Sampling

Measure the **density** (the number of individual plants per quadrat i.e. Daisies). From this, it is possible to calculate how many individual species there are in the entire area.

Results:

We will now predict the number of daisies in the area!

Written Example below:

- The chosen study area measured 10m x 10m ($100m^2$).
  20 quadrats are placed, each 0.5cm x 0.5cm (a total of $5m^2$).
  A total of 12 daisy plants were found in the quadrats.
  So there were 2.4 daisies per m^2 and so there are 240 in the entire area (predicted).

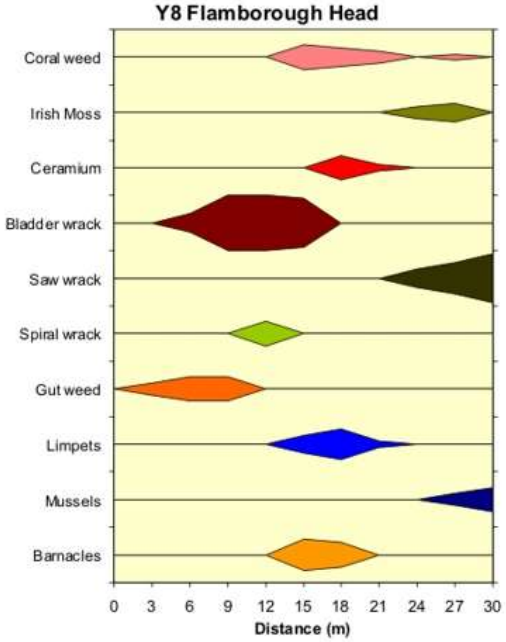
Ecology – Belt Transects

A method of determining Abundance is by using Belt transects:

- Run a string along the area and secure with two stakes
- Lay a parallel line 0.5m away to make a run way
- At every 0.5m along the lines, lay a quadrat (100 squares, each square representing a percentage)
- Record the cover (%) in each square, accurate naming all the plants within the quadrat and bare areas
- Record the height of the taller plants
- Repeat this until the transect line length ends (20 quadrats are ideal)
- Record the orientation of transect using a compass

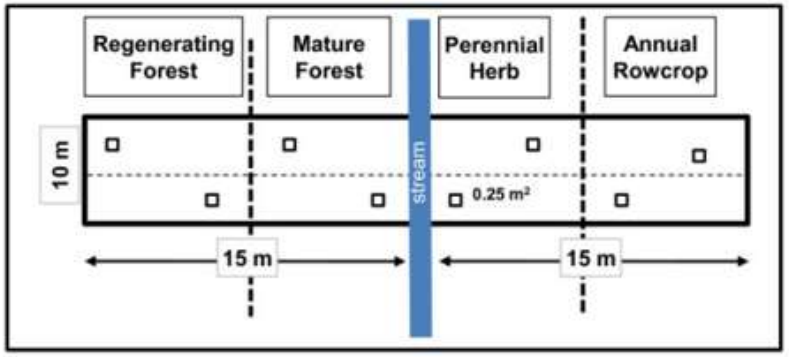
Ecology – Belt Transects

Results can be represented in a variety of ways;
Tabulated, pie chart, bar charts, kite diagrams etc.



http://www.heckgrammar.co.uk/web_files/departments/biology/Image/KS3/Y8_kite.jpg

(accessed 5th July 2017)



https://openi.nlm.nih.gov/imgs/512/157/3298783/PMC3298783_1750-0680-7-4-1.png

(accessed 5th July 2017)

Risk Assessment

When outside, look for hazards:

- Sharp objects (i.e. broken glass)
- Biological hazards (animal poo, plant sap, insects (stings and bites))
- Slip / trip / fall hazards
- Environmental conditions (weather).

Conclusion and Summary

In this presentation, it was important to explore the How and Why in biology, to hypothesize and investigate at first hand the simple questions asked when researching the subject.

Of course, there are more experiments that can be considered to enhance our knowledge.

There is also scope to review and alter the practical experiments discussed in this presentation to suit the application, equipment, environment or resources at hand.

Thank you very much for taking part!