Introduction

Biology - Paper 5 notes

Planning

Independent variable	- List min 5 different values of the independent variable: E.g. 5 different concentrations with regular intervals apart/ 5 different temperatures. - State how you are going to set up these independent variables. - Remember to include the apparatus used: E.g. simple or serial dilution / use a thermostatically-controlled water bath. - State how you make sure the experiment is well-controlled / less error-prone: E.g. randomise sampling to avoid bias / make sure participants do not know which drug they are getting as results can be affected by subject expectation.
Dependent variable	- This must be something that's measurable. E.g. rate is not measurable, but time is measurable and used to calculate rate. - State how you are going to measure the dependent variables: E.g. take a reading every 10 minutes. - Remember to include the apparatus used: E.g. length is measured using a ruler/ dry the plant in an oven until the mass becomes constant. Measure dry mass using a weighing scale. - State how you make sure the experiment is well-controlled / less error-prone: E.g. incubate enzyme and substrate separately, then start timer immediately after enzyme is added to substrate / use yeast without methylene blue as a colour standard. - State how you are going to analyse the results: E.g. compare between group A and B / plot a graph / calculate using this formula.
Controlled variables	- Min 3 constant variables: E.g. Temperature / pH / volume of solution. - State how you are going to keep these variables constant. - Remember to include the apparatus used: E.g. use pH buffer/ use a thermostatically-controlled water bath.

Control	- Control experiment ≠ controlled variable.
	- Control experiment is important to ensure validity of the experiment. To make sure that the independent variables are the ones causing the change in the dependent variable.
	- Two types:
	    - Negative control = results should be always negative: E.g. use boiled enzyme, so no rate of reaction.
	    - Positive control = results should be always positive: E.g. have petri dish without antibiotics for bacteria, bacteria will definitely grow.

Safety	- State the hazard + why it is dangerous + precaution:
	  - Strong acid – corrosive – wear gloves and goggles.
	  - Enzymes / reagents – harmful– wear gloves and goggles.
	  - UV light – mutagen – wear goggles.
	  - Electrical appliances – danger of electrocution – wear rubber gloves / do not touch with wet hands.
	  - (In field experiments) Pollen / insect bites – allergies – wear mask / protective clothing
	  - For human experiments, make sure they are aware of health risk, get consent before testing and allow test subjects to stop if they feel unwell.

Repeat	- Repeat experiment at least 3 times and obtain a mean to remove anomalies [R average]

Dealing with data

Math Skills Required

- Mean, $\overline{x}$
- Median
- Mode
- Range
- Interquartile range
- Ratio
- Percentage change = $\frac{\text{new} - \text{old}}{\text{old}} \times 100$
- Standard deviation, s = $\sqrt{\frac{\sum(x-\bar{x})^2}{(n-1)}}$
- Standard error, $S_M = \frac{s}{\sqrt{n}}$
- 95% confidence interval / error bars = mean ± 2 S_M
- Respiratory Quotient (RQ) = $\frac{[CO_2]}{[O_2]}$
- Rf value for chromatography = $\frac{\text{distance travelled with pigment}}{\text{distance travelled by solvent}}$
- Mark release-recapture method
  Estimated population size = $\frac{\text{no. of individuals in first sample x no. of individuals in second sample}}{\text{no. of individuals marked in second sample}}$
- Simpson's Index of Diversity
  $D = 1 - \left(\Sigma\left(\frac{n}{N}\right)^2\right)$
  where n = number of individuals of each species present in the sample
  N = the total number of all individuals of all species
- The Hardy-Weinberg equations:
  $p^2 + 2pq + q^2 = 1$
  $p + q = 1$

Statistical Tests

1. Chi-squared test
2. t-test

} To test for significance of difference between 2 data sets

3. Pearson's linear correlation
4. Spearman's rank correlation

} To test for correlation

Null Hypothesis

There is no significant difference / relationship between ____________ and ____________.

Test	Chi-squared test, χ^2	t-test	Pearson's linear correlation	Spearman's rank correlation
Purpose	To show if the observed results are significantly different from the expected results	To test whether data from 2 samples are significantly different	To test for correlation between 2 paired sets of data	To test for correlation between 2 paired sets of data
Requirements	• **Discrete** / nominal data • Discontinuous distribution • Usually to test the results of: - Breeding experiments - Ecological sampling	• **Continuous** / interval data • Data is normally distributed • Standard deviations are approx. the same • Two samples have <30 values each	• **Continuous** / interval data • Both sets of data are normally distributed • Scatter graph indicates a linear / skewed relationship with no obvious outliers • There are 5 or more pairs of data	• Data is **discrete** / nominal • Discontinuous distribution • Scatter graph shows that there is a relationship (not necessarily linear / skewed) • There are 5 or more pairs of data • Data points within samples are independent • All individuals must be selected at random from a population
Formula	$\chi^2 = \sum \frac{(O-E)^2}{E}$	$t = \frac{\lvert \bar{x}_1 - \bar{x}_2 \rvert}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$	$r = \frac{\sum xy - n\bar{x}\bar{y}}{(n-1)s_x s_y}$	$r_s = 1 - \left(\frac{6 \times \sum D^2}{n^3 - n}\right)$ (D = difference in rank)
Degree of freedom	$v = c - 1$ (c = number of classes)	$v = (n_1 - 1) + (n_2 - 1)$	$v = n - 2$ n is often shown in table directly, so you may not need to calculate v at all!	$v = n - 2$ n is often shown in table directly, so you may not need to calculate v at all!
Analysis	If χ^2 / t value is **more than** critical value at p = 0.05 • The two data sets are **significantly** **<u>different</u>** • Probability of the **difference** occurring by chance is less than 5% • The null hypothesis is rejected • The differences are not due to random error / chance		If r / r_s value is **more than** critical value at p = 0.05 • The two data sets are **significantly** **<u>correlated</u>** • Probability of the **relationship** occurring by chance is less than 5% • The null hypothesis is rejected • The relationship are not due to random error / chance	

Test	Results	Information
t-test	(Use a t-test table to look up your value of t) Obtained value > t value for a probability of 0.05 (the critical value), there is significant difference between A and B	Whether two sets of continuous data are significantly different from one another
Chi-squared (x2) test	(Use a χ 2 table to look up value of χ^2) Obtained value > χ^2 value for a probability of 0.05, there is significant difference between observed results and expected results	Whether observed results differ significantly from your expected result
Pearson's linear correlation	Value close to +1 indicates a positive linear correlation Value close to −1 indicates a negative linear correlation Value close to 0 indicates no correlation	Whether there is a linear correlation between two paired sets of data
Spearman's rank correlation	(Use correlation coefficient table to look up value of r_s) Obtained value of $r_s > r_s$ value for a probability of 0.05, there is a significant correlation between your two values	Whether there is a correlation between two random paired sets of data
Simpson's index of diversity, *D*	0 (lowest species diversity) – 1 (highest species diversity)	To find species diversity after collecting data on species abundance

Standard deviation

$$s = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$$

Standard error (S_M)

$$S_M = \frac{s}{\sqrt{n}}$$

t-test

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

Chi-squared test

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

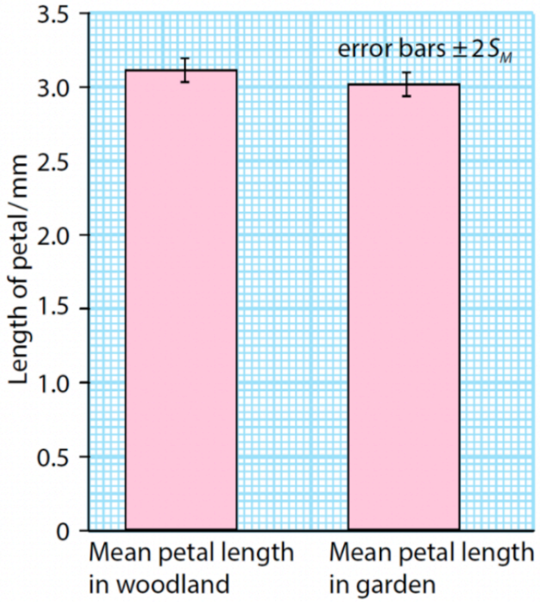
where: Σ = sum of
O = observed value
E = expected value

Pearson's linear correlation

$$r = \frac{\sum xy - n\bar{x}\bar{y}}{ns_x s_y}$$

Spearman's rank correlation

$$r_s = 1 - \left(\frac{6 \times \sum D^2}{n^3 - n}\right)$$



Evaluation

- Describe = state your observations
- Explain = explain why the observations are as such
- Compare / contrast = state similarities or differences between 2 sets of data
- Always read the x and y axis first!
    - x-axis = independent variabl
    - y-axis = dependent variable

Describing graphs

1. General trend
    - First, describe how the independent variable and dependent variable changes overall: E.g. when x increases, y increases, but tails off when x increases above 10.
    - Then, if needed, split the graph into several parts to describe and explain separately.

2. Comparative data quote
    - Compare 2 points to support your statement
    - Provide the x and y coordinates with the correct units
    - You can also quote the maximum and minimum values where appropriate
    - You can include some manipulative figures: E.g. the number of cases reduced by half in 2010 compared to 2008

Describing the graph / table when there are multiple groups present

1. General trend
    - Look for similarities and make general statements overall: E.g. Both increase as x increases.

2. Comparative data quote

3. Compare within the group
    - Does it increase / decrease as independent variable increases?

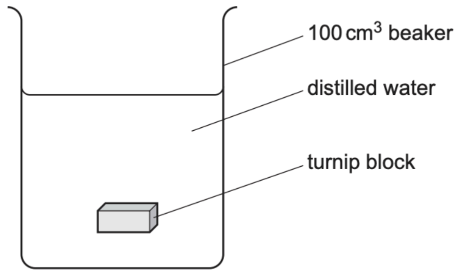
4. Compare between groups
    - Compare the end points
    - Compare the max / min points
    - Compare the range
    - Compare steepness of the graphs

When standard error bars given

- If they don't overlap, significant difference.
- If they overlap, no significant difference

Planning Experiments

Osmosis

Method to investigate the effect of temperature solution on rate of osmosis.  *Do not include the risk assessment or how to calculate the rate of osmosis from the results.*
1. Control variables - same / stated variety / age of turnips. - same / stated dimensions of turnip block.
2. Method - Use a minimum of five stated temperatures: 10,20,30,40,50. - Method to maintain constant temperature for each replicate / experiment: thermostatically controlled water bath. - Apparatus for obtaining same size of turnip blocks: ruler. - For each temperature, place turnip blocks in a beaker of (fresh / new) distilled water. - At each temperature, measure the initial mass and the final mass after a set / stated time. - For each temperature, use at least three different blocks and calculate a mean. - Method to remove excess water from turnip block (before measuring final mass: soak up excess water using tissue paper.

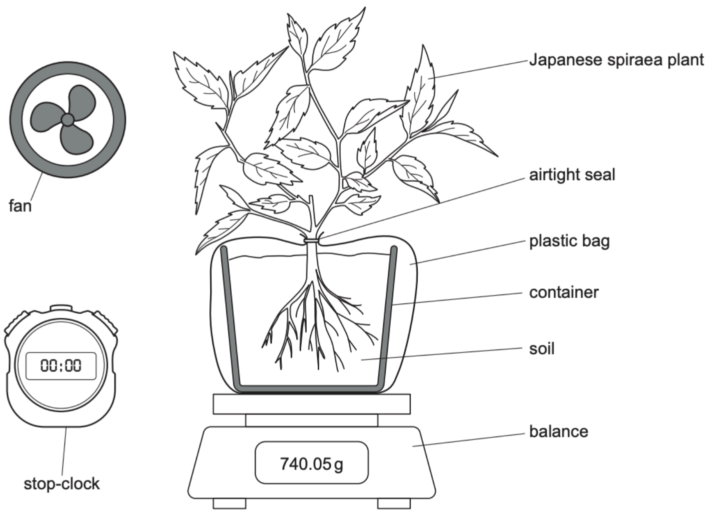
Germination and growth of seeds

Method to investigate the effect of a solutiom solution on: - *percentage of seeds that germinate* - *growth of each seedling 10 days after the appearance of the seedling above the soil.*
3. Control variables - Standardise the exposure of seeds to solution: place the seeds on identical filter paper or cotton wool soaked with solution, making sure each seed is equally wetted. Spread seeds out so they are not overlapping. - Use the same seeds- same type, age, variety. - Maintain constant/same temperature by placing setup in a

thermostatically controlled water bath/ temperature-controlled room. - Maintain constant/same light intensity by placing the setup a fixed distance from a light source/ lamp. - Maintain constant pH using a buffer solution.
4. Method - Count/note/record the number of seeds planted, and count the number of seeds that germinate after a set time (10 days). - Measure the length of the seedling above the soil after 10 days using a ruler/ vernier calipers. - Use a large number of seeds and calculate the mean growth for each group (with solution and without/ control). - Reapply solution (to seedlings) after germination.
5. Safety hazard + risk + precaution - Solution | irritant/toxin/allergy | gloves/mask/PPE - Seeds/plants | irritant/allergy | gloves/mask/PPE - Soil | biohazard/pathogens/allergy/irritant | gloves/mask/PPE

Transpiration

Method to investigate the effect of wind speed on rate of transpiration



- *Japanese spiraea plant growing in a container of soil*
- *added 200cm3 of water to the soil*
- *plastic bag around the container of soil to prevent water loss from soil*
- *placed the plant and container on the balance*
- *switched on the fan to a low setting.*

1. Control variables:
    - Standardised variable for using the fan: Place the fan the same fixed distance away from plant.
    - Maintain constant/same temperature using temperature controlled

room. - Maintain constant/same light intensity by placing setup fixed distance from light source/ lamp.
2. Method - Allow equilibration / acclimatisation of plant / apparatus. - Method to use fan(s) to obtain a minimum of five different wind speeds: change the speed setting on fan. - Method for measuring wind speed: use anemometer. - At each wind speed, measure / note / record the initial mass and final mass, in a fixed time. - Use at least three measurements for each wind speed <u>and</u> calculate a mean. - Replace water lost from plant before each new measurement.
3. Safety hazard + risk + precaution - Plant | irritant/allergy | gloves/mask/PPE - Soil | biohazard/pathogens/allergy | gloves/mask/PPE - Fan | hair/clothing trapped in fan | method to control loose hair/clothing

<u>Biodiversity</u>

Using belt transects to collect the data needed to determine Simpson's index of diversity (D) of the undergrowth in plots of plantations.
1. Control variables - Selecting a start point for the transect / placement of tape / line / string OR all transects same lengt in plots / plantations / repeats. - Use quadrats of the same size. - Sample at / use quadrat at regular intervals / stated distances along the tape / line / string / transect OR continuous belt transect
2. Method - Use of tape / line / string and quadrat to create belt transect. - Method to identify each of the plant species in the quadrat: use key/ diagrams. - Count / record / note the number of individuals of each plant species (n) in each quadrat / at each sampling point OR estimate percentage (%) cover of each plant species in each quadrat. - Use at least 3 different transects in each plot.
3. Safety hazard + risk + precaution - Plants/fungi | falling trees/branches | hard hat Plants/fungi | thorns | gloves/goggles/mask/long trousers/PPE Plants/fungi | irritant/allergy/infection (spores) | antihistamines/cover skin/first aid equipment

- Named animal(s)/parasite | bites/infection/attack/stings/allergy | antivenom/antihistamine/trained professionals/medics available/insect repellent/gloves/goggles/mask/PPE/cover skin - Plantation/terrain | trip hazard | correct footwear/watch where you are walking/use walking sticks - Plantation/terrain | getting lost | work in group/maps/use GPS/guide - Poachers | being attacked/shot | trained professionals/medics available

Investigate changes in the species diversity of plants in a grey dune ecosystem during ten-year period after conservation practices were introduced.
1. Control variables - use same / stated size (frame / point) quadrat.
2. Method - use quadrats. - method of random / systematic placement of quadrats: on transect line (systematic) OR generating random coordinates (random). - method of identifying (plant) species: use key/diagrams. - count the number of individuals of each (plant) species. - take care to note low growing / small / easy to miss species. - sampling 10 or more separate points / quadrats in the grey dune habitat. - measure biodiversity of plant species at regular intervals over the 10-year period and at the same time of the year. - repeat whole investigation over 10 years at different times of year / seasons.
3. Safety hazard + risk + precaution - plants / pollen | allergy / irritation / injury | gloves / eye protection / mask / PPE / protective clothing - animals | allergy / irritation / injury | gloves / eye protection / mask / PPE / protective clothing - grey dunes / ecosystem | getting lost / trip hazard / injury | wear suitable shoes / clothes / gloves / PPE; stay in a group / with expert / ranger

Strength of blood vessels

Blood vessels must be able to withstand and maintain varying blood pressures.

There are several methods that could be used to determine the strength of a blood vessel. One method is called the circumferential tensile strength (CTS) test, as shown in Fig. 1.1.

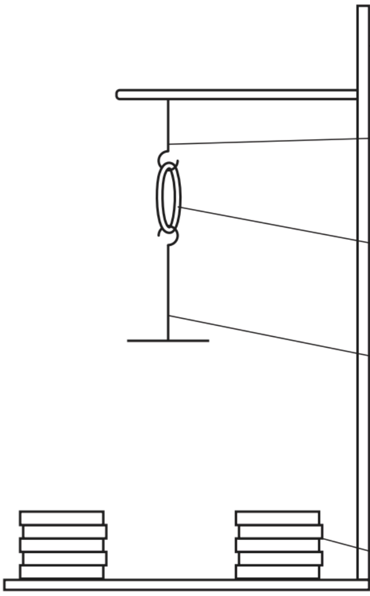


Fig. 1.1

The ring of blood vessel, shown in Fig. 1.1, was cut from a length of artery or vein that was prepared by removing surrounding tissues.

A student wanted to use the apparatus in Fig. 1.1 to determine the mass needed to break an artery and the mass needed to break a vein.

Describe how to use the CTS test to determine the mass needed to break an artery and the mass needed to break a vein.

1. Control variables
    - Use artery and vein from the same species/ source.

2. Method
    - Method to cut blood vessel: use a scalpel/knife.
    - Method to measure blood vessel of same length: use vernier calipers.
    - Discard samples that are damaged.
    - Wait to see if the blood vessel breaks before adding the next 10g mass.
    - Add 10g masses to the mass hanger until the blood vessel breaks.
    - Measure mass of hanger/ include the mass of hanger.
    - For each type of blood vessel, measure/note/record mass when it broke.
    - Use at least three measurements for each type of blood vessel and calculate a mean.
    - Repeat procedure with smaller mass intervals near to breaking mass.

3. Safety hazard + risk + precaution
    - Knife/scalpel | injury | cut away from hand
    - Blood vessels | biohazard/pathogens/allergy | gloves/mask/PPE/use disinfectant/wash hands
    - Mass hanger falling | injury | suitable precaution to prevent injury

Genetic crosses

Describe a method the student could use to carry out:
- *the first cross using fruit flies from the initial two populations to produce double heterozygotes (genotype AaDd).*
- *the second cross using the double heterozygotes produced from the first cross.*
- *an analysis of the offspring phenotype ratio from the second cross.*

The method should include a description of how offspring phenotypes would be identified.

1. Control variables
    - Maintain constant temperature using incubator.
    - Use stated / same / recommended / correct dose / volume / concentration of chemical / anaesthetic.

2. Method
    - To produce heterozygotes: breed) brown-eyed /AAdd females with scarlet-eyed / aaDD males OR breed scarlet-eyed / aaDD females with brown-eyed / AAdd males.
    - Remove parent (fruit flies) from their offspring, before they are adults / before day 10 / before eggs hatch.
    - Put (anaesthetised) double heterozygotes / offspring from first cross into (new specimen) tube.
    - Cross double heterozygotes to give a large number of offspring.
    - Identify phenotype / eye colour using microscope / magnifying glass / hand lens.
    - Count / record the number of flies of each phenotype / eye colour.
    - Method to prevent double counting of flies: move already counted flies to a separate specimen tube.

3. Safety hazard + risk + precaution

hazard	risk	precaution
chemical / anaesthetic	toxic / allergy / irritant	use, gloves / goggles / mask / PPE / use fume cupboard
Drosophila / fruit flies	allergy / (spread of) pathogens / biohazard	use gloves / goggles / mask / PPE
agar jelly / specimen tube, containing food	allergy / biohazard / pathogens	use gloves / goggles / mask / PPE / aseptic technique

NOTE: Suggest a reason why the biologist removed the parent fruit flies from the specimen tubes before eggs hatching

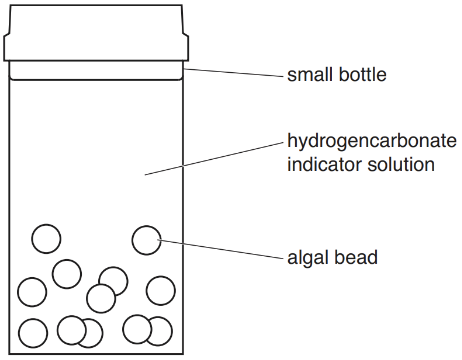
- To prevent the parent (fruit flies) reproducing / mating with the first generation / offspring

Suggest a method that the biologist could use to separate anaesthetised adult female fruit flies and anaesthetised adult male fruit flies and place four of each into a fresh specimen tube.

- use hand lens / (binocular) microscope to distinguish between male and female (fruit flies) AND
- use a paint brush / pooter / forceps to place fruit flies.

<u>Rate of photosynthesis</u>

- *Three different filters, blue, green and red, to expose two different species of algae to three different colours of light.*
- *Algae was immobilised in sodium alginate to form two sets of algal beads of the same size, one set for each species.*



- *Hydrogencarbonate indicator solution was used to estimate the rate of photosynthesis when the algal beads were exposed to each colour of light. The indicator solution changes colour with pH:*

colour of indicator solution	pH	CO_2 concentration	rate of photosynthesis
yellow	7.6	↑ increasing concentration	↓ increasing rate
yellow-orange	7.8		
orange	8.0		
orange-red	8.2		
red	8.4	atmospheric concentration (0.04%)	
red-magenta	8.6	↓ decreasing concentration	
magenta	8.8		
magenta-purple	9.0		
purple	9.2		

- *Algal beads were added to a small bottle containing indicator solution.*

- *Removed a sample of the indicator solution from the small bottle after some time.*
- *Used a colorimeter to measure the absorbance of the sample of the indicator solution.*
- *Used a calibration curve to estimate the pH of the samples of the indicator solution.*

Describe a method the student could use to collect sufficient data so that the rates of photosynthesis of both species of alga in the three colours of light can be compared. Don't include details of how to prepare the algal beads.

1. Control variables
    - Use same / stated number / mass / volume of algal beads in each bottle.
    - Place light source the same / stated distance from algal beads.
    - Use same / stated volume / concentration of indicator in each bottle.
    - Use a same / stated coloured filter / light in the colorimeter.

2. Method
    - Carry out experiment in a darkened room.
    - Method for use of filters to give blue, green, red coloured light.
    - Use a control bottle: replace the algal beads with glass / plastic beads.
    - Use red / pH 8.4 hydrogencarbonate indicator solution at start.
    - Method to take sample of indicator solution from bottle: use pipette.
    - Method to calibrate colorimeter: use a blank to set colorimeter to zero.
    - Suitable time to leave algal beads and indicator solution before measuring absorbance.
    - For each colour filter and species measure / record absorbance at same / stated time.
    - Use at least three measurements for each colour filter and species, and calculate a mean.

3. Safety hazard + risk + precaution

hazard	risk	precaution
algae / beads	irritant / allergy	gloves / mask / goggles / PPE
hydrogencarbonate indicator (solution)	irritant / allergy	gloves / mask / goggles / PPE
heat from light source	burns	do not touch bulb / turn off lamp before handling

Effect of temperature on the rate of the light-dependent stage of photosynthesis.
• Include details of how you would take accurate results.
• Do not include a risk assessment.

- *Put 10cm3 of stock chloroplast suspension into a flat-bottomed tube, add*

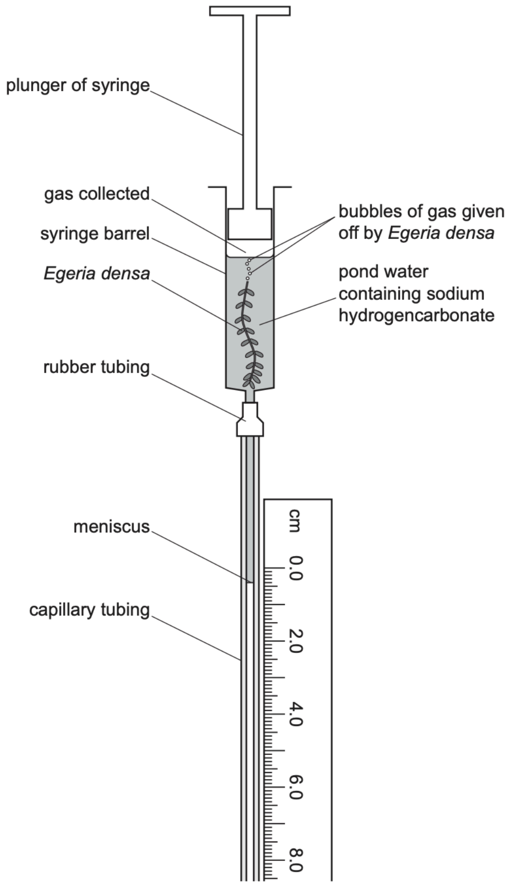
1cm3 of DCPIP solution to each tube. The chloroplast suspension is now blue-green in colour.

- *Start a timer. Remove the black plastic film from the tube. Record the time taken for the DCPIP to decolourise so that chloroplast suspension is green.*

1. Control variables
    - method of maintaining a constant temperature
    - method for ensuring same light intensity

2. Method
    - minimum of five stated temperatures within the range 10 to 60 °C.
    - allowing chloroplast suspensions to reach set temperatures (before exposing to light)
    - swirl chloroplast suspension (before exposing to light)
    - carry out experiment in dark room
    - colour of sample to match chloroplast suspension (alone) / green colour standard
    - repeats and mean

Investigate the effect of light intensity on the rate of photosynthesis:



1. Control variables
    - use same / stated length of water plant / Egeria densa

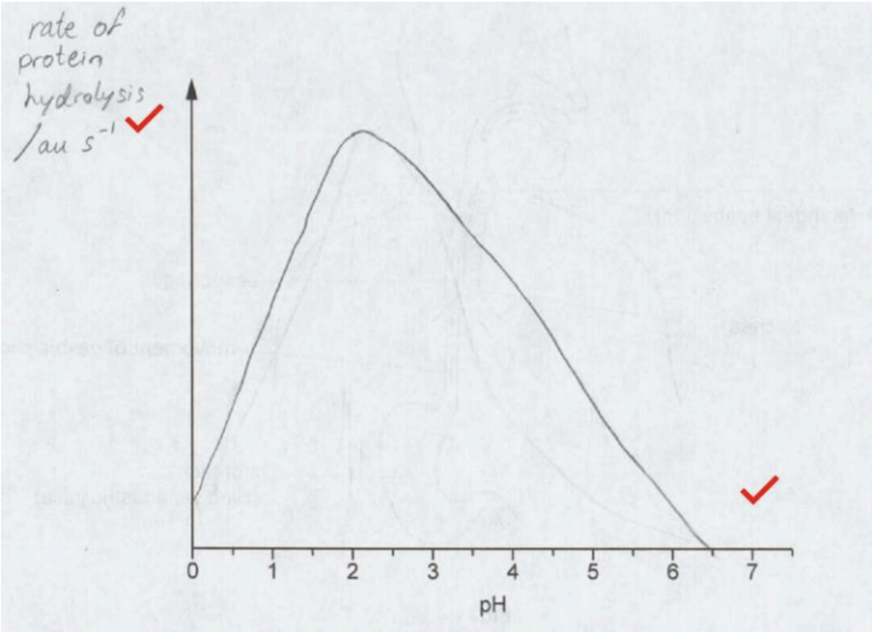
- use same / stated mass / concentration of sodium hydrogencarbonate
2. Method - use lamp at different distances from plant OR use lamp with different power ratings / wattages (at same distances). - use a minimum of five different / stated light intensities. - use a light meter / an app to measure light intensity. - carry out investigation in a darkened room / with no other light source - measure / note / record the distance the meniscus moves along the capillary tube / scale in a fixed time OR measure / note / record the time the meniscus takes to travel a fixed distance on the capillary tube / scale. - use a method to reduce heating effect of lamp. - equilibration / acclimatisation of water plant / Egeria densa / whole apparatus (to each light intensity). - use at least three measurements at each light intensity and calculate a mean.
3. Safety hazard + risk + precaution

Protein hydrolysis

Effect of pH on the rate of protein (albumen) hydrolysis by pepsin + determine optimum pH of pepsin - *Access to colorimeter and standard laboratory apparatus.* - *Pepsin is inactive at pH6.5 and above.* - *Details of how to prepare and use the colorimeter should not be included.*
1. Control variables - use the same stated volume and concentration of pepsin and albumen for each pH condition. - place pepsin, buffers, albumen in water bath at stated temperature (eg. 37˚C) to maintain constant temperature.
2. Method - prepare at least five buffer solutions with stated pH values between pH 0 and 6.5 and label them. - allow all pepsin, buffer, albumen solutions to equilibrate to the stated temperature for a fixed time, separately before mixing. - for each pH, mix pepsin with buffer solution first, then add albumen to start reaction, and place in colorimeter. - for each pH, measure/ note/ record absorbance at set/ stated time intervals (eg. every 30 seconds for 5 minutes). - use at least three replicates for each pH and calculate a mean rate of change (decrease) in absorbance / rate of protein hydrolysis. - plot mean rate of hydrolysis against pH.

- identify the pH at which rate is highest = optimum pH. - repeat experiment with smaller pH intervals around the optimum pH for improved accuracy.
3. Safaty hazard + risk + precaution

hazard	risk	precaution
albumen / eggs	allergy / irritant	gloves / goggles / mask / PPE
pH buffer	irritant / corrosive (at pH 0, 1, 2)	gloves / goggles / mask / PPE
pepsin	allergy / irritant	gloves / goggles / mask / PPE



Catalase activity

As the distance from the base of the shoot increases, the catalase activity of carrot root tissue increases.

- 1 method to cut, tissue from carrot (root) ;
- 2 minimum of 5 distances chosen (or stated distances) ;
- 3 same / stated, mass / volume, of cut carrot (used to make the extract) ;
- 4 mix / stir, liquid carrot extract (before dipping filter paper discs) ;
- 5 method of maintaining temperature (10°C – 40°C if stated) ;
- 6 same / stated, height / depth / (stated) volume, of hydrogen peroxide (solution) ;
- 7 measure / note / record, the time the filter paper disc takes to rise to the surface (of hydrogen peroxide solution) ;
- 8 use at least three measurements for each distance and calculate a mean ;
- 9 safety: hazard and risk and mitigation ;
- 10 carrying out a control with disc, dipped / AW, in water / buffer (to show rise of the discs is due to the carrot extract)

Biostatistics

Conditions for using a t-test

- comparing means from two groups
- standard deviations are approximately the same between the two groups
- data is continuous
- data is from populations that are normally distributed
- fewer than 30 values

To state the conclusion from a test:

1. State the the critical value at the degree of freedom and p = 0.05.
2. Calculated value is less than OR greater than critical value.
3. Null hypothesis is accepted OR rejected.
4. There is no significant difference at p = no 0.05 OR
   there is significant difference at p = 0.05.

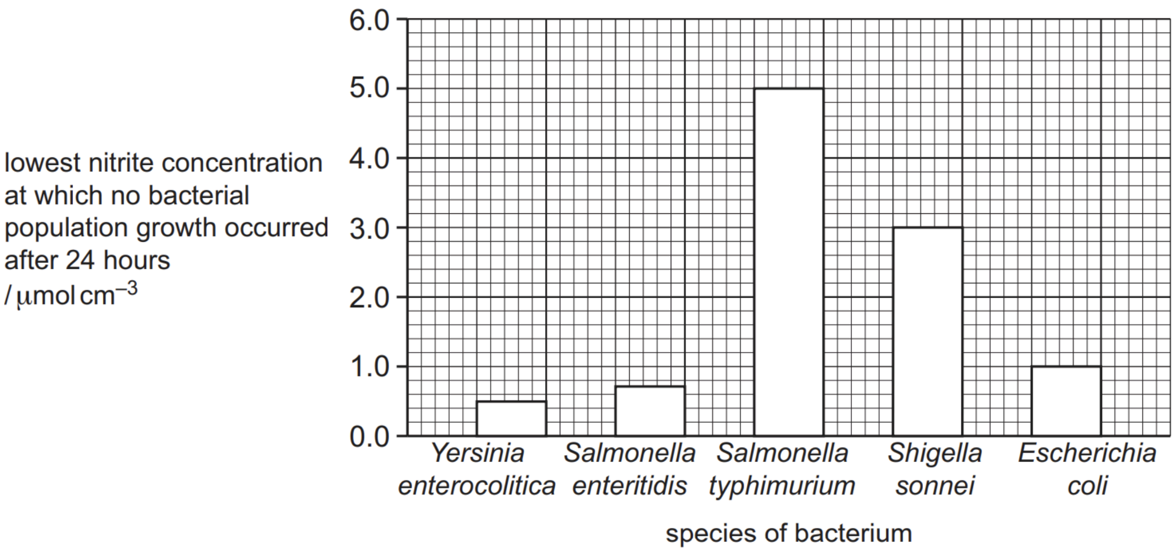
Types of data

1. Nominal (different categories with no numerical value or order; names/labels)
2. Discrete (different categories but numerical; cannot take any value in range)
3. Continuous (can take any value within a range)

Example:

Variable	**Type of Data**
Type of solution used	Nominal
Number of seeds germinated	Discrete (separate category but numerical)
Length of root growth	Continuous

(b) The results of the investigation are shown in Fig. 2.1.



Calculate the percentage difference between the results shown for the 2 species of Salmonella

- ((5 – 0.7) / 5) x 100 = 86% OR
  ((5 – 0.7) / 0.7) x 100 = 614%

NOTE: when calculating percentage change, put the – or + sign to show if the value is increasing or decreasing.

The t-value the scientists calculated had a probability (p) value greater than 0.25 (p > 0.25). State the conclusion that can be made.

- Accept null hypothesis OR there is no significant difference

NOTE:
If standard error or confidence intervals overlap = no significant difference.

20 coffee farms in an area to the south of Nagarahole National Park were chosen at random.

In March 2008, the scientists measured four variables at each coffee farm, as shown in Table 2.1.

Table 2.1

variable	how the variable was determined for each coffee farm
water availability	counting the number of ponds, lakes and water holes visible in satellite images
density of December trees	counting the number of December trees in ten circular areas, each with a radius of 10 m
grass biomass	drying and measuring the dry mass of all the grass leaves collected in 50 randomly selected areas of 0.25 m^2
distance from Nagarahole National Park	using GPS to measure the distance between the boundary of Nagarahole National Park and the boundary of the farm

Farmers from each coffee farm completed a questionnaire to record when elephants entered the farm.

The scientists decided to use Spearman's rank correlation to investigate the relationship between each of the four variables in Table 2.1 and the number of months that elephants entered each coffee farm per year.

State conditions that allow Spearman's rank correlation to be used in an investigation

- Data are paired / linked.
- Data are ordinal / can be ranked.
- There are more than 5 paired observations (there are 20).
- Data points within samples are independent of each other.
- Sampling points were selected at random.

<u>Explanation for 20 paired observations:</u>

The question states that 20 coffee farms were chosen at random.

For Spearman's rank correlation, a paired observation means:

- One value for variable 1
- One corresponding value for variable 2
  for the same subject/location (here, each coffee farm).
- In this investigation, for each farm they recorded:
  - four environmental variables
  - the number of months elephants entered the farm per year
- Each environmental variable was compared separately with number of months elephants entered the farm per year.

- Spearman’s rank correlation measures the relationship between two variables at a time.
- So the scientists would do four separate correlations:
    - Water availability vs elephant entry months
    - Tree density vs elephant entry months
    - Grass biomass vs elephant entry months
    - Distance vs elephant entry months
- For each correlation, there are 20 farms, and each farm provides: One value for the environmental variable + One value for elephant entry months
- So each correlation uses 20 paired observations.

<u>Explanation for data points within samples independent of each other:</u>
- The number of months elephants enter Farm A does not affect how often elephants enter Farm B.
- The water availability or tree density on one farm does not change the measurements taken on another farm.
- Because each farm is its own separate unit, the results from one farm do not depend on results from another.
- Each coffee farm was sampled separately and randomly, so measurements from one farm do not affect measurements from another, making the data independent.

Conditions for Pearson’s linear correlation
- Data are paired / linked.
- Data is continuous.
- There are more at least 5 paired observations.
- Scatter diagram suggests a linear relationship / correlation / association.

Table 2.2 shows the results of using Spearman's rank correlation (r_s) to investigate the relationship between each of the four variables in Table 2.1 **and** the number of months that elephants entered each coffee farm per year. The table is **not** complete.

Table 2.2

variable	r_s value	significance
water availability	0.63	
density of December trees	– 0.30	
grass biomass	0.02	
distance to Nagarahole National Park	0.38	

Table 2.3 shows some critical values for the Spearman's rank correlation at different probabilities. When comparing critical values in Table 2.3 with values of Spearman's rank correlation that are negative, the minus sign should be ignored.

Table 2.3

number of paired observations	probability			
	0.50	**0.10**	**0.05**	**0.01**
18	0.170	0.401	0.472	0.600
19	0.165	0.391	0.460	0.584
20	0.161	0.380	0.447	0.570
21	0.156	0.370	0.435	0.556

A coffee farmer living close to a different national park in India wanted to reduce the number of times that elephants entered the farm. The farmer analysed data from the investigation and concluded that reducing the number of ponds, lakes and water holes on the farm would reduce the number of times that elephants entered the farm.

Explain how the information provided and the data in Table 2.2 support and do not support this conclusion.

- Support
    - reducing water availability is significantly correlated with a reduced number of times that elephants enter the coffee farm.
    - water availability was the only variable found to be significant so reducing the number of water bodies might be the most effective action.
- Does not support

- correlation does not indicate causation (so reducing the availability of water may not affect the number of elephant visits).
- water bodies only counted once / in March / in 2008.
- investigation only in one geographical area (of India) / at Nagarahole National Park.
- size of ponds / lakes / water holes might also be important.
- questionnaire does not record actual number of times elephants entered the farms / number of elephants per month.

NOTE: Null hypothesis = no difference between x and y OR no correlation between x and y. DON'T SAY: no significant difference!!! Just no difference!

peat bog A		**peat bog B**	
species	number of individuals	species	number of individuals
L	34	L	30
M	10	M	15
N	11	N	18
O	15	O	24
P	8	P	16
Q	7	Q	8
R	4	R	20
S	1	S	4
T	3	T	0
U	3	U	0
V	2	V	0
W	1	W	0
Simpson's index (*D*) = **0.82**		Simpson's index (*D*) = **0.85**	
Shannon index = **2.03**		Shannon index = **1.96**	

The ecologists concluded that one index indicated peat bog **A** had more biodiversity, but the other index indicated peat bog **B** had more biodiversity.

Suggest how the differences in the data in the two samples, shown in Table 2.1, may have led to the different conclusions.

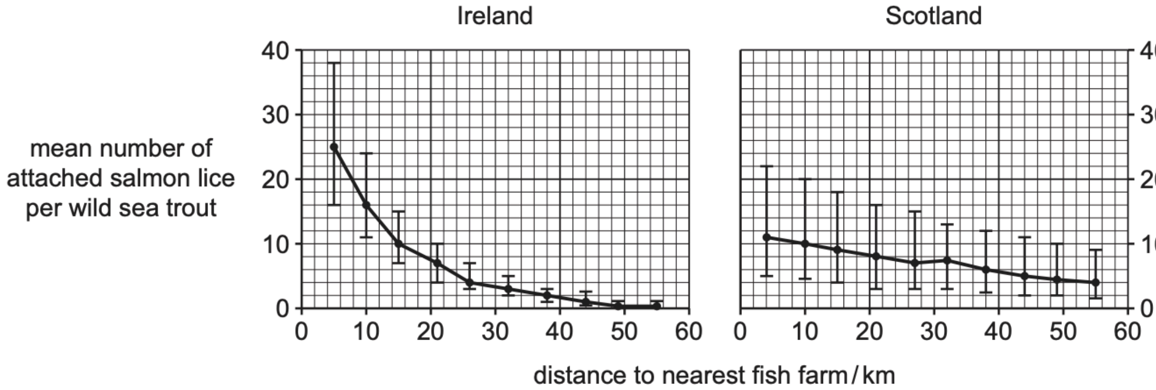
- peat bog A has a higher number of species (12 vs 8) and a higher Shannon index than peat bog B: number of species / species richness is more important for Shannon index.

- peat bog B has a smaller range of species abundance (and higher total number of individuals 135 vs 99) and a higher Simpson's index than peat bog A: range of species abundance / species evenness / total number of individuals is more important for Simpson's index

Explain what the standard error bars (SE) indicate about the data.

- How close the calculated / sample mean is (likely to be) to the true mean.
- There is overlap in the SE / error bars of the two means.
- This indicates that there is no statistically significant difference between the two means.

The error bars in Fig. 2.3 show 95% confidence intervals (95% CI).



With reference to Fig. 2.3, suggest why the scientists calculated the 95% confidence intervals for their data.

- 95% probability / confidence that / chance that the true / actual / population / parametric, mean lies within, this range / these limits OR
  To test whether the means (for number lice per trout at different distances from fish farms) are (significantly) different OR
  To see / test whether the error bars / 95% CI overlap. OR
  To get a measure of how close the calculated mean is likely to be to the true / mean OR Shorter error bars / 95% CI show true mean is likely to be closer to the calculated mean OR Longer error bars / 95% CI show true mean is likely to be further from the calculayted mean
- Exemplification: no / less overlap of (95% CI) error bars for Ireland so (difference) is / may be significant OR overlap of (95% CI) error bars for Scotland / Ireland so (difference) is not / may not be significant. OR
  Shorter / narrower error bars for Ireland the closer the calculated mean is (likely to be) to the true mean OR larger / longer error bars for Scotland, the further the calculated mean is (likely to be) from the true mean OR
  shorter / narrower, error bars / confidence limits, with increasing distance (to nearest fish farm) in Ireland / Scotland, shows the calculated mean is getting closer to the true mean.

Random Questions

NOTE: Dependent variable is always something that can be measured. For example, rate is not a dependent variable as it cannot be measured. Time is measurable so it is a dependent variable. Time is used to calculate the rate.

A student planned to investigate the effect of juglone on the cucumber plant. The student planned to compare the effect of exposing cucumber seeds to:
• juglone solution with a concentration of 1.0 × 10–3moldm–3
• a control treatment.
Identify the independent variable in this investigation.

- Presence AND absence of juglone solution
  OR presence of juglone solution AND control treatment

The student was provided with:
• a juglone solution with a concentration of 0.1moldm–3
• the mixture of solvents.
Using the solution provided, the student carried out 1 dilution to prepare a juglone solution with a volume of 500cm3 and a concentration of 1.0 × 10–3moldm–3.
State the volumes that the student used to carry out the dilution to produce 500cm3 of 1.0 × 10–3moldm–3 juglone solution.

- 5 cm3 of original / 0.1 mol dm-3 juglone solution
- 495 cm3 of the mixture of solvents

Outline improvements that could make to reduce the percentage error while carrying out dilutions.

- Use more accurate apparatus: burette/ syringe/ graduated pipette/ micropipette.
- Use volumetric flask
- Use larger volumes

Explain why a control is included in an investigation.

- to show that any effects observed are a result of the independent variable.

Compounds containing nitrite ions (NO_2^-) are present in many foods eaten by humans.

Scientists tested the effect of different concentrations of nitrite ions on the population growth of 5 species of bacterium. All 5 species are pathogenic and can infect the human digestive system.

For each species of bacterium, the scientists placed suspensions of the bacteria in a microwell plate. A microwell plate is a plastic plate containing 96 wells, which are similar to small test-tubes.

In each well, the scientists added:

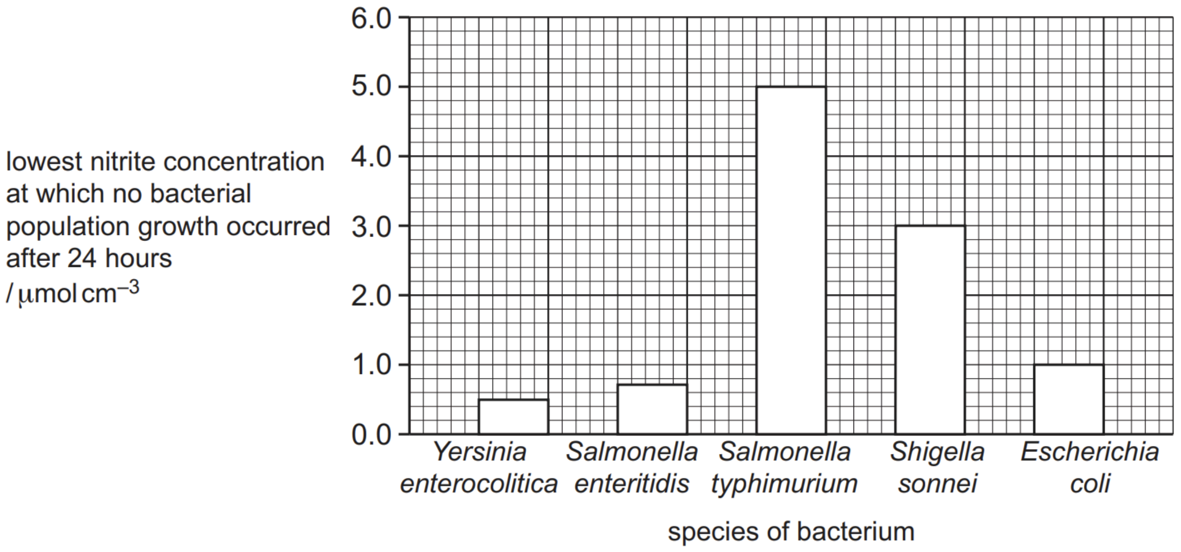
- a suspension of the bacterial species
- a solution containing nitrite ions
- nutrient broth.

The scientists incubated the microwell plate at 37 °C for 24 hours. After 24 hours, the scientists estimated the number of bacteria in each well by measuring optical density, using a microwell plate reader.

For each species of bacterium, the scientists repeated the experiment with several different concentrations of solution containing nitrite ions.

The scientists determined the lowest nitrite concentration at which no bacterial population growth had occurred after 24 hours.

(b) The results of the investigation are shown in Fig. 2.1.



A student looked at the results and stated: Consuming a lot of nitrites would improve the health of people because it would help to prevent diseases in the digestive system caused by pathogenic bacteria.

Suggest reasons why this conclusion might not be valid.

- Investigation not carried out in a person / carried out in a laboratory.
- Nitrites could cause side effects / be toxic to humans.
- pH in the digestive system / stomach / intestines is not 4.8 / is different.
- Experiment only used 5 species of pathogenic bacteria / did not use other bacteria.

- Nitrites may kill / reduce growth of beneficial bacteria in the digestive system.
- Investigation only carried out for 24 hours / no information about longer term effects.
- No evidence of control experiment.
- No statistical test / analysis.

ALWAYS state

- No statistical test
- Investigation not carried out on a person/ only using one volunteer

A student investigated the effect of wind speed on the rate of transpiration.

Identify the independent variable in this investigation.

- Wind speed

NOTE: the independent variable doesn't need to be something that is measured.

An eyepiece graticule was placed across the nucleus of one of the tumour cells.

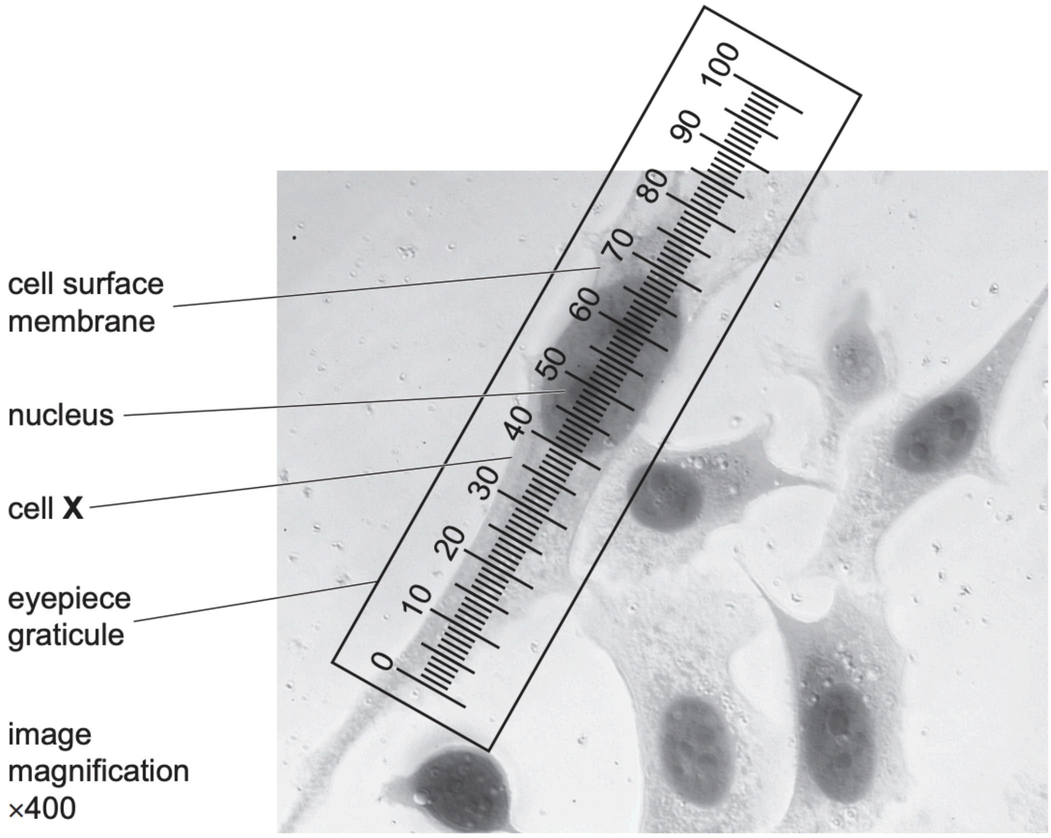


Fig. 2.1

The calibration of the eyepiece graticule scale is:

one eyepiece graticule division = 320 nm

Use the calibration of the eyepiece graticule scale to calculate the actual diameter of the nucleus of cell X, shown in Fig. 2.1. Show your working and state your answer in μm.

- Number of divisions = 28
- Diameter in nm = 28 x 320 = 8960nm
- Diameter in μm = 8960 / 1000 = 8.96μm

Suggest how the method of measuring the diameter of 100 nuclei can be standardised, so that valid comparisons can be made between benign and malignant tumour cells.

- measure the widest / narrowest / longest part of the each nucleus

Suggest and explain two disadvantages of this procedure as a diagnostic test.

- Invasive: causes pain/infection/bleeding.

- Labour intensive & time consuming: not feasible on a large scale, delayed treatment.
- Human error due to small values/ very close values: may cause misdiagnosis.

(b) The student used a different method to measure the rate of transpiration of Japanese spiraea.

Fig. 1.2 shows the apparatus used. The leaf remained attached to the plant during the investigation.

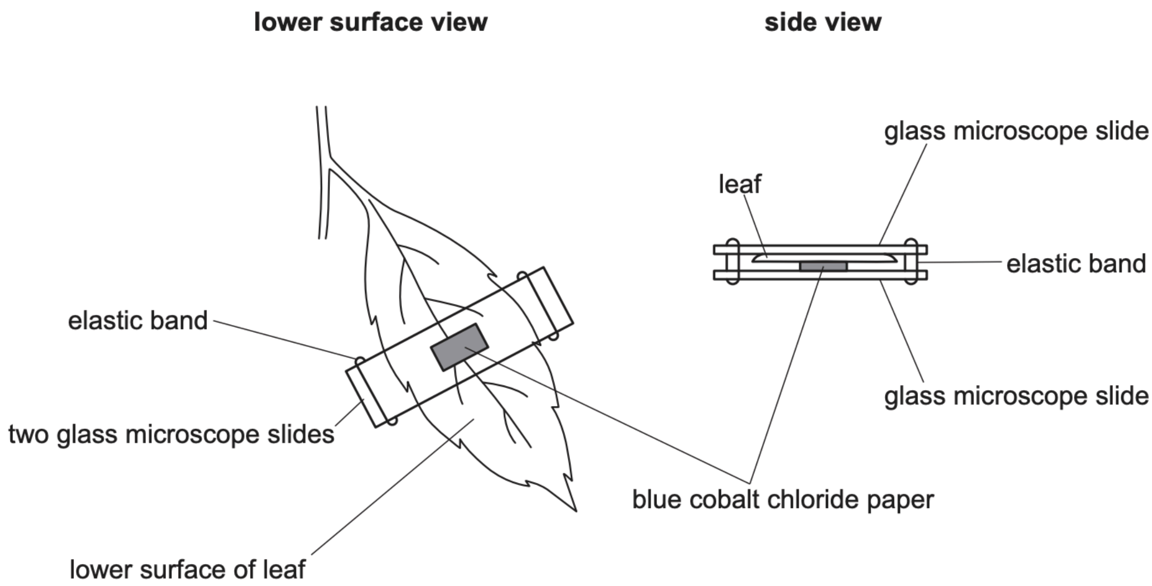


Fig. 1.2

A piece of blue cobalt chloride paper was attached to the lower surface of a leaf, as shown in Fig. 1.2. Blue cobalt chloride paper changes colour to pink if water is added.

The student measured the time taken for the blue cobalt chloride paper to change colour.

This procedure was repeated with two more leaves of the Japanese spiraea plant. The plant was kept in controlled conditions at all times.

Table 1.1 shows the results obtained using blue cobalt chloride paper.

To improve the validity of the results, the student decided to measure the time taken for blue cobalt chloride paper to change colour on a greater number of leaves of the Japanese spiraea plant.

State one other change the student could make to the method to improve the validity of the results.

- Use same / stated size of (blue) cobalt chloride paper.
- Use a (pink) colour standard to determine end point.
- Measure different positions on lower surface of leaf.
- Unbiased selection of leaves / leaf position.

NOTE: this questions asks for a change in the method! Things like comparing with results from other students are not changes, but ways to increase reliability in the same method.

State the environmental conditions in a glasshouse that should be standardised in a period of acclimatisation.

- Temperature
- Light intensity
- Duration / hours of light
- Humidity
- Volume of water added to soil
- Type / pH of soil
- Mineral ion concentration in soil
- Carbon dioxide concentration

NOTE: transpiration has a cooling effect.

A student investigated how length of a ring of vein increases as more mass is added.
Explain why the student calculated percentage increase in length of the ring of vein.

- to make a valid comparison with other blood vessels / veins.
- to allows comparison as initial / starting length of veins is not constant.

NOTE:

- arteries have a thicker wall / tunica media / muscle layer (than the vein) OR have more (smooth) muscle.
- artery withstands more (blood) pressure so stretches less (than the vein).

The student was given a stock solution of GA3 with a concentration of 2.85 × 10–4moldm–3. Describe a method the student could use to make a solution of GA3 with a concentration of 1.90 × 10–6moldm–3 and state the dilution factor used.

- 1 cm3 of stock solution to 149 cm3 of distilled water.
- Dilution factor = 150.

State how to standardise the measuring of stem length.

- easure from top of the soil / bottom of stem to top of the stem.
- straighten the stem while measuring length.
- place string along stem and measure length of string.

State and explain methods to improve confidence in results

More intermediate/ smaller values/ intervals	Better idea of the trend/ pattern/ effect

Exclude anomalous results	Improve reliability
Repeat reading for independent variable that gives anomalous result	Check the anomaly/ improve reliability
Repeat investigation at least twice	Better idea of trend/ improve reliability /reduce effect of anomalies
Calculate standard error / standard deviation / confidence intervals / statistical analysis	Check for significance / Checking accuracy
Use different apparatus / measure to nearest mm instead of cm etc.	More precise measurements
Increase the sample size	Improve reliability
Use multiple sampling sites	Improves reliability

To investigate the effect of the different root extract concentrations on the diversity of the microorganisms in soil, scientists took samples of soil from each of the containers, identified and counted the number of species of microorganism present in the samples, and calculated the Simpson's index of diversity (D) values for the species of microorganism in each of the soil samples.

State variables the scientists should have standardised when taking the soil samples.

- Same mass of soil
- Sample from same depth
- Sample from same distance from roots / position in the pot
- Sampled at the same time

Describe the mark-release-recapture method used to estimate the population size of grey bush cricket.

- Catch a sample of grey bush crickets within habitat and count total captured and mark and release them.
- Detail:
    - suitable marking so not harmful / too obvious / not removed.
    - leave for a few days to give time for populations to mix before second sample taken.
- Catch second sample and count total number of crickets and number of marked crickets.
- Use Lincoln index to estimate population size.

Abiotic factors that can be monitored in an ecosystem

- Temperature

- Light intensity
- (atmospheric) humidity
- rainfall / soil water content
- soil type
- soil pH
- mineral / ion concentration (of soil)
- salinity (of soil)
- (atmospheric) carbon dioxide concentration
- wind speed / direction

Scientists analysed the milk produced by female echidnas and identified a protein that they named EchAMP. The scientists predicted that EchAMP may have antibacterial properties.

The scientists tested the effect of EchAMP on the bacterium *Escherichia coli*.

1. 100 *E. coli* cells were added to each well on a cell culture plate with 96 wells.
2. A treatment solution that contained EchAMP was added to each well on the plate.
3. A chemical that causes living *E. coli* cells to fluoresce was added to each well.
4. The plate was incubated at 37°C.
5. Every hour for 7 hours, the fluorescence emitted by the *E. coli* on the plate was recorded as a measure of *E. coli* population growth.
6. Steps 1–5 were repeated eight times.

The scientists also carried out two control experiments.

- A negative control experiment repeated the procedure (steps 1–6), but the treatment solution did not contain EchAMP.
- A positive control experiment repeated the procedure (steps 1–6), but the treatment solution contained an antibiotic called bacitracin instead of EchAMP.

The *t* value calculated by the scientists was significant.

After reading the scientific paper published by the scientists, a student wrote the conclusion:

EchAMP would make a good treatment for bacterial infections in the human digestive system.

Suggest reasons why this conclusion might not be valid.

- Experiment is not carried out in a person / carried out in a laboratory.
- EchAMP could cause side effects / be toxic in humans OR experiment only carried out for 7 hours / no information about longer term effects.
- EchAMP may kill beneficial bacteria (in the digestive system).
- EchAMP is a protein and would be digested by enzymes in the digestive system.
- Experiment only used E. coli / did not use other bacteria.
- EchAMP might not be as effective as other treatments / antibiotics.
- EchAMP did not kill all of the E. coli.

(ii) The students were given a 20.0% stock solution of sodium chloride. The students decided to use the stock solution to make sodium chloride solutions with concentrations of 0.0%, 1.0%, 5.0%, 10.0%, 15.0% and 20.0%.

The students made 140 cm^3 of these solutions in separate beakers.

Complete Table 1.1 to show how **140 cm^3** of these solutions could be made by **proportional** dilution of the 20.0% stock solution of sodium chloride.

Table 1.1

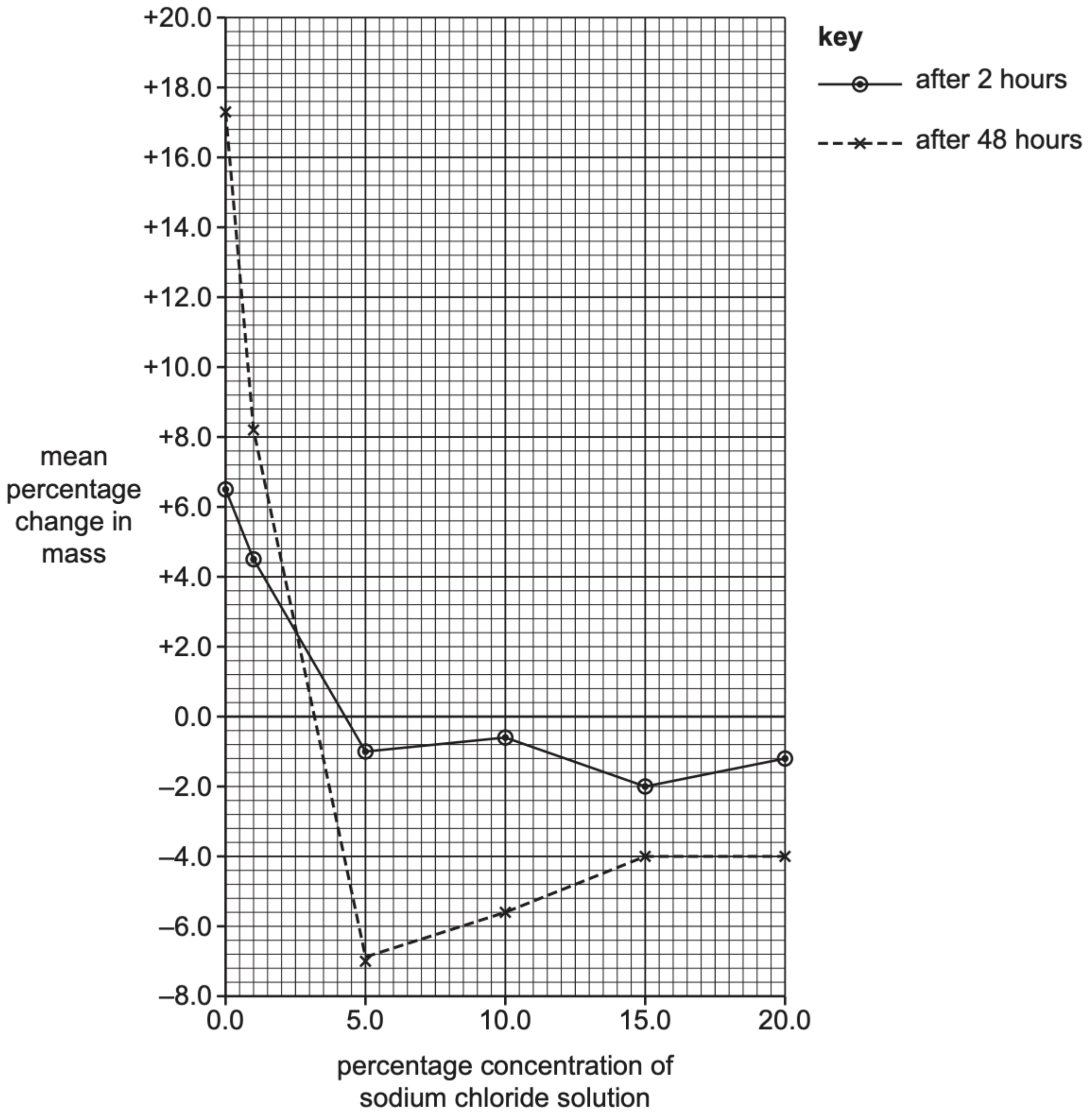
percentage concentration of sodium chloride solution	**volume of 20.0% sodium chloride solution / cm^3**	**volume of .... / cm^3**
0.0		
1.0		
5.0		
10.0		
15.0		
20.0		

(b) The students carried out this procedure.

- The dry outer layer was removed from 30 small onions.
- Five of the small onions were placed into each of the six beakers containing the sodium chloride solutions prepared by the students.
- The onions were left in the sodium chloride solutions for **2 hours**.
- The mean percentage change in mass of the five small onions in each beaker was calculated.

The students then repeated the whole procedure using a new set of 30 small onions. This time the onions were left in the sodium chloride solutions for **48 hours**.

Fig. 1.2 shows the results of the investigation.



One of the students concluded that: The water potential of the onion cells is the same as the water potential of a 4.2% sodium chloride solution.

With reference to the information provided, suggest reasons why this conclusion should not be accepted.

- conclusion is only supported for onions left for 2 hours.

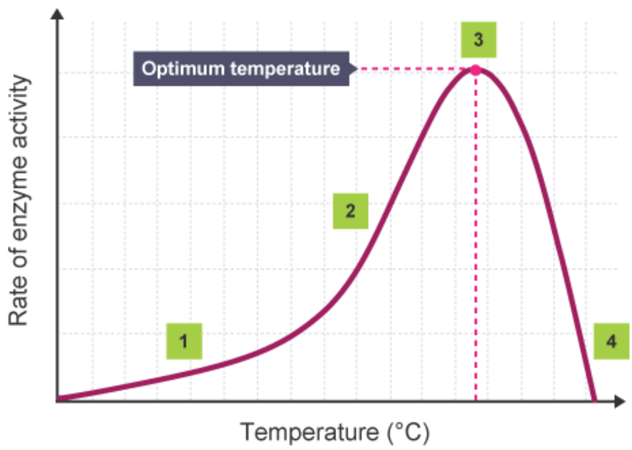
- intercept of x-axis / 0% change in mass / water potential of onions when left for 48 hours is 3.1% sodium chloride.
- ref. to qualified experimental error.
- ref. to anomalous result(s) on graph.
- line of best fit may give different value.
- intermediate concentrations of sodium chloride not tested (between 1% and 5% sodium chloride).
- no statistical analysis / standard error / 95% CI.
- not all onion cells have the same water potential.

NOTE:
A graph of rate of osmosis vs temperature usually looks like a curve that rises and then falls.

Low temperatures
- Rate of osmosis is slow
- Molecules have low kinetic energy → water moves slowly across the partially permeable membrane.

Increasing temperature
- Rate of osmosis increases
- Water molecules gain kinetic energy → diffusion happens faster.

High temperatures
- Rate reaches a maximum, then decreases sharply
- This happens because high temperatures can:
  Damage membrane proteins
  Disrupt membrane structure
  Make the membrane less selectively permeable



The biologist expected the cross to result in a phenotypic offspring ratio of 9 : 3 : 3 : 1 in the second generation.

Null hypothesis was accepted.

More recent research has shown that gene **B/b** and gene **R/r** code for polypeptides in carrier proteins. These carrier proteins are found in organelle membranes of the pigment cells of the eyes of adult fruit flies.

- Gene **B/b** codes for a polypeptide in the tryptophan carrier protein. Tryptophan is an amino acid.
- Gene **R/r** codes for a polypeptide in the guanine carrier protein.

Fig. 2.3 shows how the dark red eye colour of wild fruit flies is produced in organelles in pigment cells of the eyes.

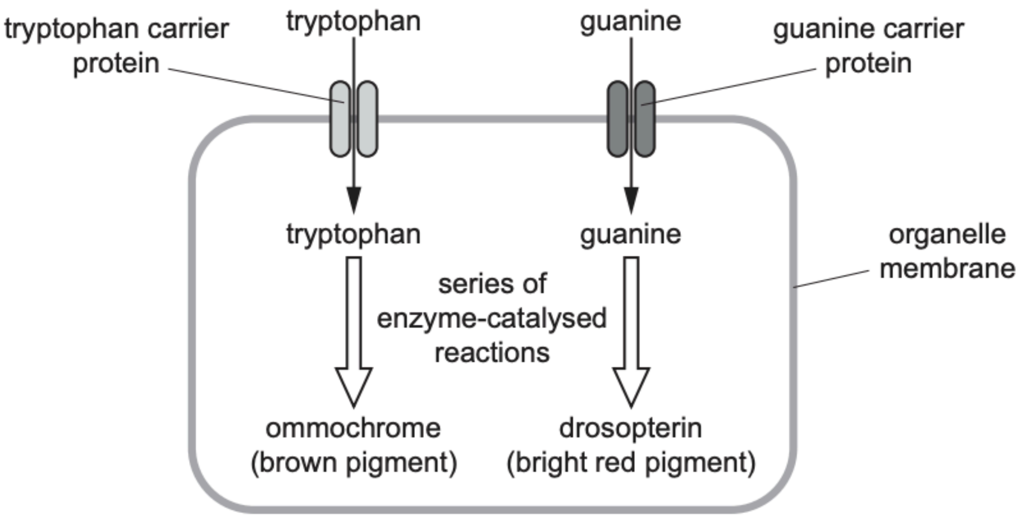


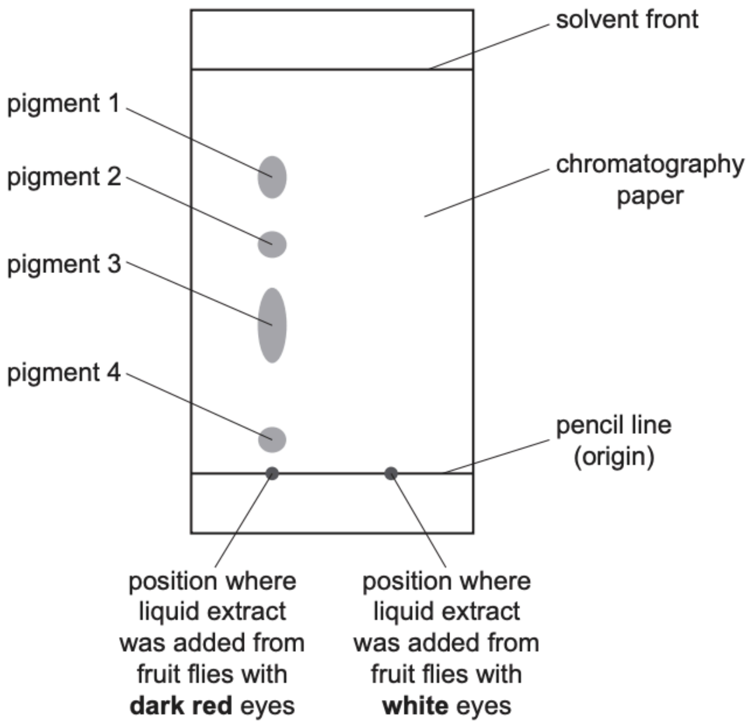
Fig. 2.3

The biologist decided to analyse all the pigments present in the eyes of the second generation of fruit flies.

The biologist started by extracting eye pigments from the adult fruit flies with **dark red eyes** and from the adult fruit flies with **white eyes**. The biologist then added a small volume of each liquid extract to chromatography paper and separated the pigments present by chromatography.

Fig. 2.4 shows the chromatography paper at the end of the procedure when viewed using visible light and ultraviolet light.

Pigment 1 and pigment 3 were visible only when viewed under ultraviolet light. Under visible light, pigment 2 was yellow and pigment 4 was bright red.



State the conclusions that can be made from the results of the fruit fly breeding experiment in (c) and from the chromatography results.

- Breeding experiment:
    - the two genes (B / b and R / r), are not linked.
    - dominant alleles code for (functional) carrier proteins / polypeptides.
    - in fruit flies with dark-red eyes entry of guanine and tryptophan (into organelle) allows pigments to be produced.
    - fruit flies with white eyes (have the genotype) bbrr / homozygous recessive (for both genes) OR fruit flies with dark-red eyes are B_R_
- Chromatogram:
    - fruit flies with dark-red eyes have 4 pigments (in their eyes).
    - fruit flies with white eyes have no pigments (in their eyes).
    - pigment 4 is drosopterin.
    - ommochrome / brown pigment, is insoluble (in the chromatography solvent) OR none of the pigments in the chomatogram are ommochrome / brown pigment.

Suggest reasons why using algal beads, instead of placing the algal cells directly into the indicator solution in the bottles, improves the validity of the investigation.

- indicator solution can be separated from algal balls.
- more accurate standardisation of mass / volume / of algae.
- colour of indicator solution is not affected by algae.

In an investigation of the rate of photosynthesis, spinach leaves were cut into small pieces, placed in a blender containing ice-cold 10% sucrose solution buffered at pH 7.0, and used to create a chloroplast suspension.
Suggest why the procedure works when 10% sucrose solution is used but does not work when distilled water is used.

- Sucrose solution has same / similar water potential as in the chloroplast.
- Water will not enter chloroplast by osmosis / as there is no water potential gradient
- Chloroplasts will not burst

Researchers carried out a study to compare the proportion of non-melanic to melanic phenotypes of an insect, in early spring vs. proportion in autumn. The data were collected over a period of 12 years.
Outline a method that could be followed to collect the data.

- Random/ systematic sampling: divide the area into coordinates, random number generator to generate coordinates OR belt transect.
- Place quadrats/ use net/ pooter.
- Count the number of individuals of each phenotype.

NOTE: Don't use mark-release-recapture for this!
Mark-release-recapture is mainly used to estimate population size, not phenotype proportions. And this investigation is done over a long time period as well.

The researchers concluded that melanic morphs warm up more quickly than non-melanic morphs. Explain how the information in graph supports this conclusion.

- Line for melanic is steeper than line for non-melanic.

Researchers made the hypothesis: Some melanics are homozygous dominant, and some melanics are heterozygous.
Outline a breeding experiment that could be carried out to test this hypothesis, and state the results you would expect if the hypothesis is supported.

- Cross melanic with non-melanic.
- Homozygous dominant parents will only have melanic offspring and heterozygous parents will have both phenotypes in offspring.
- Use many breeding pairs.
- Keep each breeding pair separate from other pairs or separate offspring from parents.
- Count the different phenotypes of offspring.

Preparing colorimeter to obtain correct measurements of absorbance:

- Select appropriate filter (usually complementary to the solution's color, e.g., red filter for blue/green solutions).

- Calibrate colorimeter.
- Use a blank (like distilled water) to set colorimeter absorbance to zero.

LPR = disease that occurs when gastric juice from stomach moves up the oesophagus and into the larynx.

Gastric juice can damage the laryngeal epithelium. Gastric juice contains hydrochloric acid and pepsin.

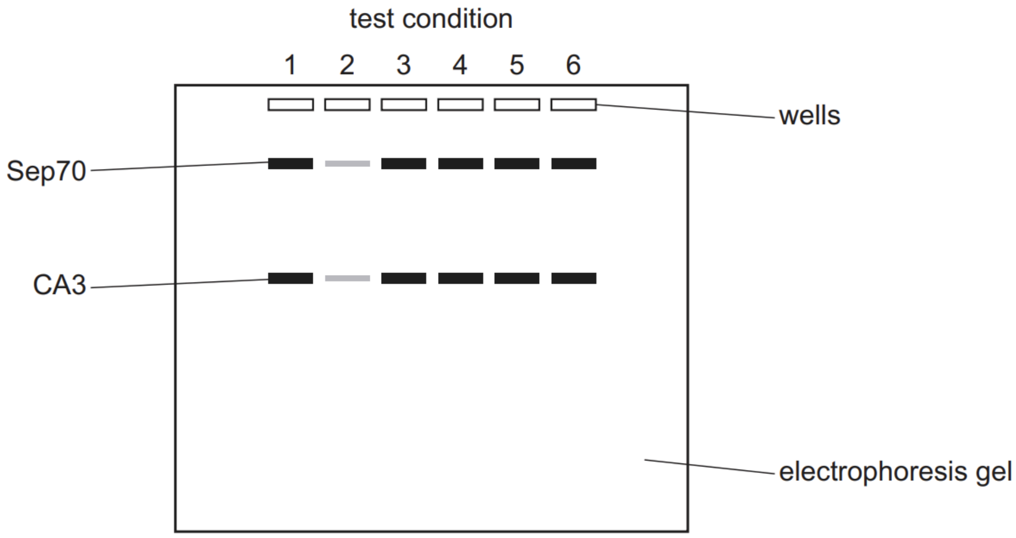
Reduction in the quantity of two proteins – CA3 and Sep70 (present in laryngeal epithelium) – was identified in people with LPR.

6 sections of mammalian laryngeal epithelium were obtained to investigate how different test conditions affect the quantity of CA3 and Sep70 in the laryngeal epithelium.

Each section of laryngeal epithelium was exposed to different test conditions for 20 minutes.

Pepsin is inactive in the presence of the inhibitor, pepstatin.

treatment used	**test condition**					
	1	**2**	**3**	**4**	**5**	**6**
pepsin	yes	yes	yes	yes	no	no
pepstatin (inhibitor)	no	no	yes	yes	yes	yes
pH buffer	7.4	4.0	7.4	4.0	7.4	4.0



*Sep70 and CA3 are proteins.

Conclusions from the electrophoresis
- test condition 2 decreases / hydrolyses CA3 and Sep70.
- acid / pH 4.0 with no active pepsin does not change CA3 and Sep70.
- mass / size CA3 is smaller than Sep70 (because it travels more along the gel).

A student who read the scientific paper concluded that pepsin causes damage to the laryngeal epithelium in people with LPR. Suggest why the results of this investigation might not support this conclusion.

- pepsin only causes damage to the laryngeal epithelium if hydrochloric acid / pH 4.0 is also present.
- investigation only carried out once / was not repeated.
- no information on species of mammal used in investigation OR investigation not carried out in a person / carried out in a laboratory.
- reduction in quantity of Sep70 and CA3 may not cause damage to the laryngeal epithelium.

An investigation was conducted on how the colour and pattern of spots on female golden orb weaver spiders help to attract insects to their webs. 5 webs were used, with 5 models of spiders having different colours and patterns. Each model was placed in the centre of the web, video camera was placed 1m away and web + model were filmed for 6 hours. Insect attraction event was recorded whenever an insect flew towards the model, touched the model, or touched the web.

Suggest how this investigation could be improved to increase confidence in the results

- do investigation in more than one forest / geographical area.
- do investigation at different times of the day.
- do investigation in more than one month / season / year.
- only count insects that touch the model / web.
- use more models with different colours / patterns OR use 3–dimensional model (instead of flat / 2–dimensional model).
- idea of (video) living spider on web.

NOTE: sodium hydrogencarbonate = source of carbon dioxide. (used in photosynthesis experiments)

Students are investigating the effect of different light intensities on the rate of photosynthesis a plant. **Suggest a suitable control for this investigation.**

- Replace the plant with glass beads of the same volume OR with dead plant of the same volume

Describe difficulties with paper chromatography that could explain why the Rf values calculated by the students were not exactly the same as the published data.

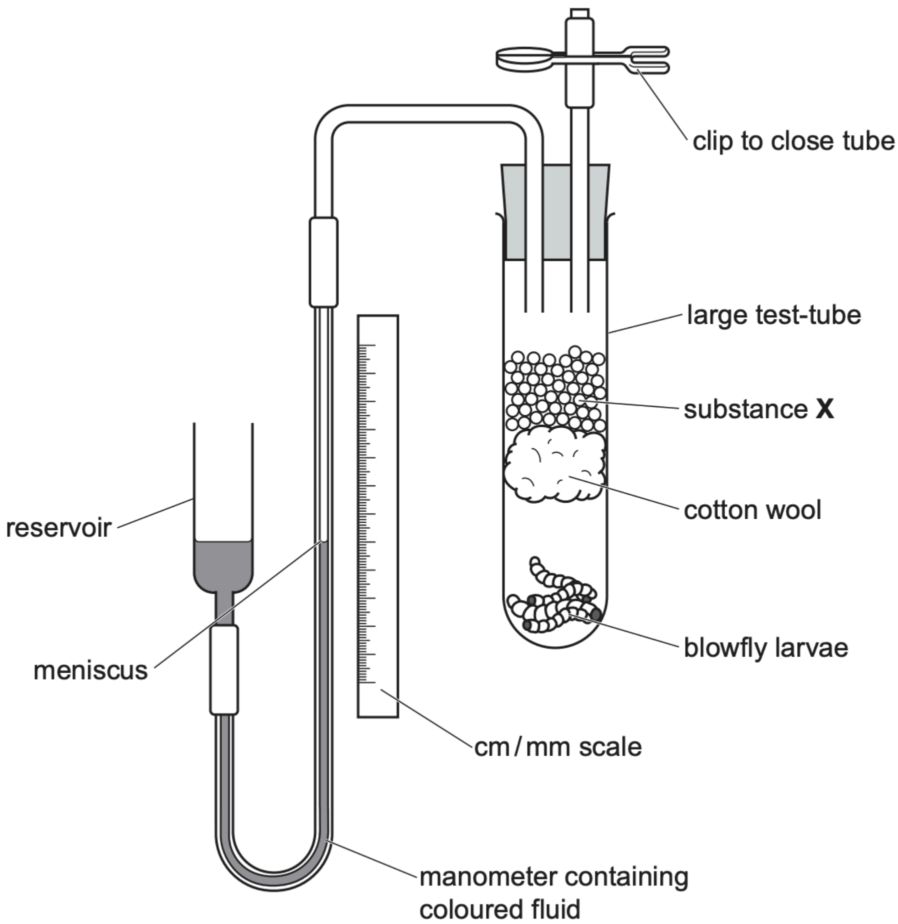
- difficulty in achieving concentrated / not spread out spot of extract.
- each pigment spreads out during migration / gives long streak / runs into each other / overlaps so difficult to measure accurately.
- pigment may fade / be too pale to see clearly so difficult to measure accurately.

- uniformity / quality / grade of chromatography paper.
- type of solvent.
- different laboratory / ambient conditions.

Explain the conclusion of lower rodent diversity in woodland with only saltcedar (invasive species) trees than in woodland with mixture of saltcedar and native trees.
- fewer food sources for rodents
- saltcedar trees provide less protection / shelter / nesting sites / niches

NOTE: when using a centrifuge, standardise:
- Speed of centrifuge
- Time in centrifuge

NOTE: to improve confidence in results when comparing between 2 ecosystems:
- Check that abiotic factors are similar between the 2 ecosystems
- Check that biotic factors are similar between the 2 ecosystems



X= soda lime to absorb carbon dioxide

Describe how the apparatus is modified so that the volume of carbon dioxide produced can be determined.

- Remove substance X
- Replace X with an inert material/ glass beads

Variables to be standarised each time experiment is carried out

- temperature
- number of (blowfly) larvae
- mass of (blowfly) larvae
- age of (blowfly) larvae
- food source for (blowfly) larvae
- mass / number of substance X / inert material / beads
- time to equilibrate
- time for measurement (of meniscus movement)

replicates	1	2	3	4	5
distance moved by manometer fluid in Fig. 2.1 when measuring oxygen uptake in 10 min / mm	15	48	61	56	68
volume of oxygen taken up / $mm^3 min^{-1}$	1.18	3.77	4.79	4.40	
volume of carbon dioxide produced / $mm^3 min^{-1}$	3.30	2.75	3.31	2.83	3.00
respiratory quotient (RQ)	2.80	0.73	0.69	0.64	

The result for replicate 1, ringed in Table 2.1, is anomalous. Suggest two possible causes for this anomaly other than measurement error.

- the larvae were not fully adjusted / equilibrated
- the larvae were at a lower temperature (than the apparatus)
- the larvae were less active

1. **State one reason for keeping mitochondria in cold buffer solution during isolation.**
    - cold buffer prevents mitochondrial enzymes / proteins denaturing.
    - cold temperature prevents damage to mitochondria by enzymes.
    - buffer prevents osmotic lysis of mitochondria.

2. **State two ways that the student could have processed the results to allow for these possible anomalies.**
    - do not include anomalies and calculate a more accurate mean.
    - calculate standard deviation / standard error / 95% confidence limits for each trial

3. **A student decided to make a proportional dilution using a 2.0% stock solution of phenolphthalein. The student made 50cm3 of each diluted solution. Describe a method the student could use to make a proportional dilution of the 2.0% stock solution of phenolphthalein to get a range of concentrations.**

1 five stated concentrations between 2 % and 0 % **and** units, % / percentage **;**

2 correct method for dilution shown for two intermediates **;**

concentrations %	**2 % stock / phenolphthalein / cm^3**	**distilled water / cm^3**
2.0	50	0
1.6	40	10
1.5	37.5	12.5
1.2	30	20
1.0	25	25
0.8	20	30
0.5	12.5	37.5
0.4	10	40
0.25	6.25	43.75
0.0	0	50

4.

Fig. 2.1 shows the apparatus used to measure the oxygen consumption of the isolated mitochondria.

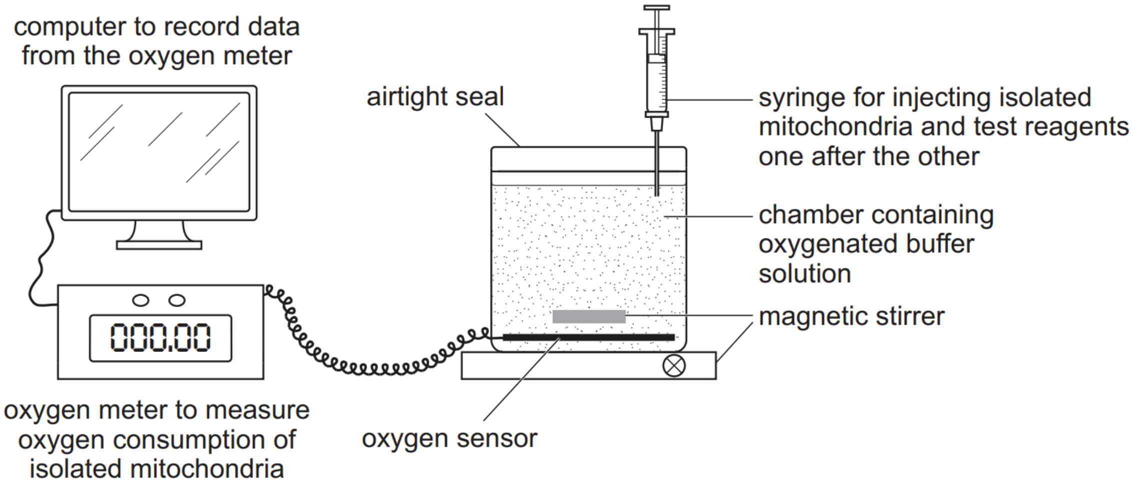


Fig. 2.1

The apparatus was used as follows.

- The apparatus was set up as shown in Fig. 2.1 and left for 1 minute to equilibrate.
- At 1 minute, a standard volume of mitochondria in buffer solution was injected through the seal.
- At 2 minutes, a solution of succinate, a Krebs cycle intermediate, was injected through the seal.
- At 5 minutes, a solution of ADP was injected through the seal.
- At 6 minutes, a solution of cyanide was injected through the seal.

During this procedure the oxygen concentration in the chamber was measured continuously and displayed as a trace on the computer screen as shown in Fig. 2.2.

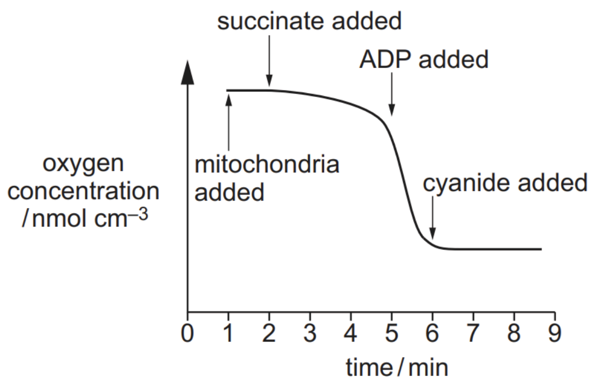


Fig. 2.2

Using the figures from the trace, the computer calculated the rate of oxygen consumption following each addition. This procedure was repeated three more times using fresh samples of mitochondria.

Table 2.1 shows the rates of oxygen consumption for each of the four trials.

Table 2.1

	rate of oxygen consumption / nmol min^{-1}				
	trial 1	**trial 2**	**trial 3**	**trial 4**	**mean**
mitochondria alone	0.03	0.02	0.03	0.01	0.02
mitochondria with succinate	50.22	49.10	48.53	50.15	
mitochondria with ADP and succinate	139.23	170.10	142.67	138.10	147.53
mitochondria with cyanide, ADP and succinate	0.00	0.10	0.00	0.01	0.03

Explain the results shown in Fig. 2.2 and Table 2.1

- in mitochondria alone: oxygen concentration does not change OR do not use oxygen OR rate of oxygen consumption is very low.
- adding succinate (and no ADP) increases the rate of aerobic respiration / Krebs cycle / oxidative phosphorylation.
- adding succinate and ADP gives a larger increase in the rate of aerobic respiration / Krebs cycle / oxidative phosphorylation.
- oxygen acts as the final electron acceptor in aerobic respiration / oxidative phosphorylation.
- adding cyanide stops aerobic respiration / oxidative phosphorylation.

5. **The intensity of the pink colour of phenophthalein is an indication of its concentration. To estimate the concentration of phenolphthalein produced by a reaction, the student decided to make a proportional dilution using a 2.0% stock solution of phenolphthalein. The student made 50cm3 of each diluted solution.**

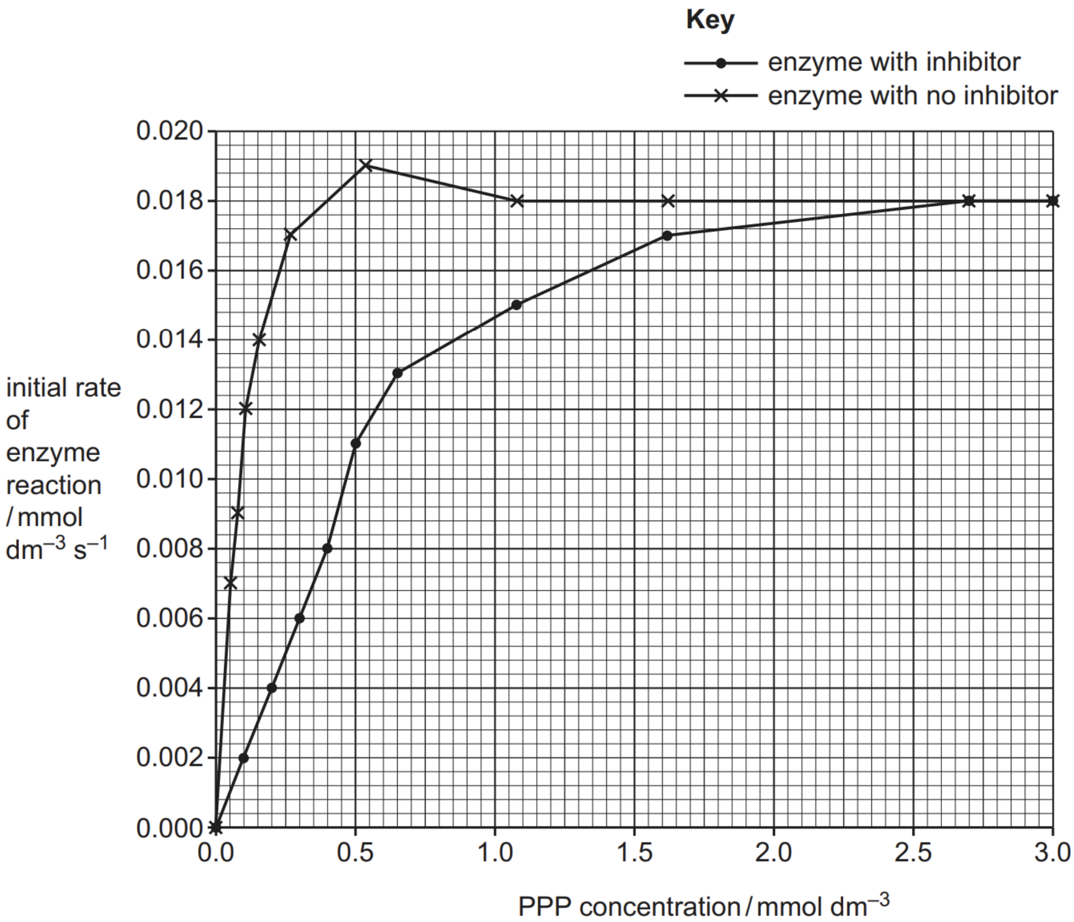
Describe how the student could use these dilutions and a colorimeter to estimate the concentration of phenolphthalein in a reaction mixture.

- Measure absorbance for the dilutions <u>and</u> extract / reaction mixture.
- Use the calibration curve / graph to determine phenolphthalein / extract concentration.

6.

(f) The student investigated the effect of a competitive inhibitor on the activity of a phosphatase.

Fig. 1.3 shows a graph of the initial rates of enzyme reaction against PPP concentration for the enzyme with no inhibitor and the enzyme with inhibitor.



One of the data plots is anomalous. Circle the anomalous data plot and explain why you think it is anomalous.

- point 6 on top line is circled
- higher than the Vmax / 0.018 (mmol dm−3 s−1) / the plateau

Use the figure to determine the Michaelis–Menten constant (Km) for the enzyme with no inhibitor and for the enzyme with inhibitor. Include the correct units in your answer.

- With no inhibitor: 0.075
- With inhibitor: 0.43 / 0.425
- Unit: mmoldm−3

<u>Explanation:</u>

- As substrate concentration increases:
    - The rate increases rapidly at first
    - Then levels off into a plateau → this is Vmax

- This happens because:
    - At low substrate concentration → many free active sites
    - At high substrate concentration → all active sites are occupied (enzyme is saturated)

- Effect of a competitive inhibitor (why the curves differ)
    - Inhibitor competes with substrate for the active site
    - Can be overcome by increasing substrate concentration
    - At low PPP concentrations:
        - Rate with inhibitor is much lower
        - Because inhibitor occupies many active sites
    - At high PPP concentrations:
        - Both curves reach the same maximum rate
        - Substrate outcompetes the inhibitor

- Competitive inhibition because:
    - Vmax is unchanged
    - Km increases

- Vmax= maximum rate of reaction when all enzyme active sites are occupied by substrate.
- Km = substrate concentration at which the reaction rate is half of Vmax.

7.

Scientists sampled the marine habitats along a length of coastline in Algeciras Bay in southern Spain to study the species diversity of sponges.

The scientists selected 12 sampling stations, **A** to **L**, as shown in Fig. 2.2. The scientists noted the land use or human activity along the coast next to each sampling station.

Key to diagram:

sampling station	type of land use or human activity
A	natural habitat
B	natural habitat
C	tourist beach
D	housing
E	shipping port
F	tourist beach
G	thermal power station
H	oil industry
I	ship building and repairs
J	tourist boats
K	tourist boats
L	natural habitat

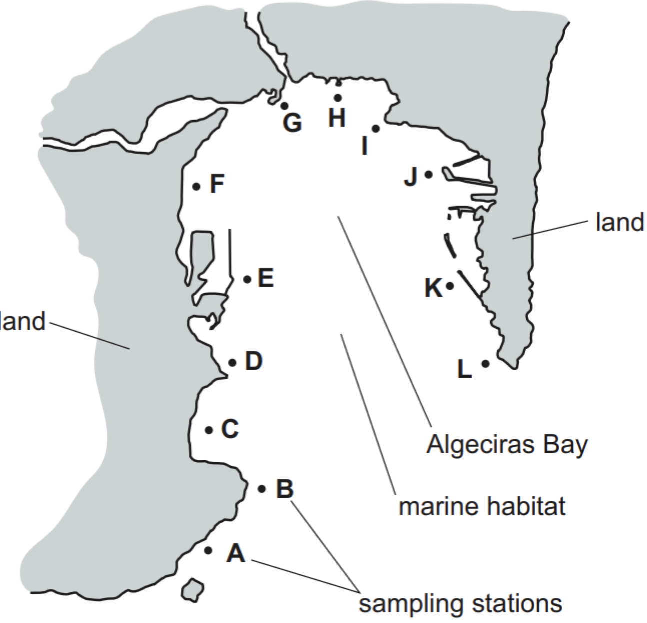


Fig. 2.2

The sampling stations were chosen to compare the effects of land use or human activity on the species diversity of sponges in the bay.

At each marine sampling station, the scientists:

- placed permanent line transects, 50 m in length, on the seabed
- photographed all sponges sighted at a distance of 1 m either side of the transect
- sampled each transect for the same length of time
- sampled each transect four times a year.

State two other variables that the scientists should standardise in this investigation.

- (same) time of the year OR (same) seasons OR (same) months.
- same depth of water / sea / sponge / seabed / water pressure.
- same orientation of transect.

REMEMBER

- When sketching a graph, always label the axes and give the state symbols if there are

Difficulty in paper chromatography:

- Uniformity/quality of chromatography paper.

Why pH meter is used instead of indicator solution and colour chart:

- more accurate
- more precise
- can continuously record data on a computer

1.

Table 1.1

concentration of GA / mol dm^{-3}				
0	1×10^{-7}	1×10^{-6}	1×10^{-5}	1×10^{-4}
mean shoot length / mm ± SE				
120 ± 10	150 ± 5	175 ± 15	210 ± 12	180 ± 8

Explain what standard error (SE) shows about the reliability of the results in Table 1.1.

- smaller standard error (SE) shows that the mean is more reliable.
- smaller SE shows that calculated / sample mean is closer to the true / actual mean.
- example of overlap: SE for 1 x 10^{-7} ABA concentration and 1 x 10^{-6} ABA concentration do not overlap therefore mean shoot length may be significantly different OR SE for 1 x 10^{-6} ABA concentration and 1 x 10^{-4} ABA concentration overlap, therefore mean shoot length is not significantly different.

Format

1. Independent variable:
    - Method to change the independent variable (including apparatus + any reagents used)
    - At least 5 different values of independent variable: state the values used.
2. Method: Details of method/ procedure to be followed.
3. Control variables: Method to keep control variables constant.
4. Dependent variable: Method to measure dependent variable (including apparatus).

5. Control experiment: Describe conditions of control experiment.
6. Record at set time intervals.
7. Repeat experiment at least 2 or 3 times and calculate mean/ plot a graph.
8. Safety hazard + risk + precaution

When a species of plant or animal is involved, use the following control variables:

- same species
- same age
- same sex

<u>To find the effect of different concentrations of ABA on the germination of wheat grains.</u>

1. Independent variable
   a. Use atleast 5 different concentrations of ABA
      1 x 10^{-3} mol dm^{-3}, 1 x 10^{-4} mol dm^{-3}, 1 x 10^{-5} mol dm^{-3}, 1 x 10^{-6} mol dm^{-3}, 1 x 10^{-7} mol dm^{-3}. Prepare each concentration using serial dilution (10cm3 solution to 90cm3 water).
   b. Use the same volume of each prepared ABA concentration (100cm3); measure volume using measuring cylinder.
2. Method
   a. Place wheat grains in a container with soil/ paper.
   b. Carry out experiment in a dark room.
3. Dependent variable
   a. For each ABA concentration, count the number of wheat grains that have germinated after the same time OR measure the length of shoot after the same time.
4. Control variables
   a. Maintain a constant temperature - at 20˚C, using a temperature-controlled room/ thermostatically controlled water bath.
   b. Add the same volume of water to each container of grains for germination.
5. Carry out a control experiment for one group of 50 grains: replace the ABA solution with distilled / deionised / pure water.
6. Safety hazard:
   a. Wheat grains – irritant/allergy – gloves/mask/PPE
   b. ABA – irritant/allergy/toxic – gloves/mask/PPE
   c. Soil – biohazard/pathogens/allergy/irritant – gloves/mask/PPE

<u>To determine the optimum pH for phosphatase (enzyme).</u>

(The student read that the optimum pH for phosphatase extracted from mung bean seedlings is less than pH7.0.)

$$\text{PPP} \xrightarrow{\text{phosphatase}} \text{phenolphthalein + inorganic phosphate}$$

1. Independent variable:
    a. Prepare 5 solutions with stated pH values up to pH7.
2. Method:
    a. Equilibrate PPP substrate, buffer solution, and phosphatase extract separately to fixed temperature, before mixing.
    b. Mix buffered PPP substrate with extract / enzyme solution.
3. Dependent variable:
    a. Calibrate colorimeter OR use a blank to set colorimeter absorbance to zero / reset colorimeter and measure absorbance after fixed time.
    b. at each pH, stop the reaction after a set time and measure / note / record absorbance.
4. Repeat experiment with smaller pH intervals around the optimum pH / values with the highest absorbance / concentration (of phenolphthalein).
5. Repeat experiment at least twice and calculate the mean absorbance, for each pH / their means.
6. Hazards + precautions:
    a. phosphatase enzyme / extract, PPP, sodium carbonate – allergy / irritant – gloves / eye protection / PPE
    - pH buffer – irritant pH 3–7 OR corrosive below pH 4 – gloves / eye protection / PPE
    - phenolphthalein — toxic / irritant / allergy — gloves / eye protection / PPE; flammable keep away from naked flame