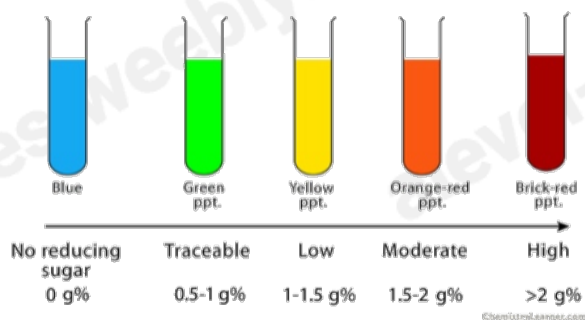# Paper 3 (practical) notes

2.1 Testing for biological molecules

Reducing sugars

- add equal volumes of the food sample and Benedict's reagent
- heat in a water bath at 80°C
- colour changes from blue, green, yellow, orange, brick red ppt
- for accuracy, make sure the tubes are left in the water bath for the same amount of time

Non-reducing sugars

- first hydrolyse the food sample with hydrochloric acid and heat
- then neutralise the sample with an alkali (e.g., NaOH, Na_2CO_3)
- add an equal volume of Benedict's reagent to the test tube, heat in a water bath at 80°C and observe the colour change (refer to the above diagram)

Iodine test for starch

Add a few drops of iodine solution to the sample

- if a blue black colour is quickly produced, starch is present
- if the iodine solution remains yellow-brown, starch is absent

Emulsion test for lipids

- shake sample with ethanol till it dissolves
- add water
- if lipids are present solution turns into a cloudy white suspension

Biuret test for proteins

- take 2 cm^3 of food sample to be tested in a test tube
- add 2 cm^3 of 5% sodium hydroxide solution and shake
- then add a few drops of 1% copper sulphate solution and observe colour change
- or if biuret reagent is available then add equal volumes of that to the food sample and observe colour change
- if the colour remains blue, no proteins are present
- if the colour turns lilac/purple, proteins are present

Qualitative data

Descriptive data from observations and not measurements such as colour changes

Qualitative food test errors

1) difficult to judge colours, especially if concentrations are low resulting in light colours or small colour changes
2) temperature of the water bath may not remain constant throughout heating

Improvements for qualitative tests

1) use a thermostatically controlled water bath
2) use a colourimeter
3) place a white card or tile behind the test tubes

Quantitative data

Numerical data such as measurements

Quantitative food test errors

1) difficulty in comparing colours
2) when determining an unknown concentration, it may be between 2 concentrations
3) temperature not being constant for all samples
4) DCPIP test for vitamin C – drops fall on the sides of the test tubes

Improvements for quantitative tests

1) carry out more experiments with a narrower or wider range of concentrations
2) plot a graph with results to estimate the unknown
3) use a colourimeter to help compare colour changes better
4) use a white card/tile to observe the colour changes better
5) use a thermostatically controlled water bath
6) in the DCPIP vitamin C test, use a wider test tube, or a wide-mouthed one
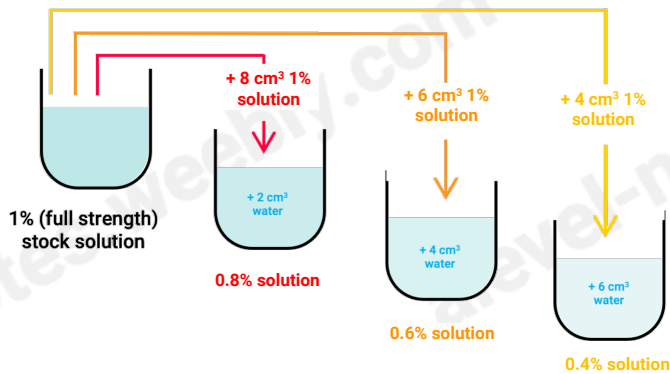7) repeat and take the average

Dilutions

$$C_1 V_1 = C_2 V_2$$

starting concentration — C_1
starting volume — V_1
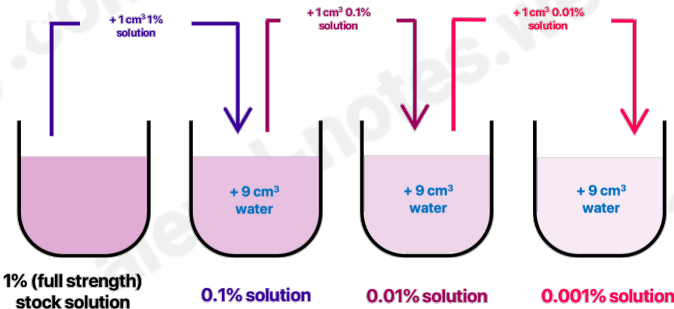final concentration — C_2
final volume — V_2

a) Simple dilutions

E.g., reducing the concentration by a factor of 2 to make a 10 cm^3 solution (this differs depending on the question)

volume of solution / cm^3	volume of distilled water / cm^3	concentration / %
10	0	1.0
8	2	0.8
6	4	0.6
4	6	0.4

+ 8 cm^3 1% solution

+ 6 cm^3 1% solution

+ 4 cm^3 1% solution

1% (full strength) stock solution

+ 2 cm^3 water

0.8% solution

+ 4 cm^3 water

0.6% solution

+ 6 cm^3 water

0.4% solution

b) Serial dilutions

Reducing the concentration *by a factor of 10* to make a 10 cm^3 solution

+ 1 cm^3 1% solution

+ 1 cm^3 0.1% solution

+ 1 cm^3 0.01% solution

1% (full strength) stock solution

+ 9 cm^3 water

0.1% solution

+ 9 cm^3 water

0.01% solution

+ 9 cm^3 water

0.001% solution

3.1 Mode of action of enzymes

Errors in enzyme experiments

1) counting bubbles is inaccurate due to different bubble sizes, bubbles being too fast to count, bubbles too small so they're missed
2) temperature or pH not constant (if they aren't the factors being investigated)
3) difficulty in judging endpoint e.g., with renin the coagulation may not be clear

Improvements in enzyme experiments

1) use a gas syringe to measure volume of gas instead of counting bubbles
2) control temperature using a thermostatically controlled water bath
3) control pH by using buffers
4) use a colourimeter/put a white tile or card to judge colour change

3.2 Factors that affect enzyme activity

Effect of heavy metals such as copper sulphate and lead nitrate

- can act as inhibitors
- may cause protein to clot or coagulate
- may denature proteins
- may breakdown bonds so that can alter tertiary and quaternary structures

Immobilising enzymes

Errors

1) beads are not of equal sizes
2) beads stuck to the sides of the tube and to each
3) other
4) forceps may cause damage to the beads
5) difficulty to introduce drops using syringe
6) test tube is not vertical and test tubes are not of equal sizes
7) temperature/pH is not controlled (if they are not the factors under investigation)

Improvements

1) use sieve with equal size holes to produce beads with equal diameter
2) use wider test tubes
3) stain the beads for clearer movement
4) use retort stand to make the test tube vertica
5) use test tubes with equal sizes
6) use spatula or spoon instead of forceps
7) control temperature using thermostatic waterbath
8) control PH using buffer

4.2 Movement of substances into and out of cells

Effect of changing surface area to volume ratio on diffusion using agar blocks of different sizes

Errors

1) difficulty in cutting the agar squares into equal dimensions
2) difficulty in judging the colour change
3) agar is not of equal depth
4) pigmentation of the agar is not even
5) agar may be damaged during cutting

Improvements

1) use moulds for preparing agar squares with equal dimension
2) use a different indicator with clearer endpoint

3) use wider or narrower range of concentrations (in case of determining unknown)
4) place a white card below the beaker for better judgment of colors

Effects of immersing plant tissues in solutions of different water potentials

How to keep fair comparison in osmosis experiments?

- when using petri dishes, use petri dishes of the sme size
- when using sucrose or any other solution, put the same volume in each dish
- when using onion epidermis or potato, must be of the same size
- leave the potato or onion epidermis in the solution for the same period of time

General precautions in osmosis experiments

- cover the petri dishes during the experiment to avoid evaporation of water which can affect concentration of the solution
- when using droppers or syringes, use separate droppers for each solution or wash and dry after each step
- while preparing a slide of onion epidermal cells, lower the coverslip gently to avoid any air bubbles

Errors

1) time tissues left in solutions is not enough to observe complete plasmolysis
2) evaporation of solution may take place and therefore change the concentration
3) difficulty in judging degree of plasmolysis
4) difficulty in cutting the samples into correct dimensions
5) parallex error may occur during reading the lengths

Improvements

1) leave for longer time in the solutions
2) prepare more concentrations with narrower/wider range (give examples and method of dilution)
3) cover the petri dish to avoid evaporation of solution
4) prepare more than one sample in each solution
5) ensure that the tissues are completely immersed in the solutions

4.2.2 Expectations for each mark category (Paper 3)

Making decisions about measurements or observations

Decide how the independent variable should be changed within a suitable range to provide accurate results.

- concentration – simple/serial dilution
- temperature – water bath/freezer
- pH – use buffers
- moving air – fan
- humidity – plastic bag/calcium hydroxide

Decide the number of values at which measurements are recorded

- a minimum of 5 measurements,
- replicates or more measurements around a specific value

Appropriate controls

- replacing a solution with the same volume of water
- denaturing an enzyme by boiling

Decide which variables to standardise

- volume (must be suitable for apparatus)
- concentration
- temperature
- pH
- biological material (e.g., same species, age, storage conditions, time of year, mass)
- humidity (plastic bag/Ca(OH)$_2$)
- apparatus

Measuring area

- count areas covering half or more of a grid square as one whole square
- don't count areas less than half of a square

Constructing a table

- the first column should be the independent variable followed by the dependent variable
- the heading should have the units
- units should be in "standard form" i.e., not mol/dm^3 instead mol dm^{-3}
- the slash (/) should only be used to separate the heading from the unit e.g., concentration / mol dm^{-3}
- all the figures in the table should be the same number of significant figures or decimal places

independent variable / unit	dependent variable / unit

Line graphs

dependent
variable (x-axis)

independent
variable (x-axis)

types of points
(x) crosses
(☉) circle with dot inside it
(+) plus
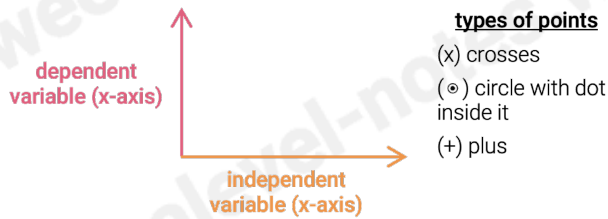
- each axis should be scaled using multiples of 1, 2, 5, 10 for each 20 mm square on the grid
- never use multiples of 3
- do not extrapolate unless they've asked you to

Presenting anomalous results

Put a circle on the graph away from the line and put a key to state that the circled point(s) represents ana anomalous result

How to present the curve

- **straight line** – e.g., effect of enzyme concentration on rate of enzyme catalysed reaction, if obvious that points lie on a straight line
- **smooth curve** – only if intermediate values fall on the curve
- **single results** – draw straight lines between points; this indicates uncertainty about the result for the values of independent variable

Bar graphs

- bars should not be shaded
- they need to be clearly labelled
- y-axis usually starts from 0
- all bars should be of equal thickness
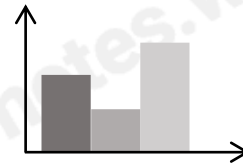- leave equal spacing between bars (including a space between the first bar)
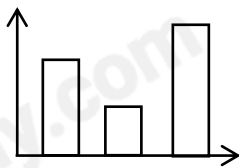
- if the bar chart is for two classes, make the bars of the 2 classes attached to each other but leave even spacing between intervals
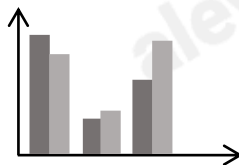
Histograms

Bars should be attached to each other, have the same thickness and the x-axis should have intervals e.g., 12-14, 14-16 and not categories.

Rate of reaction

- 1/t or 1000/t
- or the gradient of a line
- the unit used is s^{-1}

Types of errors

a) **systematic errors** – from apparatus, doesn't affect the trend in results
b) **random errors** – e.g., from variability of biological material, may affect trend and accuracy

Calculating error

$$\text{error} = \frac{\text{smallest division}}{2}$$

$$\% \text{ error} = \frac{\text{error}}{\text{measurement}}$$

- when 2 readings are taken (e.g., syringe, burette, change in something), the error is multiplied by 2.

Suggesting improvements to a procedure/modification

Improvements

1) standardise relevant variables
2) use a measurement method for the dependent variable which is more accurate
3) collect more data by taking replicates to obtain mean

Modifications

1) change a different independent variable
2) standardise all other variables including a previously used independent variable
3) use an improved method to obtain accurate and precise results for dependent variable

Taking measurements from photographs

Measure in mm not cm.

Plan diagrams

- show outlines only
- make proportions of tissues/cells in the diagram the same as the observed section

High power drawings (of cells)

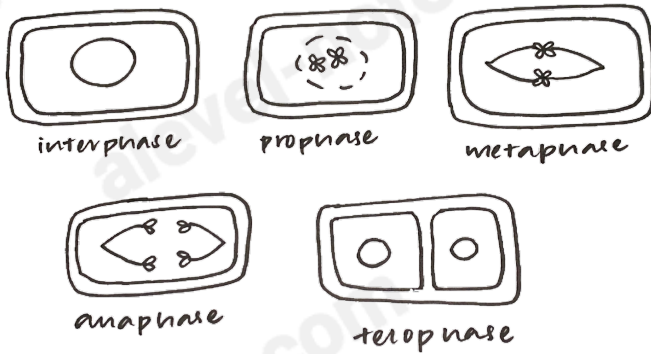Plant cell walls should be shown as double lines with a middle lamella between cells

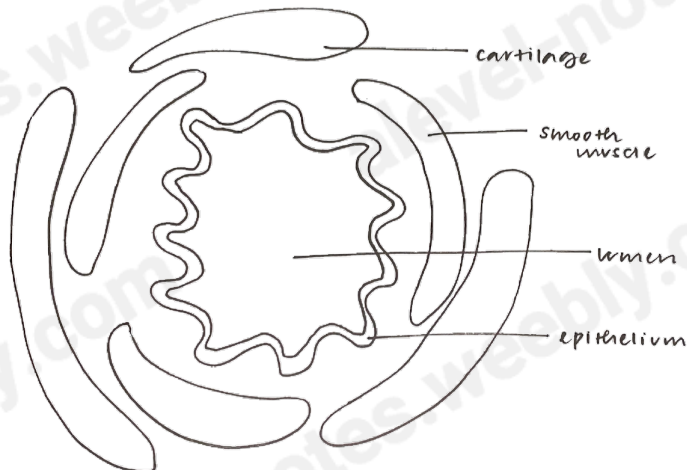Show any details of content of cells; **draw what you see not what you know should be present**.

- use a sharp pencil
- no overlapping lines
- no shaky lines
- never use a ruler
- don't press too hard on the pencil as this makes it difficult to erase in case of mistakes

Disclaimer: *The following examples of plan diagrams are generalised drawings. In the actual question, the drawing that should be drawn may differ, and these are examples for your assistance when attempting such questions.*
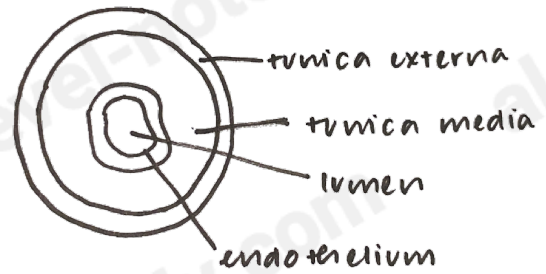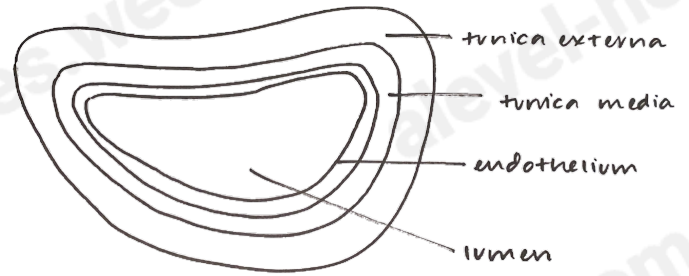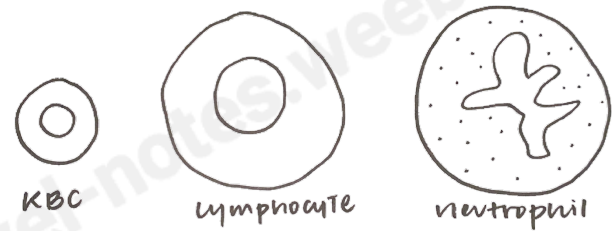
Stages of mitosis

interphase   prophase   metaphase

anaphase   telophase

Bronchus

cartilage

smooth muscle

lumen

epithelium

Artery

tunica externa

tunica media

lumen

endothelium

Vein

tunica externa

tunica media

endothelium

lumen

Blood smear

RBC   lymphocyte   neutrophil

Pseudo-stratified columnar ciliated epithelium cells

cilia

goblet cell

cell membrane

cytoplasm

basement membrane

nucleus

Alveoli

Trachea

- cartilage
- epithelium
- lumen
- smooth muscle

Bronchiole

- epithelium
- lumen
- smooth muscle

Xerophyte leaves
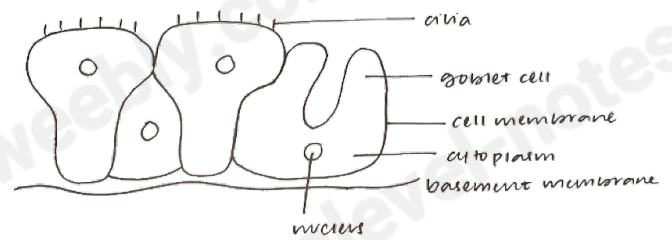
- cuticle
- lower epidermis
- vascular bundle
- upper epidermis
- mesophyll
- parenchyma
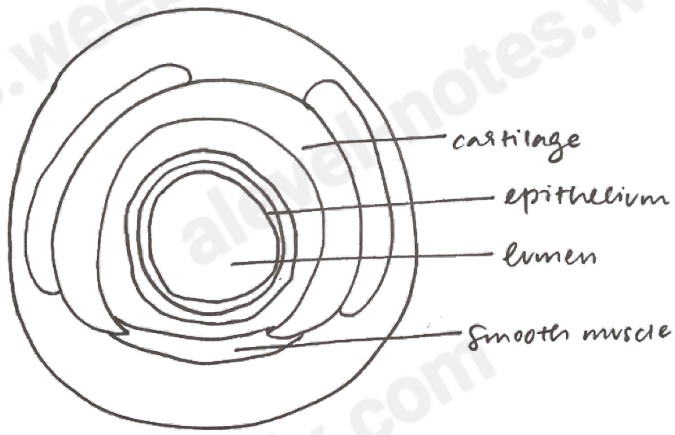
Longitudinal section of a root

- zone of maturation
- zone of elongation
- zone of division
- root cap
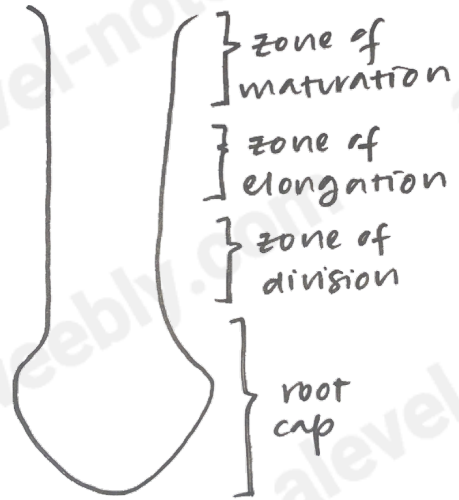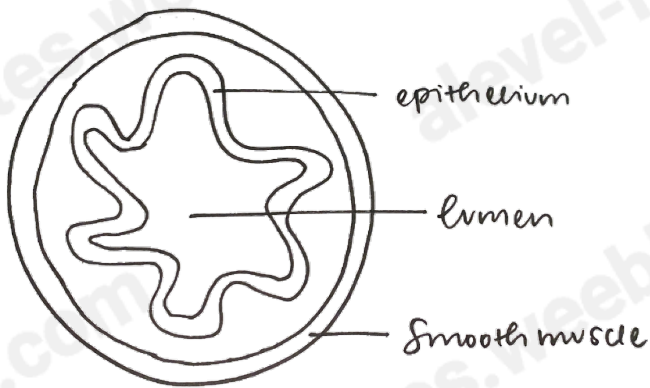
What to avoid when drawing plan diagrams

overlapping lines

uneven cell wall size when drawing plant cells.

② Transverse Sections (plant)

PLANT

STEM

epidermis
cortex
phloem
xylem
pith
cambium

ROOT

dicotyledonous mesophyte (e.g. Ligustrum / Prunus)

epidermis
cortex
xylem
phloem
root hair
pericycle
endodermis

LEAF

cuticle   upper epidermis   palisade mesophyll   spongy mesophyll
collenchyma
xylem
phloem
lower epidermis

maize
monocot

root

root hair
cortex
endodermis
pericycle
xylem
phloem

stem

cuticle
epidermis
xylem
cambium
phloem

cuticle
upper epidermis
mesophyll
xylem   phloem   bundle sheath cells
lower epidermis

rice
monocot

cuticle
epidermis
phloem
xylem