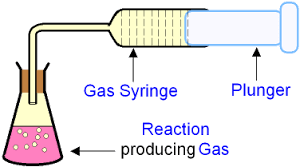


Practical techniques revision booklet for AS



NB 15% of marks on each exam paper will be based on this. Learn it and test yourself!

There are 5 sections:

1. Experimental terms
2. Equipment and techniques
3. Safety
4. Practicals you need to read through again, and look carefully at the graphs
5. Revision quiz

**1) Experimental terms**

a) The reliability of an experiment can be increased by repeating the experiment several times and then finding the mean of the results found.

b) Accurate results are close to the true value. The accuracy of an experiment can be increased by using more sensitive and accurate apparatus/equipment. You could also take results from values closer to a particular value with smaller intervals to find out, for example, exactly the temperature at which an enzyme denatures.

c) The precision on an experiment can be increased by increasing the decimal places used in the experiment (use a smaller unit / division, eg use mm instead of cm).

d) Valid test = ‘fair test’, ie only ONE variable is changed. The validity of an experiment can be ensured by controlling all variables that could affect the experiment other than the independent variable.

e) Biological variability is how the cells, for example, differ, biologically. This might be due to the fact that they have a different age, or are damaged in some way. The cells might also be different due to genetic variability. In order to reduce genetic variability, it is advisable to use clones, as this ensures that all cells have exactly the same genetic information, thereby reducing genetic variability. Also all cells must have the same age approximately, and must be handled carefully so that the minimum number of cells are damaged. If clones are not available, use a large sample size to minimise the effect of genetic variation.

f) A quantitative test is a numerical measurement, and a qualitative test is a description of an observation, eg colour of test solution.

g) Standard curve - it is a graph that displays quantitative results. Multiple samples with known properties are measured and graphed, which then allows the same properties to be determined for unknown samples.

h) Processed results are results that are the final figures from an experiment that have been calculated from your RAW values e.g. an average of your results.

i) Random sampling: carried out to avoid bias

j) Variables: Independent = I change

Dependent = I measure [DIM]

k) A control variable is a factor in an experiment that may influence the results and is therefore controlled to standardise its effect, eg temperature.

l) The purpose of a ‘control’ in an experiment is to show that the independent variable is what actually causes the changes seen in the experiment. It shows what would happen if the variable tested was not changed. So, a control is something you use in an experiment to discount its effect – e.g. growing bacteria in sugar solutions – the control would be to grow them in water so you would know it was the sugar not the water having the effect. Always quote a control.

m) The calibration of an instrument is the process of configuring an instrument to provide a result for a sample within an acceptable range and to maintain the instruments accuracy. Eg when we used colorimeters we set a water sample to read 100 % transmission.

1. **Equipment and techniques**
2. Food tests

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Food | Reagent | Heat? | Positive Result | Negative Result |
| Reducing sugar | Benedict’s | ✓ | yellow/orange/brick red | light blue |
| Non-reducing sugar | 1. Do reducing test | ✓ |  |  |
| 1. 1cm3 HCl, boil | ✓ |  |  |
| 1. Excess sodium bicarb to neutralise | x |  |  |
| 1. Do reducing test | ✓ | yellow/orange/brick red | light blue |
| Starch | Iodine | x | Blue/black | Yellow |
| Protein | NaOH, CuSO4 | x | lilac | Blue |
| Fat | ethanol, H2O shake | x | cloudy white emulsion | stays separate |

b) Indicators

**e.g. Universal Indicator for measuring pH.**

There are also indicator species, which is the use of an organism which is sensitive to a particular abiotic factor. The presence or absence of this species can therefore be used as an indicator of environmental conditions:  
e.g. Stonefly nymphs need high [O2], therefore their presence indicates high [O2] in a stream/river.  
 Lichens – some species are very sensitive to levels of SO2 in the air. Their presence indicates low SO2 concentrations.

1. Water baths

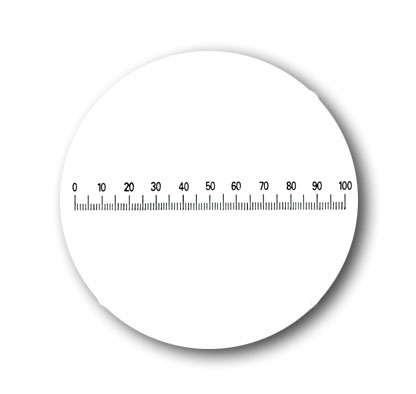
When doing an experiment temperature is often controlled as a higher temperature will initially speed up metabolic reactions (ie enzymes) and later slow them down as denaturation starts.

Water has a higher specific heat capacity than air, so it cools down/warms up more slowly than air. Therefore if temperature needs to be controlled the experiment should be done in a water bath. The temperature of the water bath should be checked using a thermometer, rather than ‘believing’ the dial.

1. Colorimeter

Colorimeter- this is a piece of equipment which can measure either light absorption or light transmission. This is calibrated at the beginning to a known sample eg water. It can tell us how much pigment has been released from a beetroot cell, or how ‘cloudy’ a substance is (e.g. measuring the growth of a bacterial culture). You can also choose which colour of filter, ie light, to shine through the substance in the colorimeter.

1. Graticule

A graticule is a scale put ‘in’ the lens of a microscope which allows you to accurately measure the image size of whatever you are looking at. This lets you find the actual size of the image, after dividing the magnification of the microscope from the image size you have measured, helping you identify what you are looking at. If you know what each small division represents (e.g. each small division = 100µm) then you can simply read off the scale. However, you can also find out what each small division represents when using different objective lenses. You can do this by using a stage micrometer: look down the eyepiece graticule at the small scale, and read off how many small divisions it takes for 100µm, and you can use this scale for that magnification. Graticules are used when measuring the size of a cell, any organelle in a cell, the size of a pollen grain, or the length of cells in an onion. Look it up in Toole and Toole p64

1. Serial Dilution

This can be used e.g. if a reagent needs to be diluted by a known amount or if you want to assay a sample, eg see how many bacteria there are in some river water – you dilute it down, culture the bacteria, count them, then multiply it up again.

1cm3

1cm3

1cm3

10g sample



100cm3 sterile

water

9cm3 water

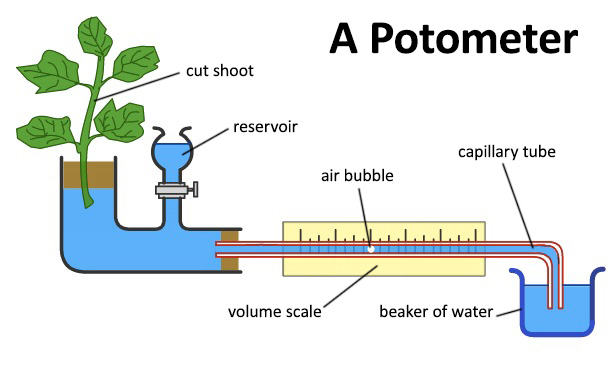
9cm3 water

9cm3 water

* How much will each one be? (e.g. 1/100 etc)

1. Potometer

A potometer is used for measuring the uptake of water from a leafy plant shoot, most often from transpiration. They can be used to measure how changing the atmospheric surroundings (humidity, heat, wind) can effect transpiration.

 To use the potometer you must set it up as shown, and once an air bubble is created allow it to reach the beginning of the volume scale, and then measure how far along the volume scale the bubble moves in a given time, repeating under different conditions to see how these effect transpiration.

1. Light Microscope

These are also referred to as optical microscopes. They use light and several lenses to magnify a sample. Light comes through the condenser lens through the sample, where certain wavelengths are filtered to produce an image. The light is then focused by the objective lens, which can be changed to alter magnification, and then through the eyepiece lens onto the eye so the image is seen. The maximum magnification of a light microscope is usually x1500 and the maximum resolution is 200nm, as the wavelength of light is relatively long, compared to an electron microscope.

A sample is prepared for examination by light microscope as follows:

|  |  |
| --- | --- |
| Action | Reason |
| Prepare / cut *thin* section | *Light* can pass through |
| Place on slide: add a drop of *stain* | Stain will make specific substances *show up* |
| Place coverslip on at 45⁰ | See below |
| Allow coverslip to drop/ press firmly | As the coverslip drops it *expels air bubbles* |

i) Mitotic Index and how you calculate it

Mitotic index is the ratio between the number of cells undergoing mitosis divided by the number of cells.

**http://www.tiem.utk.edu/~gross/bioed/webmodules/mitotindeq.gif**

The number of cells in each cell is proportional to the time spent in each stage.

So, to work out the time spent in each stage:

time spent in each stage = Number of cells in stage x time for one cycle

Total number of cells observed

Obviously, you may need to convert hours to minutes, depending on what the question asks for.

j) Centrifugation

This is a piece of equipment which is used to separate out particles of different size and density by spinning them at high speed. The densest particles will separate out at the lowest speed. They will separate out in this order: first nucleus, then mitochondria, then ribosomes.

k) Graphs

In an exam, you will need to interpret graphs. Usually you won’t have to draw a graph, but you may need to explain which graph you would draw / how you would work out rate of reaction, etc, as you have been asked to do in tests so far. Look at p36-59 in your Maths for Biology textbook.

l) Agar jelly for microbial culture

* Agar is used as a medium for growing bacteria.
* It works since it contains nutrients at optimum levels to promote bacterial growth.
* Also, it creates a flat ‘platform’ for them to grow on, is clear which is useful for observing growth of bacteria, doesn’t easily change the pH of the bacteria, and it won’t be degraded (eaten) by bacteria.

m) Broth for microbial culture

* Broth is a nutrient-infused liquid medium used for growing bacteria.
* Broth is used because most bacterial samples can be grown in this type of medium.
* Although broth contains nutrients which help the growth of bacteria, it is not as good of a culture medium as agar jelly, because the bacteria are mixed up in the broth, rather than growing on the surface (which makes them easy to observe).
* Broth may be in a conical flask / test tube and may have a stopper / cotton wool.
* The advantage of broth is that bacteria can grow in it that have a variety of oxygen needs: anaerobes will grow at the bottom and aerobic bacteria at the top.
* The disadvantage is that you can’t see individual colonies growing (a colony is a little population of bacteria that have arisen from a single bacterium and should therefore be identical)



n) Aseptic technique

- Minimises contamination by unwanted microorganisms

- Everything used must be sterilized

* Petri dish, Agar jelly (or other culture medium) must be sterilized e.g. by heat or radiation
* Inoculating loops and neck of agar bottle used to transfer the desired microorganisms / agar is passed through a Bunsen burner flame
* Lid of the petri dish is only ever lifted slightly, and then 2 pieces of sellotape used to lightly seal to stop microorganisms in the air contaminating it but to allow oxygen in

In schools temperatures are kept below 25 ⁰C to stop harmful pathogens from replicating.

Like in GSCE, you want to grow just one type of microorganism and so must not contaminate it with any other microorganisms. Imagine a diesel engine car, you mustn’t put petrol in lol

(Joke by Tim P…)

o) Working out volume of stock required for a particular concentration

e.g. 2M stock, you want 40ml of 0.3M solution

**Volume needed of stock = Concentration required x volume required**

**Concentration of stock**

Volume needed of stock = 0.3 x 40 = 6ml stock.

2

Then to make it up to 40 ml, add 40-6 = 34 ml water.

p) Calculating the size of an image / magnification

See p14-15 in your Maths book. Practise!

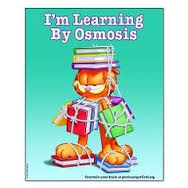
1. **Safety**

State the HAZARD and the CONTROL MEASURE.

eg

|  |  |
| --- | --- |
| HAZARD | CONTROL MEASURE |
| Acid may splash into eye | Wear goggles when handling acid |
| Bag = trip hazard | Put bags under desks |
| Pathogenic bacteria more likely to grow above 25⁰C | Incubate microbial cultures at 25⁰C or lower |

1. **Practicals** you must re-familiarise yourself with:
2. Effect of temp on beetroot
3. Effect of alcohol on Bryophyllum cell membranes
4. Osmosis in potato cylinders: determination of molarity.
5. Plasmolysis in onion cells: determination of water potential
6. Effect of heat on amylase
7. Effect of CuSO4 on catalase
8. Root tip mitosis
9. Effect of antimicrobial substances on microbial growth – antibiotic resistance of *E. coli* determined using discs.
10. **Revision quiz:** These are based mostly on ‘required practicals’. Look up any equations you need.
11. Think through what we did when we determined the molarity of potato cells. How would you increase the reliability of this experiment? (2)
12. How would you find the temperature that the amylase denatured with more accuracy in the experiment *‘Effect of heat on amylase’*? (1)
13. Think through what we did when we investigated the effect of CuS04 on catalase. How would you increase the precision when collecting the results (dependent variable)? (1)
14. A criticism of the experiment we did using liver as a source of catalase, would be that it isn’t truly valid because of biological variability. Explain this statement. (4)
15. How could we have improved the validity? (1)
16. Describe how we made the Benedict’s test i) qualitative ii) quantitative. (2)
17. Describe how we used a standard curve to determine how much starch had been digested (in the ‘*Effect of heat on amylase’* practical) (4)
18. What are processed results? (1)
19. What is the advantage of *random* sampling? (1)
20. When we investigated the *Effect of alcohol on Bryophyllum cell membranes*, what were the independent and dependent variables? (2)
21. What is the purpose of a control? (1)
22. What was the control experiment in the ‘*Effect of CuSO4 on catalase’* experiment? (1)
23. How and why did we calibrate the colorimeter? (2)
24. How did you test for non- reducing sugars? (5)
25. How did you test for protein? (1)
26. How did you test for fat? (1)
27. Why do we carry out experiments in a water bath? (2)
28. How would you use a graticule to measure the diameter of a cell? (1)
29. Why would you need to use a micrometer? (see p64 T&T) (1)
30. Look at the serial dilution section in this booklet. Write down the fraction (concentration) for each dilution. Write down the % concentration for each dilution. (2)
31. When using a potometer, why is it important to cut the shoot freshly when putting it into the potometer? Where should vaseline be used? Why does it not matter that the bubble isn’t at zero each time? Does a potometer measure water uptake or water loss? (6)
32. Calculate how much water was taken up by the shoot in 1 hour, given that: a bubble moved 7mm in 5 minutes. The diameter of the capillary tube was 1 mm. (2)
33. Write down how you would prepare a slide of muscle tissue, and the reason for each step. (3)
34. If 10 cells out of a total of 50 were observed to be in metaphase, and the cycle took 10 hours, how long will the cell be in metaphase for? (2)
35. A solution is used to suspend cells for centrifugation. What 3 properties does this solution need to have and why? (3)
36. Give 3 reasons why agar jelly is a suitable medium for microbial growth. (3)
37. Name an advantage and a disadvantage that broth has compared to agar jelly. (2)
38. Give 3 steps with reasons for aseptic technique. (3)
39. Write down 2 ways that we can use in schools to prevent the growth of pathogenic micro-organisms when incubating. (2)
40. Work out the volume of stock and water required to make up 25 ml of a 0.2M NaCl solution. You are provided with 1M NaCl. (2)
41. Calculate the magnification of an image where the scale bar is given as 20 nm long, actually measures 10mm. (2)
42. What is a hazard and what is a control measure? (2)



Answers

1. Repeat; take mean / average;
2. Repeat experiment more times at smaller intervals around the denature temperature;
3. Use a measuring cylinder with smaller divisions;
4. The experiment is not truly valid because the variable of biological variability has not been controlled. The tissue will vary genetically; and in terms of age; and degree of damage; this may affect the amount of catalase each piece produces;
5. Use pure catalase extract of uniform strength;
6. i) Recorded colour; ii) Recorded mass of precipitate;
7. Take colorimeter (transmission) readings for a range of known starch concs; plot starch conc on x axis and % transmission on y axis; draw best fit line; starch conc can then be determined from reading off graph for any unknown transmission;
8. Any results that have been manipulated / used in calculations and are not ‘raw’;
9. Prevents bias;
10. Independent = alcohol conc; dependent = colour;
11. Control used to ensure any changes seen are due to the independent variable;
12. As others but no CuS04;
13. We used water to set transmission to 100%; eliminates any effect of water;
14. Boil with 1ml HCl; add sodium bicarb to neutralise, test pH; carry out reducing test by adding 1 ml Benedict’s; heat; orange / green is +ve, blue is -ve;
15. Protein test – add NaOH and ^6 drops of CuS04, lilac is +ve, blue is –ve;
16. Fat emulsion test – mix alcohol and shake. +ve result: cloudy emulsion. –ve test = stays separate;
17. Keeps temp constant; important not to change this variable as it affects rates of reactions;
18. To calculate a measurement under the microscope, multiply the number of divisions on the graticule by the actual length of the division; Eg if a graticule division under x400 is 2.5µm,

a cell under x 400 that is 20 divisions long, would be 20 x 2.5 = 50 µm.

1. You can use a micrometer to calibrate the graticule for a particular microscope / objective;
2. 1/10, 1/100, 1/1000, 1/10000; and 10%, 1%, 0.1%, 0.01%;
3. To cut off the last section in case there is an air bubble in the xylem which would prevent water uptake; Vaseline can be used to seal any joints between plant and equipment; Bubble doesn’t need to be at zero because we are just measuring change in bubble position; Measures water uptake; This may then be used in p/s or metabolic reactions or to fill vacuoles; To measure loss, don’t give it water and weigh before / after;
4. Vol of cylinder = π r² h = π 0.5² x 7 = 5.5mm³ multiply by 12 to get volume in 1 hour (because there are 12 lots of 5 minutes in 1 hour); = 66mm³/hr;
5. Cut thin section -so light can pass through; place on slide in drop of water / named stain -to more easily see structures; Place cover slip at edge of drop at 45⁰, allow to drop to exclude air bubbles;
6. Time spent in metaphase = 10/50 x 600; = 120 minutes;
7. Ice cold – to minimise metabolic reaction rate which may destroy cells / organelles / metabolites; Isotonic – to stop water movement resulting in differences in water potential; buffered – to prevent changes in pH which may denature enzymes;
8. Optimum levels of nutrients for bacterial growth; acts as a smooth platform for bacteria to grow on as they won’t break down the agar; clear nature allows colonies easy to observe;
9. Broth advantage: bacteria can grow in which ever O2 condition suits them (less O2 at bottom of test tube); Disadvantage: cannot see individual colonies in broth;
10. Aseptic technique: equipment and culture medium (agar / broth) sterile; neck of agar bottle and inoculating loop flamed to sterilise; lid of petri dish only ever lifted slightly to minimise micro-organisms from air entering;
11. Incubating temp below 25⁰C; only 2 pieces of sellotape used to seal petri dish, to allow in O2.
12. 0.2/1 x 25 = 5 ml stock, 20 ml water
13. Magnification = image size / actual size = 10,000,000 / 20 = x 500,000
14. The hazard is the danger; a control measure is the action taken to reduce risk of harm from the hazard;

The end. Now do some past questions, and use the mark scheme to mark them.