CORE BIOLOGY PRACTICALS

You will need to know these practicals as the exam board may ask you questions based on them. Below is a summary of each one.

Name of practical and independent & dependent variables	Other variables to be controlled	Other equipment	Method and outcome	Possible evaluation issues
Effect of caffeine on Daphnia heart rate Independent: caffeine concentration Dependent: heart rate of Daphnia	• Temperature • Volume of solutions • Stress of Daphnia • Size of Daphnia • Time of acclimatisation	Microscope, counter, cavity slide, dropping pipettes, stop clock, distilled water, test tubes, stop clock	**Method:** Remove 1 Daphnia and place in cavity slide. Remove pond water and replace with distilled water. Leave for 5mins to acclimatise then observe & count heart rate under microscope for 30s, multiply number by 2 to calculate beats/min. Repeat with 2 more Daphnia. Repeat again, this time with small concentration of caffeine solution in place of distilled water. Carry out for 5 concentrations of caffeine = 3 repeats at 3 concentrations. **Outcome**: as caffeine concentration increased, heart rate increased	• Ensuring Daphnia were same size • If left too long under microscope, temp increases (due to lamp) = increased heart rate • Ensuring enough data is collected • Too high concentration of caffeine kills Daphnia • Counting of heart beat can be inaccurate
Measuring the content of Vitamin C in fruit juice Independent: fruit juice Dependent: volume of juice required to decolourise $1cm^3$ of DCPIP	• Temperature • Concentration of DCPIP solution (1%) • Shake each tube same number of times • Same end point colour. i.e. until the blue colour of DCPIP just disappears	1% DCPIP solution, 1% vitamin C solution, range of fruit juices, test tubes/conical flasks, beakers, pipette accurate to $1cm^3$, burette, safety goggles	**Method:** pipette $1cm^3$ blue DCPIP into test tube. Using burette (or accurate pipette) add 1% vitamin C solution drop by drop. Shake tube gently after each drop. Continue until the blue colour just disappears. Record volume of solution needed to decolourise the DCPIP. Repeat further 2 times and calculate mean result. Repeat procedure with different fruit juices. **Calculations:** $1cm^3$ of 1% vitamin C solution contains 10mg Vitamin C, therefore *mass in $1cm^3$* = 10mg x volume of 1% vitamin C to decolourise $1cm^3$ of DCPIP. *Mass in sample* = mass of vitamin C to decolourise $1cm^3$ DCPIP ÷ volume of sample required to decolourise $1cm^3$ DCPIP	• Difficulty in controlling temperature • Amount of shaking (too much adds oxygen which will slightly restore the DCPIP to blue) • End point difficult to judge as needs to be just when blue colour disappears especially in highly coloured juices • Some loss of solution when transferring from one beaker to another • Accuracy of measuring equipment
The effect of temperature on cell membranes Independent: temperature of water Dependent: % transmission of light through resulting solution	• Volume of distilled water • Time left in water • Size of beetroot piece	Raw beetroot, size 4 cork borer, white tile, knife, ruler, beaker, forceps, water baths, boiling tubes, thermometer, colorimeter & cuvettes, stop clock, distilled water, syringe	**Method:** using cork borer and knife, cut pieces of beetroot into 1 cm length cylinders. Place in distilled water overnight to remove any dye released on preparation. Wash and blot dry. Place 8 boiling tubes of distilled water into 8 water baths of different temperature. Once at temperature, add a piece of beetroot to each and leave for 30 mins. Remove beetroot and shake tubes to disperse dye. Set colorimeter to % absorbance on blue/green filter. Calibrate using distilled water in a cuvette first then add 2cm3 of beetroot solution from the first temp to a new cuvette. Place into colorimeter to read % absorbance. Repeat for all other pieces. **Calculations & outcome:** to calculate % transmission = 100-%absorbance. As temperature increased, % transmission slightly increased to a point at which it greatly increased due to membrane molecules gaining more heat energy, vibrating more to a point where the vibrations caused large gaps in the membrane enabling the release of dye also proteins in membrane denatured leaving large pores.	• Some beetroot may have skin on affecting surface area. • Difficulty in maintaining temperature • Accurate reading of the colorimeter • Accurate size of beetroot • From the different parts of the root • Ensuring same amount of time at the different temperatures
The effect of changing enzyme concentration on rate of reaction. Independent: concentration of enzyme dependent: time taken for enzyme to break down substrate	• Temperature • Volume of enzyme • Volume of substrate • Concentration of substrate • pH	Protease e.g.1% trypsin, casein solution, small beakers, thermometer, distilled water, syringes, stopclock, large beaker	**Method:** make up different concentrations of enzyme using distilled water. Ensure different syringes for different chemicals to prevent cross contamination. Set up water bath for temperature to keep constant. Place 1 test tube of $5cm^3$ casein solution into water bath alongside second tube containing $2cm^3$ of 0.2% trypsin. Allow to acclimatise for 3 mins so that at same temperature then add trypsin to casein, start stop clock. Time how long it takes for casein solution to turn transparent. (mark a 'X' on the other side of tube, as soon as seen through solution stop clock). Repeat a further 2 times then repeat for next concentration. **Calculations & outcome:** rate = 1÷time As concentration of enzyme increases, rate of reaction increases until a plateau point where all enzyme has metabolised all substrate immediately.	• Maintaining constant temperature • Accurately making up the different concentrations • Identifying end point consistently • Difficult to see the cross through the solution
		Using catalase in yeast and hydrogen peroxide	**Method:** using first concentration of yeast solution, acclimatise to desired temperature alongside separate tube of hydrogen peroxide. Set up gas syringe and set to 0. Quickly add peroxide to yeast and attach gas syringe. Read off the volume of oxygen gas produced every 10 mins until 3 readings are the same. Repeat 3 times for each concentration of yeast solution. **Calculations & outcome:** rate = initial rate of reaction = gradient at steepest point from graphs of volume against time for each concentration. Outcome as protease above	• Attaching syringe can be slower allowing loss of gas • Inaccurate reading of gas syring • Inaccurate reading of syringes in making up dilutions • Reaction going too quickly to read

Name of practical and independent & dependent variables	Other variables to be controlled	Other equipment	Method and outcome	Possible evaluation issues
Observing Mitosis Chromosomes stained blue using orcein ethanoic stain		Garlic roots, sharp knife, 1M hydrochloric acid, Ethanoic alcohol, Orcein ethanoic stain, ice-cold distilled water, water bath @ 60˚C, 2 watch glasses, test tube, 2 pipettes, microscope slides, forceps, mounted needle, filter paper, microscope with mag x100 & x400	**Method:** place test tube of 2cn3 1M HCl into 60˚C waterbath. Cut off 1-2cm of root tip from garlic root. Put in watch glass containing 2cm^3 of acetic alcohol for at least 12 mins. Remove then place into another watch glass containing 5cm^3 ice cold distilled water. Leave for 4-5 mins, then remove and dry. Place tips into heated HCl for 5mins then repeat process again by placing tips back into acetic alcohol etc. Tips will be very fragile at this point. Transfer 1 tip to microscope slide, cut 4-5mm from growing tip (site of mitosis) and keep the tip. Gently break up (macerate) with mounted needle, add 1 small drop of orcein ethanoic stain and leave for 2 mins. Add coverslip and blot with filter paper. View under microscope and identify the stages of mitosis. **Calculations:** percentage of cells in each stage of mitosis Mitotic index: number of cells containing visible chromosomes ÷ total number of cells in the field of view	• Resolution of microscope • Human error in counting numbers of cells • Enough time in the solutions to enable successful maceration or staining.
Totipotency & Tissue Culture		Seeds of white mustard, agar, distilled water, damp sponge, cling film, McCartney bottles, weighing scales, plastic tray, 250ml beaker, glass rod, scissors, sunny window sill	**Method:** sprinkle seeds on damp sponge and allow to germinate. Use when just starting to unfold their cotyledons (seed leaves). Make up Agar gel and pour 2cm height of gel into McCartney bottles and allow to set. With sharp scissors, cut the tops off just below the shoot apex (including the cotyledons). This is called an explants. Push the stem of the explant into the gel (making sure cotyledons don't touch agar) cover with cling film and place on sunny windowsill. Observe over 10 days. **Outcome:** explant grows roots and leaves continue to grow. You need to be able to explain why they are covered in cling film and why they continue to grow even when covered. Also why they shouldn't be opened again.	• Unwanted pathogens growing in the gel as it is a good source of water and nutrients • Wrong part of the plant cut and inserted into gel.
The strength of plant fibres Independent: source and type of fibre Dependent: mass that can be held	• Length of fibre • Size of each individual mass	Stems of stinging nettles or celery, bucket, gloves, paper towels, clamp stands, slotted masses and holders, white tile, sharp knife	**Method:** plant material should be left to soak in a bucket of water for about a week in order for the fibres to be easily extracted (called retting). Or celery stalks should be left in beaker of coloured water in order for fibres to be easily seen and pulled out. Once fibres removed, connect between 2 clamp stands and gradually add mass in the middle until the fibre snaps. Try with individual fibres from different plants and different ways of combining fibres eg twists and plaits. Can also compare stem to individual fibres. **Outcome:** the more fibres combined together the stronger it is.	• Maintaining length of fibres • Ensuring consistency when twisting or plaiting • Using fibres of the same age (as they get older they become more brittle) • Extracting whole fibres that are useful
Investigating plant mineral deficiencies Independent: minerals present Dependent: physical characteristics of the plant	• Volume of mineral solution • Species of plant • Size of container • Amount of light received	Mexican hat plantlets or geranium leaves, 7 test tubes, test tube holder, different mineral solutions:- each lacking 1 nutrient and 1 containing all, aluminium foil	**Method:** half fill a tube with the 'all nutrients present' solution. Cover the top of the tube with foil or paraffin and push down on covering so that there is a well in the centre. Gently push the geranium stem/roots of Mexican hat plantlet through the hole so it is in solution below. Repeat with solutions lacking in nitrogen or phosphate or potassium or magnesium or calcium or lacking all. Wrap all tubes in aluminium foil and place in tube holder on sunny window sill. Observe regularly. **Outcome:** the 'all nutrients present' plant will look healthy whereas the others will all have some abnormality. Make sure you know what nutrient deficiencies affect plants.	• Ensuring accurate measurement of solutions • No air bubble caught in xylem of geranium • possible microorganism growth in nutrient solution • insufficient time to see an effect.
Effect of garlic and mint on bacterial growth Independent: presence of garlic or mint Dependent: zone of inhibition around disc	• concentration of plant material • lawn of bacteria on petri dish • contamination of petri dish by other microbes • same volume of plant material on each disc	Agar plate seeded with bacteria, plant material e.g. garlic & mint, pestle & mortar, 10cm^3 industrial denatured alcohol, sterile pipette, paper discs, sterile petri dish, sterile forceps, hazard tape, marker pen.	**Method:** make plant extract by crushing 3g of plant material with 10cm^3 industrial denatured alcohol. Shake occasionally for 10 mins. Pipette 0.1cm3 of extract onto sterile paper disc. Allow to dry on sterile petri dish. Meanwhile label agar plates with date and split into 4 sections. 1 for each type of plant extract. Place 1 disc of each extract in each quadrant of the agar plate, close and tape with hazard tape. Leave to incubate over night and observe zone of inhibition. Carry out controls with just distilled water on discs. **Outcome:** the control discs completely covered with bacteria, some plant extracts will create larger zones of inhibition than others, meaning they are more effective at lower concentrations.	• Growth of unwanted microbes on agar plates due to bad aseptic techniques • Not shaking extract enough to ensure enough active ingredient • Inconsistency when adding plant extract to paper discs. • Contaminating controls • Using wrong species of bacteria for lawn