AQA, OCR, Edexcel

A Level

A Level Biology

Required Practical Methods, Questions and Mark Schemes

Name:

M M E

Mathsmadeeasy.co.uk

Total Marks:

A-level Biology required practical No. 1

Student Sheet

Investigation into the effect of a named variable on the rate of an enzyme-controlled reaction

The effect of temperature on the rate of the reaction catalysed by trypsin

Casein is a protein found in milk. Trypsin is an enzyme that digests casein. When trypsin is added to a dilute solution of milk powder, the casein is digested and the solution goes clear.

Method

You are provided with the following:

- 0.5% trypsin solution
- 3% solution of milk powder
- pH7 buffer solution
- a large beaker to use as a water bath
- test tubes
- test tube rack
- stop watch
- marker pen
- pipettes or syringes
- thermometer.

You are required to find the rate of reaction at **five** different temperatures.
You should read these instructions carefully before you start work.

1. Using a marker pen write an 'X' on the glass halfway down one side of each of three test tubes.
2. Add 10 cm^3 of the solution of milk powder to each of these three test tubes.
3. Add 2 cm^3 of trypsin solution to 2 cm^3 of pH 7 buffer in another set of three test tubes.
4. Stand the three test tubes containing the solution of milk powder and the three test tubes containing trypsin and buffer in a water bath at 20 ^{o}C.
5. Leave all six tubes in the water bath for 10 minutes.
6. Add the trypsin and buffer solution from one test tube to the solution of milk powder in another test tube and mix thoroughly.
7. Put the test tube back into the water bath.
8. Repeat steps 6 and 7 using the other test tubes you set up.
9. Time how long it takes for the milk to go clear. Do this by measuring the time taken to first see the 'X' through the solution.
10. Record the time for each of the three experiments.
11. Using the same method, find out how long it takes the trypsin to digest the protein in the solution of milk powder at 30 ^{o}C, 40 ^{o}C, 50 ^{o}C, 60 ^{o}C.
12. Record your data in a suitable table.
13. Process your data and draw a graph of your processed data.

Alternative method using colorimeter:

5. Leave all six tubes in the water bath for 10 minutes. While you are waiting set up a colorimeter. Use the trypsin solution as a blank to calibrate the colorimeter to zero absorbance.
6. Add the trypsin and buffer solution from one test tube to the solution of milk powder in another test tube and mix thoroughly.
7. Put the test tube back into the water bath. Time the reaction for **exactly** 4 minutes. Pour the contents of the tube into a cuvette and measure the absorbance **immediately**.
8. Repeat steps 6 and 7 using the other test tubes you set up.
9. Record the absorbance for each of the three experiments.

Questions on the practical

1. a. What is your independent variable?
   b. What is the range of your independent variable?
   c. When the technicians made up the trypsin and milk powder solutions before the experiment, they decided to make up the solutions in one large batch, rather than weighing out the trypsin and milk powders for each group separately. Would their method result in more or less accurate dilutions – explain your answer.
   c. What is your dependent variable?
   d. What are your control variables and how did you control them?
   e. Why are all the test tubes left in the waterbaths for 10 minutes before the trypsin and milk are added together?

2. Why does the risk assessment for this practical instruct you to wear safety goggles and gloves?

   3. Why did the milk become clearer in this investigation?

   4. There are two possible ways of measuring the dependent variable in this particular method..
   a. One method involves timing how long it takes for a cross to become visible through the milk. What are the limitations of this method?
   b. The other method involves using a colorimeter to measure the absorbance of the mixture after exactly four minutes.
      i) What is a cuvette and how should they be handled carefully?
      ii) Why is it necessary to use a 'blank' in the colorimeter?
      iii) Why does the absorbance of the mixture change after the milk and enzyme solutions are mixed together?
      iv) Why are you told to take a reading of absorbance immediately you add the mixture to the cuvette after 4 minutes?
      v) Why is using a colorimeter likely to give more accurate results than the first method?
   5. Describe the pattern of results that you obtained.
   6. What is the optimum temperature for this trypsin enzyme – what additional readings do you need to take to identify this temperature with greater accuracy?
   7. Explain the pattern of results using A Level enzyme science – make sure you include why the rate of breakdown increases as temperature increases up to the optimum, & why the rate decreases as the temperature increases above the optimum.

ISA Questions

   1. You used a buffer solution in your investigation. What are buffer solutions used for? 1 mark
   2. You left the test tubes in the water bath for 10 minutes before you added the enzyme to the milk powder solution. Explain why.   1 mark
   3. Did you use a water bath at room temperature? Give a reason for your answer   1 mark
   4. Describe and explain what you did to make sure the temperatures of the waterbaths were as reliable as possible. 2 marks
   5. Explain why you set up three experiments at each temperature.   2 marks

6. A student decided to improve this investigation with control experiments. At each temperature she set up a test tube containing a solution of milk powder and buffer. She did not add trypsin. What would these control experiment show?   1 mark
7. (8) It is difficult to decide when the milk solution goes clear. Suggest one better way of determining when the milk solution goes clear.   2 marks

ISA Markscheme

1   Maintain constant pH; 1
2   To equilibrate/reach temperature at which reaction will take place; 1
3   Credit 'yes' only together with valid reason – temperature variation greater in air than in water/room air temperature may fluctuate/water bath keeps test tubes at constant temperature; 1
4   Measure temperature of water bath at beginning and end of reaction period (as a minimum number of times);
To assess the effect of any temperature changes during the reaction/to show that there was no/little variation in temperature;
OR
Measure temperature (several times) and add hot or cold water as appropriate;
To try to keep the temperature close to that required; 2
5   Enables calculation of a more reliable mean;
So that anomalous data can be identified; 2
6   Controls show that the casein digested was due to the action of enzyme / not due to temperature changes; 1
8   Use a colorimeter;
Record time taken to reach constant/set value (of absorbance / transmission);
OR
Set up a standard / solution where complete digestion has occurred for   comparison;
Measure the time taken to reach same colour/transparency as standard; 2

Another Enzyme practical from an ISA - Catalase on piece of card - Questions

Calculate the mean time taken for the card to rise to the surface at each concentration of hydrogen peroxide.   *(1 mark)*

Use the graph paper provided to plot a graph of your processed data. Write a suitable title for your graph.   *(6 marks)*

1 You were told to remove the card from the catalase extract and shake off any surplus liquid (step 3). Explain why it is necessary to shake off surplus liquid.   *(2 marks)*
2 It would have been better if you had kept temperature and pH constant in this investigation.
2 (a) Describe how you could keep temperature constant.   *(2 marks)*

2 (b) Describe how you could keep pH constant.   *(1 mark)*

3 You were told to repeat the measurements at each concentration of hydrogen peroxide (step 7).
How many repeats did you carry out at a concentration of 100% hydrogen peroxide?
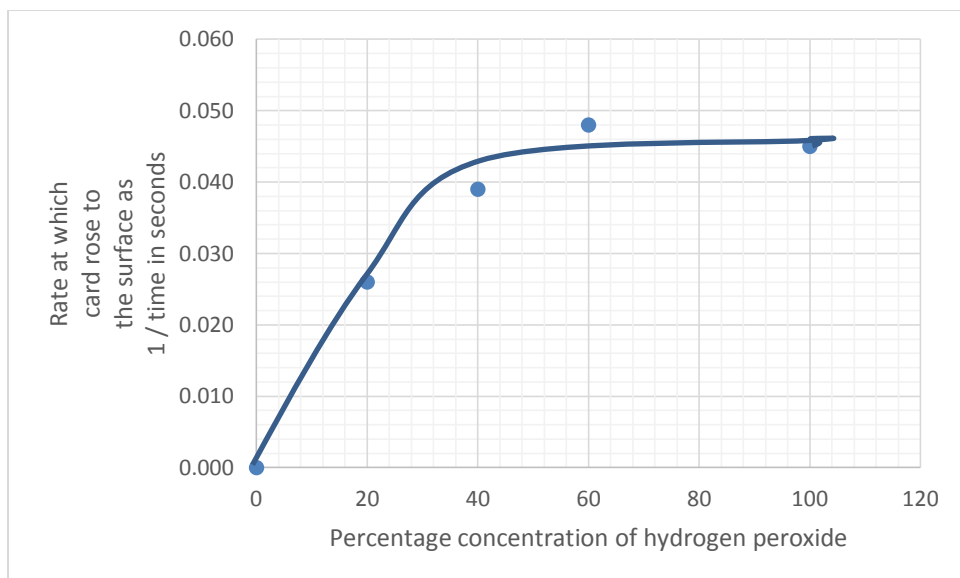Explain why you carried out this number of repeats.
Number of repeats
Explanation   *(1 mark)*

4 In this investigation, you found the time taken for the card to rise to the surface.
Explain why this is a valid measure of the rate of the reaction.   *(3 marks)*

5 Another student carried out the same investigation as you did. She obtained the results shown in the graph.

5 (a) Describe the results that the student obtained. *(2 marks)*

5 (b) What factor limited the rate of reaction between hydrogen peroxide concentrations of 0% and 30%? Give the evidence for your answer.
Factor
Evidence *(2 marks)*

6 A student carried out a similar investigation to yours. He decided to carry out repeats using the same piece of card in the same tube. Each time the card reached the surface, he immediately pushed it back down again. He noticed that the card took longer to return to the surface each time. Explain why the card took longer to return to the surface. *(2 marks)*

Markscheme

1. 1 (Liquid) contains enzyme;
   2 Need to add same amount of enzyme each time / extra enzyme may increase rate of reaction / extra enzyme may affect rate of reaction;

2. A. 1 Water bath;
   2 Monitor temperature with thermometer / use data-logger to record temperature;
   3 Adjust temperature with hot/cold water;
   2 max The basic principles are
   1 using a water bath and 2 describing how the temperature is monitored.
   These points should be marked independently

   2.b. Use buffer;

3. One repeat because readings very similar/concordant;
   OR
   Two or more because first repeat not concordant / variation in values / so that anomalies can be identified / so that mean is more reliable;

4. 1 Bubbles/oxygen make card rise;
   2 (The faster the rate of reaction) the greater the amount of oxygen released;
   3 (The faster the rate of reaction) the more bubbles/oxygen on card;
   4 (The faster the rate of reaction) the quicker the card will rise to the surface;

5. A. 1 Rate increases then remains constant;
   2 At concentration of 42% / at rate of 0.045;

   5b. 1 Concentration of hydrogen peroxide/substrate/percentage
   concentration;
   2 As concentration increases so does the rate (at which card
   rose/of reaction) / positive
   correlation between
   concentration and rate (at which
   card rose/of reaction);

Alternative Investigation – the Effect of pH on the rate of protein digestion by a protease enzyme.

A student set up boiling tubes containing 2cm^3 of protease and 5cm^3 of buffer solution (of differing pHs). A glass capillary tube containing solidified egg white – the protein albumin – was placed in each tube. The length of the egg white in the tubes was measured before being placed in the solutions. The boiling tubes were stoppered and placed in a water bath for 12 hours at 30°C. After this time the capillary tubes were removed and the lengths of the egg whites remaining were measured.

pH	Initial length of egg white in tube/mm	Final length of egg white in tube/mm
4.8	52	48
5.8	50	45
6.6	54	32
7.6	53	9
8.6	48	25
9.0	52	38
9.6	54	47

1. Why is it important to calculate % of egg white digested rather than simply change length of egg white in mm?
2. Suggest a suitable control for this investigation (nb this is not the same as a control variable). Explain why a control is needed.
3. Why are the boiling tubes stoppered before being placed in the water bath?
4. Why are buffer solutions used in this investigation, rather than just adding the correct volume of an acid or alkali to produce the correct pH at the start of the investigation (think about what is produced as the protein is digested)?
5. One student concluded that the optimum pH for this enzyme is 7.6. Do you agree with this conclusion – explain your answer.
6. Use your knowledge of enzyme science to explain the results of the investigation.

A-level Biology required practical No. 2

Student Sheet

Preparation of stained squashes of cells from plant root tips; set up and use of and optical microscope to identify the stages of mitosis in these stained squashes and calculation of a mitotic index

Root tip squash using onion root meristem tissue

You are provided with the following:

- 100 ml beaker
- hydrochloric acid (5 mol dm^{-3})
- microscope slide and cover slip
- toluidene blue stain
- filter paper
- mounted needle
- scalpel
- distilled water
- watch glass
- forceps
- root tip of onion or garlic
- microscope and light source.

You are required to prepare a microscope slide of the meristem tissue from an onion root. You will add a stain to the material which allows you to see the chromosomes. You will look at the slide under the microscope to identify any cells showing stages of mitosis. You will then calculate the mitotic index.

Safety

Hydrochloric acid (5 mol dm^{-3}) is corrosive and should be handled with caution. Eye protection must be worn.

The beaker must be stood on a bench mat. Do not carry the beaker with acid in it.

N.B. Do not leave root tips for investigation lying about on the bench top prior to staining. Cut your root tip immediately before you put it into the acid. This will stop any reactions and hopefully some cells will be in a stage of division.

You should read these instructions carefully before you start work.

Making your slide

1. Stand the beaker on a bench mat before adding approx. 10ml of hydrochloric acid (5 mol dm^{-3})
2. Place about 2 cm of root tip in the acid and leave for 15 minutes.
3. Set up your microscope while you are waiting.
4. Rinse the root tip in distilled water in the watch glass.
5. Cut off the root tip (1mm) and place on a microscope slide.
6. Cover the section with toluidene blue stain and macerate with the mounted needle to separate the cells.
7. Continue to macerate until the tissue is well broken and the cells are stained dark blue.
8. Add a cover slip and with gentle finger pressure 'spread' the material and blot at the same time by using a folded filter paper between finger and slide.
9. Look carefully at all slides for cells undergoing mitosis. Chromosomes should stain dark blue. Repeat for several fields of view.
10. Record your data in a suitable table.
11. Calculate the mitotic index.

Questions

1. Why are you told to observe cells that are found at or just behind the root tip in a region called the meristem?
2. If you take a section of tissue from too far along the root, the cells appear elongated and there are no visible chromosomes. Explain this observation.
3. In one method after the root tip has been cut it is placed in ethanoic alcohol to 'fix' the tissue. What does this mean?
4. In the method above, the root tips are placed in concentrated hydrochloric acid. This breaks down chemicals called pectins in the middle lamella – the layer between cells that cements them together. Explain why this stage is important in your method.
5. Why is the toluidine blue necessary?
6. Why is it necessary to spread the material on the slide? How would the slide appear when viewed under the microscope if you fail to do this?
7. Why is it suggested that look at several fields of view?
8. How do you calculate the mitotic index?
9. Garlic has a diploid number of 16. What does this mean?
10. Some plants have diploid numbers that are much higher than garlic. Suggest why garlic roots may be more suitable to use than roots from these other bulbs.

Related Questions

Q1. The graph shows information about the movement of chromatids in a cell that has just started metaphase of mitosis.
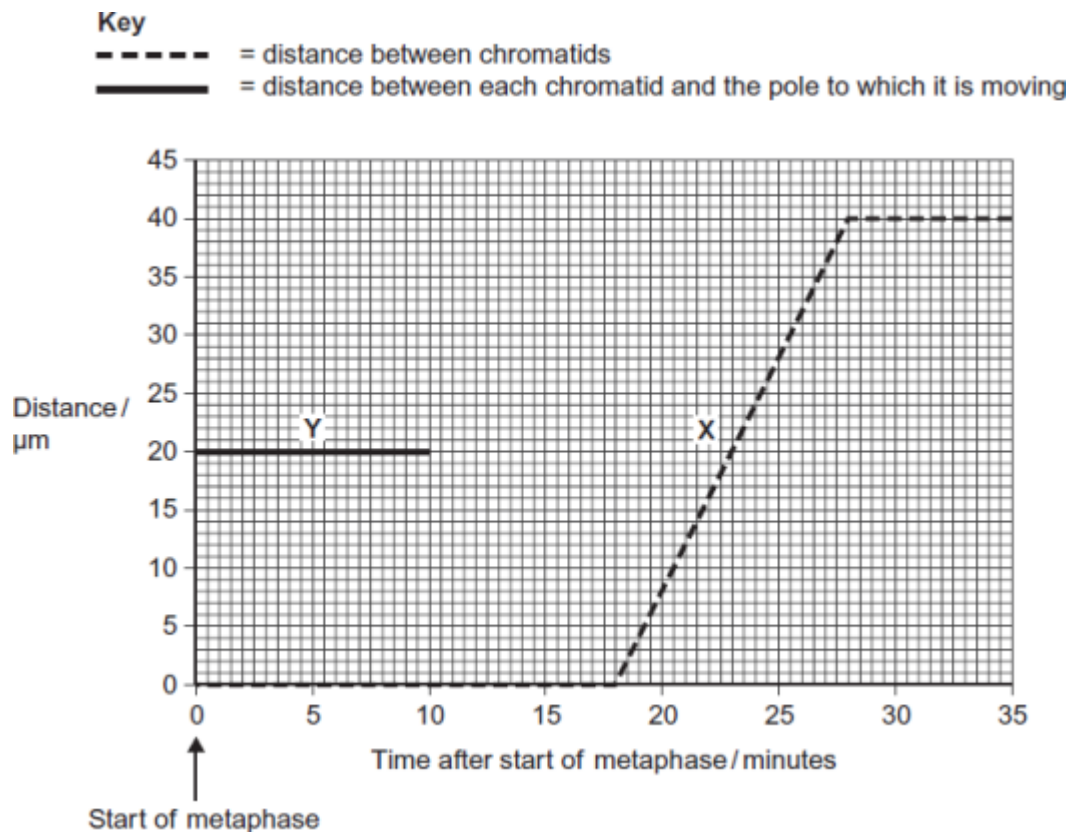
(i) What was the duration of metaphase in this cell?

(ii) Use line **X** to calculate the duration of anaphase in this cell.

(iii) Complete line **Y** on the graph.

(c)   A doctor investigated the number of cells in different stages of the cell cycle in two tissue samples, **C** and **D.** One tissue sample was taken from a cancerous tumour. The other was taken from non-cancerous tissue. The table shows his results.

Stage of the cell cycle	Percentage of cells in each stage of the cell cycle	
	Tissue sample C	Tissue sample D
Interphase	82	45
Prophase	4	16
Metaphase	5	18
Anaphase	5	12
Telophase	4	9

(i)   In tissue sample **C**, one cell cycle took 24 hours. Use the data in the table to calculate the time in which these cells were in interphase during one cell cycle. Show your working.

Time cells in interphase .... hours

(ii)   Explain how the doctor could have recognised which cells were in interphase when looking at the tissue samples.

(iii) Which tissue sample, **C** or **D**, was taken from a cancerous tumour? Use information in the table to explain your answer.

A-level Biology required practical No. 3

Student Sheet

Production of a dilution series of a solute to produce a calibration curve with which to identify the water potential of plant tissue

Determining the water potential of potato tuber cells

You are provided with the following:

- large potato tuber
- potato chip cutter
- 1 mol dm^{-3} sucrose solution
- distilled water
- boiling tube rack
- six boiling tubes,
- marker pen
- thermometer
- 10 cm^3 graduated pipette and pipette filler
- White tile
- scalpel or small kitchen knife
- ruler
- paper towels
- timer
- digital balance
- forceps.

You should read these instructions carefully before you start work.

Preparing the dilution series

1. Label six boiling tubes 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mol dm^{-3} sucrose.
2. Use the 1.0 mol dm^{-3} sucrose solution and water to make up 20 cm^3 of sucrose solution of each of the following concentrations:

   0.2 mol dm^{-3}
   0.4 mol dm^{-3}
   0.6 mol dm^{-3}
   0.8 mol dm^{-3}
   1.0 mol dm^{-3}

Complete **Table 1** to show the volumes of 1.0 mol dm^{-3} sucrose solution and water that you used to make up each concentration.

3. Stand the boiling tubes containing the sucrose solutions in a water bath set at 30 ^{o}C. Use a thermometer to check the temperatures in all tubes reaches 30 ^{o}C.
4. Using the chipper, cut six chips from your potato tuber. Make sure you remove any peel on the potatoes. Use a ruler, scalpel and tile to cut all of the chips to the same length. Blot the potato chips dry with a paper towel, i.e. roll each chip until it no longer wets the paper towel and dab each end until dry. **Do not squeeze the chips**. Put each potato chip onto a clean square of paper towel which you have numbered in the same way as the boiling tubes.
5. Weigh each potato chip. Record these initial masses in a suitable table.
6. At the water bath, set the stop clock to zero. Quickly transfer each potato chip from its square of paper towel to its own boiling tube with the same number.
7. After precisely 20 minutes, remove the chips from the boiling tubes. Blot the chips dry, as before. Then reweigh them. Record these final masses in your table.
8. Calculate the change in mass and then calculate the percentage change in mass.

9. Plot a graph of your processed data and use this to determine the concentration of sucrose which is which has the same water potential as the potato tuber cells.

Questions
1. Complete the table below to show how you made up the different solutions

Concentration of sucrose solution/mol dm^{-3}	0	0.2	0.4	0.6	0.8	1.0
Volume of 1.0 mol dm^{-3} sucrose solution / cm^3	0					20
Volume of water/cm^3	20					0

2. Name the apparatus that you would use to make up the different concentrations accurately & explain how you would use it.
3. Why is important that the potato pieces are all placed in the same volumes of solutions.
4. If the solutions were kept at 20°C rather than 30°C, the potatoes would have to be left in the solutions for longer. Explain why.
5. Why are the potatoes cut using a chipper into equal size pieces?
6. Why are the potato chips dried carefully with a paper towel after being removed from the solutions?
7. Different pieces of potatoes placed in the same solution may change in mass by different amounts. Suggest a reason for this. Would this affect the validity of your results?
8. Why is it necessary to calculate a % change in mass?
9. Explain how you could use your graph to identify the concentration of sucrose that has the same water potential as the potato tuber cells.
10. Explain the change in mass of the potato tissue using your understanding of water potential.
11. How could you identify this concentration more precisely – give details of any further work you would carry out.

Further Questions

Q1. A group of students carried out an investigation to find the water potential of potato tissue.
The students were each given a potato and 50 cm^3 of a 1.0 mol dm^{-3} solution of sucrose.
- They used the 1.0 mol dm^{-3} solution of sucrose to make a series of different concentrations.
- They cut and weighed discs of potato tissue and left them in the sucrose solutions for a set time.
- They then removed the discs of potato tissue and reweighed them.
The table below shows how one student presented his processed results.

Concentration of sucrose solution / mol dm^{-3}	Percentage change in mass of potato tissue
0.15	+4.7
0.20	+4.1
0.25	+3.0
0.30	+1.9
0.35	−0.9
0.40	−3.8

(a)   Explain why the data in the table above are described as **processed** results. (1)

(b)   Describe how you would use a 1.0 mol dm⁻³ solution of sucrose to produce 30 cm³ of a 0.15 mol dm⁻³ solution of sucrose.   (2)

(c)   Explain the change in mass of potato tissue in the 0.40 mol dm⁻³ solution of sucrose. (2)

(d)   Describe how you would use the student's results in the table above to find the water potential of the potato tissue.   (3)

Q2. Strawberries may be dehydrated by removing most of the water they contain. Dehydrated strawberries have many different uses in the food industry.

Food scientists investigated the effect of using osmosis to dehydrate strawberries.

1.   The scientists weighed a sample of strawberries and then cut them into 10 mm thick slices.
2.   They put the strawberry slices into a 1.2 mol dm⁻³ solution of sucrose at a temperature of 25 °C.
3.   After 1 hour, they removed the slices from the sucrose solution and washed them in water. They dried the slices by blotting them and then weighed them.
4.   They also measured the texture of the strawberry slices.
5.   The scientists repeated steps 1 to 4, but they left the strawberry slices in the sucrose solution for different amounts of time.

The results of the investigation are shown in the table.

Length of time in sucrose solution / hours	Percentage loss in mass	Texture / arbitrary units
0	Not applicable	1.2
1	15.96	0.9
2	22.88	0.7
4	32.36	0.7
6	38.78	0.7

(a)   (i)   In this investigation, the scientists cut the strawberries into slices (step 1). Explain the advantage of this. (2)

         (ii)   The scientists blotted the strawberry slices dry before weighing them (step 3). Explain why. (2)

(b)   In the second column of the table, the percentage loss in mass for one of the values has been recorded as not applicable. Explain why. (1)

(c)   Use the table to describe how the length of time in the sucrose solution affected the strawberries. (3)

(d)   You could use the data in the table to predict the time that strawberries should be left in sucrose solution to dehydrate them fully. Describe how you could use a graph to do this. (3)

Q3.   (a)   A plant cell was observed with an optical microscope. Describe how the length of the cell could be estimated. **(2)**

(b)   The water potential of a plant cell is –400 kPa. The cell is put in a solution with a water potential of –650 kPa. Describe and explain what will happen to the cell. **(3)**

(c)   A group of students investigated the effect of sucrose concentration on the change in length of cylinders of tissue cut from a young carrot. They measured the initial lengths of the carrot cylinders, then placed one in each of a number of sucrose solutions. After 18 hours, they removed the carrot cylinders and measured their final lengths. Some of the results are shown in the table.

| Concentration of sucrose / mol dm^{-3}	Percentage decrease in length of carrot cylinder
0.4	4.2
0.5	8.7
0.6	13.0
0.7	16.8
0.8	18.1
0.9	18.1
1.0	18.1

(i)   The carrot cylinders were left for 18 hours in the sucrose solutions. Explain why they were left for a long time. **(1)**

(ii)   Explain how you would use a graph to predict the concentration of sucrose that would result in no change in length of the carrot cylinders. **(2)**

(iii)   Young carrots store sugars in their tissues but, in older carrots, some of this is converted to starch. How would using cylinders of tissue from older carrots affect the results obtained for a sucrose solution of 0.6 mol dm^{-3}? Give a reason for your answer. **(2)**

Q4.   Six cylinders of a standard size were cut from a single large potato. One cylinder was placed in distilled water and the others were placed in sucrose solutions of different concentrations. The length of each cylinder was measured every 5 minutes for the next 50 minutes.

The graph shows the changes in length at each sucrose concentration.
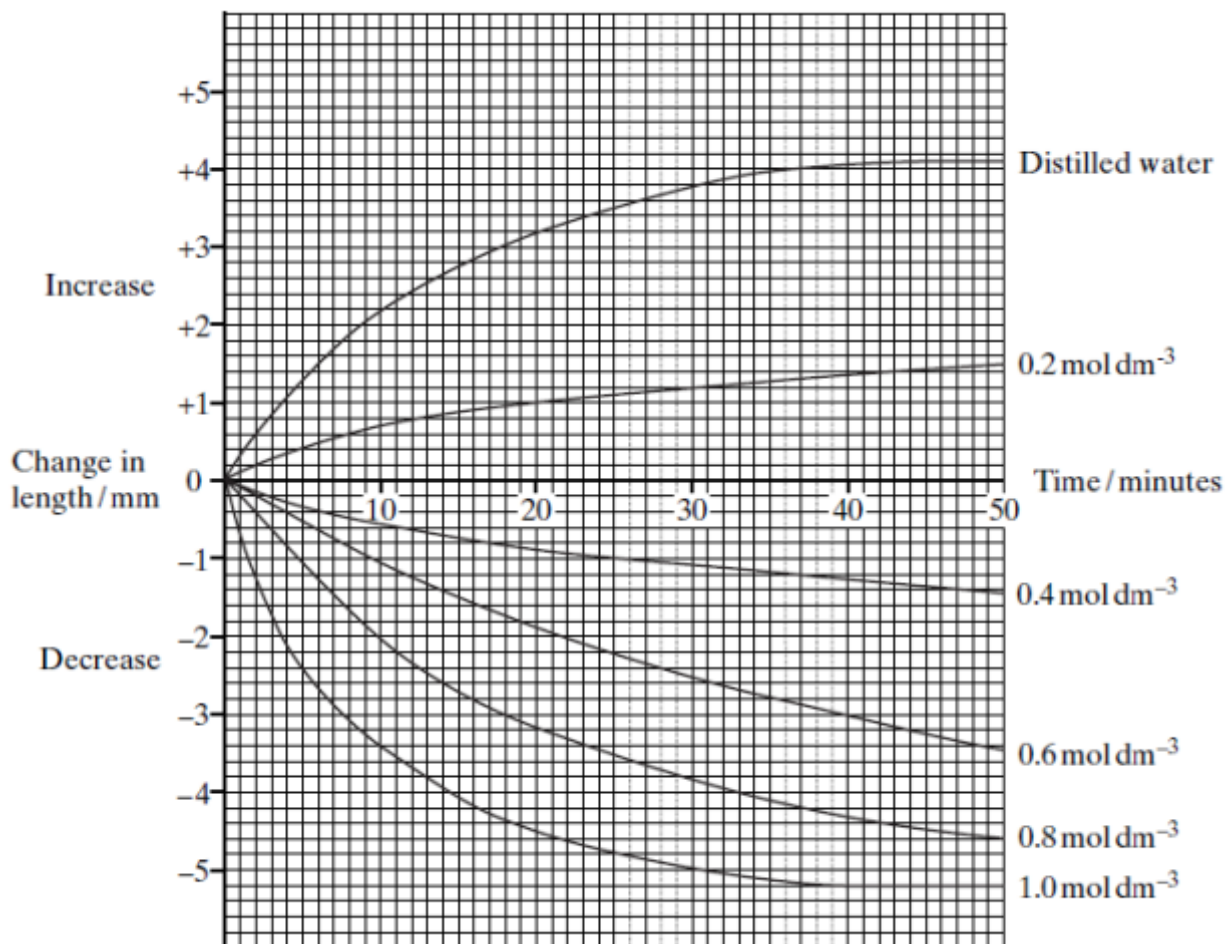
(a)   Explain why   (i)   the potato cylinder in distilled water increased in length;   **(2)**

         (ii)   the potato cylinder in the 1.0 mol dm⁻³ sucrose solution showed no further decrease in length after 40 minutes.   **(2)**

(b)   (i)   Describe the difference in the rate of decrease in length during the first 10 minutes between the cylinder in the 0.4 mol dm⁻³ and the cylinder in the 0.8 mol dm⁻³ solution.   **(1)**

         (ii)   Use your knowledge of water potential to explain this difference.   **(1)**

(c) After 45 minutes the potato cylinder in the 0.8 mol dm⁻³ solution was removed and blue dye added to this solution. Some of this blue-stained solution was drawn into a syringe. A drop was then released, slowly, halfway down a test tube of fresh 0.8 mol dm⁻³ sucrose solution as shown in the diagram. The blue drop quickly moved to the surface of the liquid in the test tube.
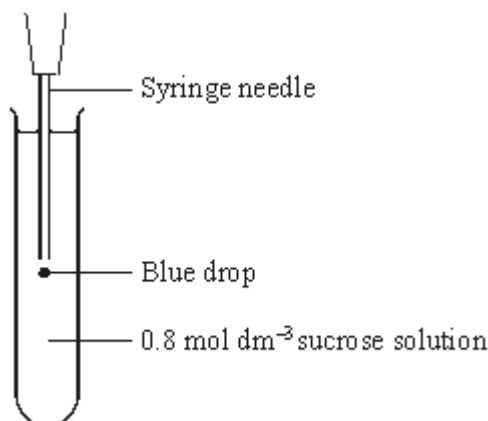
(i) The density of a solution depends on its concentration. The more concentrated the solution the greater its density. Explain why the blue drop had a lower density and therefore moved up. **(2)**

(ii) A sucrose solution of concentration 0.3 mol dm⁻³ has a water potential which is equivalent to that of the potato cells. Describe and explain what would happen to the blue drop from this solution. **(2)**

Markscheme for AQA Osmosis Questions

M1.(a) Calculations made (from raw data) / raw data would have recorded initial and final masses;**1**

(b) Add 4.5 cm³ of (1.0 mol dm⁻³) solution to 25.5 cm³ (distilled) water;

       If incorrect, allow 1 mark for solution to water in a proportion of
       0.15:0.85   **2**

(c) 1. Water potential of solution is less than / more negative than that of potato tissue;   *Allow Ψ as equivalent to water potential*

    2. Tissue loses water by osmosis;   **2**

(d) 1. Plot a graph with concentration on the *x*-axis and percentage change in mass on the *y*-axis;

    2. Find concentration where curve crosses the *x*-axis / where percentage change is zero;

    3. Use (another) resource to find water potential of sucrose concentration (where curve crosses *x*-axis);   **3**

M2.(a) (i) 1. Increases (surface) area / inside surface exposed / more cells exposed / shorter distance for water to move;

        2. Producing water loss;
        Accept better answers, such as diffusion or osmosis relating to
        water loss.   **2**

    (ii) 1. Sucrose solution / water / liquid (on the slices) would add to the mass / weight of the slices;

2.   Would vary; *Ignore references to reliability*   **2**

(b)   This is initial mass / the time is too short for water to have left / the time is too short for osmosis / have not been treated;   **1**

(c)   1.   Percentage loss in mass increases with time;

2.   Texture decreases then levels out;
        Only credit answers that refer to decreasing <u>and</u> levelling out.

3.   (Texture levels out) after first 2 hours;   **3**

(d)   1.   Plot graph of percentage loss in mass against time;

2.   Draw curve (of best fit);

3.   Extrapolate / record when no further change in mass / record when curve flattens out;   **3**

M3.   (a)   Measure diameter of field <u>with</u> ruler; And proportion taken up by the cell; or Measure length with (eyepiece) graticule / eyepiece scale;
Calibrated against stage micrometer / something of known length; *Reject divide apparent length by magnification*   **2**

(b)   Membrane / cytoplasm shrinks / pulls away from cell wall / cell plasmolysed / goes flaccid; Water moves down water potential gradient / to lower / more negative water potential; By osmosis;   **3**

(c)   (i)   Reaches equilibrium / no further / maximum change in length; *Reject osmosis takes time*   **1**

(ii)   Line / curve of best fit; Extrapolate (and read off) / find where it crosses x-axis; **2**

(iii)   Greater decrease / length smaller; More water removed;
Greater difference in water potential / cell with higher / less negative water potential;
Starch is insoluble / has no effect on osmosis   **max 2**

M4.   (a)   (i)   potato more negative water potential / hypertonic; *(accept more concentrated)*

water enters by osmosis causing <u>cells</u> to extend / become turgid; **2**

(ii)   little / no water remaining in potato / fully plasmolysed / all water has moved out;
cell wall prevents further shrinkage / sucrose solution moves in;

<u>or</u>, water potentials are equal / equilibrium / isotonic;
no <u>net</u> <u>movement</u> of water / no further osmosis; **2**

(b)   (i)   faster rate (of decrease) in 0.8 mol dm^{-3}; **1**

(ii)   bigger water potential gradient / greater difference in water potentials (between potato and surrounding solution);   **1**

(c)   (i)   water moved into the solution from the potato;
solution diluted / becomes less concentrated; **2**

(ii)   no net movement of water (in or out);
drops move up / less dense;

<u>or</u>, no net movement of water (in or out); drop would not move / densities the same; **2**

A-level Biology required practical No. 4

Student Sheet

Investigation into the effect of a named variable on the permeability of cell-surface membranes

The effect of alcohol concentration on the leakage of pigment from beetroot cells

Introduction

Beetroot contains high concentrations of betalin. This is a purple pigment found inside the vacuoles of the cells. The pigment cannot move across undamaged plasma membranes. You will investigate the effect of alcohol concentration on the amount of pigment leaking through beetroot plasma membranes.

In **Part 1** of the investigation you will produce a set of standards. In **Part 2** you will use these standards to compare the colour of the solutions obtained when beetroot discs have been soaked in different concentrations of alcohol.

Method

You are provided with:

- stock solution of beetroot extract
- five concentrations of alcohol labelled 100%, 80%, 60%, 40%, 20%
- discs cut from a beetroot and rinsed thoroughly in water
- graduated pipettes or syringes
- test tubes
- bungs to fit some of the test tubes
- thermometer
- large beaker to use as a water bath
- stop watch
- test tube rack
- small beakers
- permanent marker pen
- water.

You should read these instructions carefully before you start work.

Technicians Information
A stock solution of beetroot extract has been prepared in the following way (quantities per candidate). Measure 20 cm3 of the 100 % alcohol into a beaker and add 20 discs of beetroot tissue. Leave the discs for 10 minutes, shaking the beaker every minute. Remove the beetroot discs leaving a concentrated solution of betalain.

Part 1 Making the colour standards

1. Use the extract and water to prepare a series of six test tubes containing 5 cm^3 of different concentrations of extract. The concentrations should be equally spaced and cover a range from pure water (0%) to pure extract (100%). These will be your colour standards.
2. Label these standards 0, 2, 4, 6, 8, 10.
3. Complete **Table 1** to show the concentration of extract in each tube.
4. Complete **Table 1** to show how you made the colour standards in **Part 1** of the investigation.

Table 1

Volume of beetroot extract/cm3						
Volume of water /cm3						
Concentration of extract/%	0					100
Absorbance reading from colorimeter						

Part 2 The Investigation

5. Set up a water bath at 30 ^{o}C.
6. With a second set of test tubes add 2 cm^3 of 100% alcohol to a test tube and put a bung in the tube.
7. Label the tube with the alcohol concentration.
8. Repeat steps 5 and 6 with alcohol concentrations of 80%, 60%, 40% and 20%.
9. Put the tubes of alcohol in the water bath until temperature of the alcohol reaches 30 ^{o}C.
10. Blot 10 discs of beetroot with a paper towel to remove excess water.
11. Gently put two discs of beetroot in each of the five tubes. Replace the bungs as soon as possible after doing so.
12. Leave the tubes in the water bath for five minutes. Shake the tubes gently once every minute. Then remove the tubes from the water bath.
13. Immediately pour each solution into a clean test tube, being careful to label the tubes appropriately. Throw the beetroot discs away.
14. Compare each of your solutions with the colour standards you made in **Part 1**. Note which standard has the same colour as each of your solutions. If the colour of the solution falls between two of the values you can use the intermediate number. For example, if the colour value is between 2 and 4 record the colour value 3.
15. Record your results in a suitable table.

Part 1. Making the colour standards – for use in colorimeter

1. Use the extract and water to prepare a series of six test tubes containing 5 cm3 of different concentrations of extract. The concentrations should be equally spaced and cover a range from pure water (0 %) to pure extract (100 %). These will be your colour standards.
2.   Set up a colorimeter. Use water to calibrate the colorimeter to zero absorbance. Measure the absorbance of each of the standards you have prepared.
3. Complete **Table 1** to show the concentration of extract in each tube and the absorbance.
4. Complete **Table 1** to show how you made the colour standards in **Part 1** of the investigation.

Volume of beetroot extract/cm3						
Volume of water /cm3						
Concentration of extract/%	0					100
Absorbance reading from colorimeter						

5. Plot a graph of concentration of extract against absorbance.

Changes to rest of method - point 13.

13. Measure the absorbance of each of your solutions with the colorimeter. Use the graph to read concentration of extract for each sample. Record your results in a suitable table.

Notes on alternative variables

The above investigation works well if detergent is used instead of alcohol. A clear washing up liquid must be used as coloured liquids will interfere with results. Dilute the detergent 50:50 with water to make the '100%' stock solution'.

Risk assessment

Risk assessment and risk management are the responsibility of the centre.

- If students cut their own discs care should be taken with use of cork borers and scalpels. Small kitchen knives could be used if available.
- Hazcard 40A covers safety issues with ethanol. No naked flames in laboratory, and ensure good ventilation to remove effects of any spillages. Wear eye protection.

Questions
1. You were provided with beetroot discs that were washed thoroughly before the start of the investigation. Explain why it was important to wash the beetroot discs. 1
2. In **Part 2** of this investigation you used five concentrations of alcohol.
   a. Describe a suitable control for your investigation. 1
   b. Explain why this control would be necessary 1
3. Explain why you were instructed to shake the test tubes every minute (step 11).
4. Explain why you were instructed to pour the alcohol *immediately* from the experimental test tube into a clean test tube (step 12).
5. You were given discs taken from fresh beetroot in your investigation.
   Explain why your results would have been different if you had used cooked beetroot. 1
6. You used a water bath in this investigation. Explain why a decrease in temperature of 5 °C would affect the results. 1
7. Two students carried out the same investigation as you did. They worked in the same practical session and followed the same procedure. They worked accurately but found their results were different. Suggest **one** reason for this. 1
8. A student carried out the same investigation as you did using the same concentrations of alcohol. He used his data to plot the following graph.
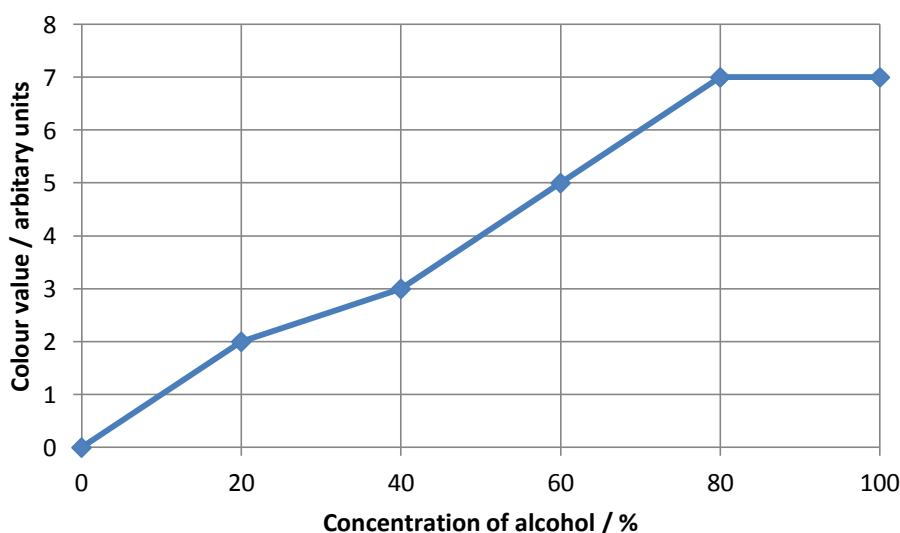
a. The student started drawing his curve from the origin, was he correct to do this? Explain why. 1
   b. Phospholipids dissolve in alcohol. Explain the shape of the curve between alcohol concentrations of 40 % and 80 %. 3
   c. The student suggested that point **A** on the graph was an anomaly. What should he do to check this? 1
   d. After further investigation the student found the point was not an anomaly. Suggest why the curve levels out. 1

9. An alternative way of carrying out the practical is to use detergent (e.g. washing-up liquid) instead of alcohol.
   a. Suggest why detergent would affect the cells in a similar way to ethanol.
   b. Explain why the detergent used needs to be colourless.

Mark Scheme for ISA Questions
1. To wash off any pigment / betalins (on the discs); To show that any pigment released is from the effect of alcohol;
2. a. (Distilled) water and (two) discs of beetroot;
   b. To show that the alcohol was causing the leakage of pigment / to compare it with the effects of the alcohol;
3. a. Increase the contact of all surfaces with the alcohol / prevent discs sticking together / maintain a diffusion gradient for the pigment;
   b. To limit the variation in time discs are in alcohol /make sure all the discs were in contact with alcohol for the same length of time / so no more pigment can diffuse out of the discs;

4. Heating / cooking would damage the (plasma) membranes; Valid explanation of damage e.g. disrupts phospholipid bilayer / denatures proteins; A lot of pigment released during cooking / less pigment present (in tissue);
5. Less kinetic energy; Molecules move slower; Lower rate of diffusion; Membranes less fluid;
   6. Variation in beetroot / variation in judging colour;
7. a. No (no mark) Curve drawn on graph should not extend beyond data collected / no control carried out with water;
   b. Phospholipids are in the membranes; Membrane breaks down faster /more at higher concentration of alcohol / (higher concentration of alcohol) increases the permeability of membranes; More betalins / pigments move out of cells / vacuoles; (Allows) faster rate of diffusion of pigment;
8. a. Repeat experiment at 100 % alcohol concentration; Check to see if results are concordant / similar;
   b. Concentration of pigment inside and outside of the tissue reached equilibrium / an equal concentration of pigment inside and outside the beetroot / pigment diffusing in and out at the same rate; Maximum membrane damage;

A-level Biology required practical No. 5

Student Sheet

Dissection of animal or plant gas exchange or mass transport system or of organ within such a system

Heart Dissection

You are provided with the following:

- a sheep's heart
- dissecting tray and board
- dissecting instruments
- labels and pins.

You should read these instructions carefully before you start work.

1. Before you cut the heart examine its external features.

- Identify the coronary arteries
- Run water into the top of the heart and see if you can see the valves in the aorta and pulmonary arteries close.
- Squeeze the heart gently and these valves should open and the water will come out.

2. Cut down each side of the heart to open up the left atrium and left ventricle and the right atrium and right ventricle.

- Look for the tendinous cords holding the atrio-ventricular valves, and lift the weight of the heart by holding one of these cords over a dissecting needle.
- Look how thin the atrio-ventricular valves are.
- Examine the thickness of the walls of the ventricles.
- Which side is thicker, and why?
- Look at the walls of the atria, they are much thinner, can you think why?
- Push the handle of the dissecting needle up behind the atrio-ventricular valves. You should notice that the aorta and pulmonary arteries cross over.

3. Make some little flags from pins and sticky labels and label the parts of the heart that you can identify. Make sure they are legible and visible as you look down on your dissection.

Ask your tutor to check your labeling and take a photograph so you can include it in your notes.

Packing away:

- Remove all pins and discard labels.
- Place pins and dissecting instruments in the beaker with disinfectant.
- Place the heart in the yellow disposal bag on the trolley.

Use the disinfectant spray to clean the dissecting board and bench, using paper towels to dry them. Dispose of the towels in the yellow disposal bag along with your plastic gloves.

Health and Safety

Lab coats should be worn by all students handling the hearts. Gloves are not necessary, but if used the teacher should ensure that they are removed immediately after the work and disposed of with the paper towels/heart remains.

Ensure cuts to skin are covered with waterproof dressings, and everyone involved in the heart dissection washes their hands thoroughly with bactericidal hand wash after the activity.

- Dissecting instruments are sharp and should be handled with care at all times. Dispose of used instruments into beaker of disinfectant. 1% VirKon or 70% IDA/ethanol (for metal instruments) should be used as the disinfectant. All instruments and surfaces used should be washed thoroughly with detergent solution, and only afterwards disinfected if considered necessary. All organic matter should be removed from instruments and surfaces immediately after the dissection.

A-level Biology required practical No. 6

Student Sheet

Use of aseptic techniques to investigate the effect of antimicrobial substances on microbial growth

Aseptic technique producing bacterial plates and use of mast ring of antibiotics

Method

You are provided with the following:

- plastic sheet to work on.
- McCartney bottle containing *Bacillus megaterium* bacteria
- Bunsen burner
- a beaker containing disinfectant
- disinfectant spray
- a prepared agar plate
- paper towels
- a chinagraph pencil or other marker
- a sterile disposable plastic spreader
- autoclave tape
- ethanol
- sterile 1 cm^3 pipette and filler
- forceps
- Multodisk antibiotic ring.

You should read these instructions carefully before you start work.

1. Spray the bench with the disinfectant and wipe down with paper towels. Place the sterile plastic sheet on the cleaned bench.
2. Place the Bunsen burner on a heat proof mat and light it.
3. Place the agar plate, the McCartney bottle and the spreader next to the Bunsen burner.
4. Write your name, the date and the name of the bacteria on the underside of the agar plate.
5. Wash your hands.
6. Remove a sterile 1 cm^3 pipette from the foil and place the filler onto it.
7. Flame the neck of the McCartney bottle.
8. Dip the pipette into the bottle and remove 0.3 cm^3 of the bacterial culture.
9. Flame the neck of the bottle again and replace the lid.
10. Lift the lid of the agar plate at an angle facing the Bunsen burner with your left hand. With your right hand, squeeze the contents of the pipette onto the surface of the agar.
11. Replace the lid of the agar plate and place the pipette into the beaker of disinfectant.
12. Take the sterile plastic spreader in your right hand. Facing the Bunsen, lift the lid of the agar plate and use the spreader to make sure that the bacteria are evenly spread around the surface of the agar.
13. Replace the lid of the plate place the spreader into the beaker of disinfectant.

The disks you will be using have eight arms, each arm containing a different anti-bacterial agent. These are coded as follows:

Code	Anti-bacterial agent	Code	Anti-bacterial agent
STR	Streptomycin	CHL	Chloramphenicol
SFZ	Sulphafurazole	ERY	Erythromycin
TET	Tetracycline	CXT	Cefoxitin
AMP	Ampicillin	PEN	Penicillin
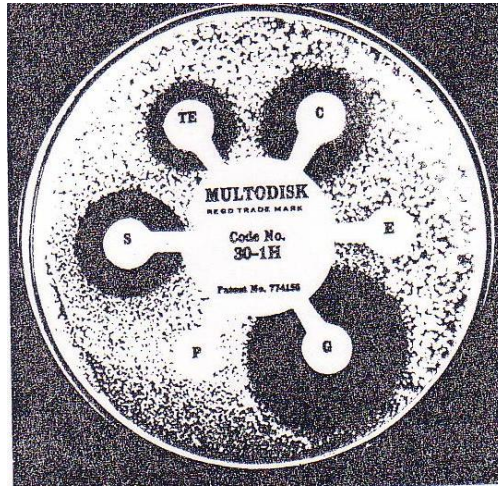
Placing the antibiotic ring

1. Take a pair of forceps. Only handle the Multodisk with the forceps.
2. Remove a Multodisk from its tin and transfer it to the centre of the agar plate.
   Do not hold the disk by one of its arms.
3. Carefully flatten the Multodisk onto the surface of the plate, using the forceps.
   Place the forceps into the beaker of disinfectant.
4. Hold the lid of the plate in place with two pieces of tape.
5. Place your plate upside down in an incubator at 25°C for 48 hours.
6. Now wash your hands.

- **After incubation Caution - plates must not be opened after they have been incubated**

7. Examine your plate and try to identify the colonies which have not been able to grow near the Multodisk arm(s). These are called zone(s) of inhibition. Turning the plate upside down and using a ruler measure the diameter of the zones of inhibition. Calculate the area of the zone of inhibition using the formula
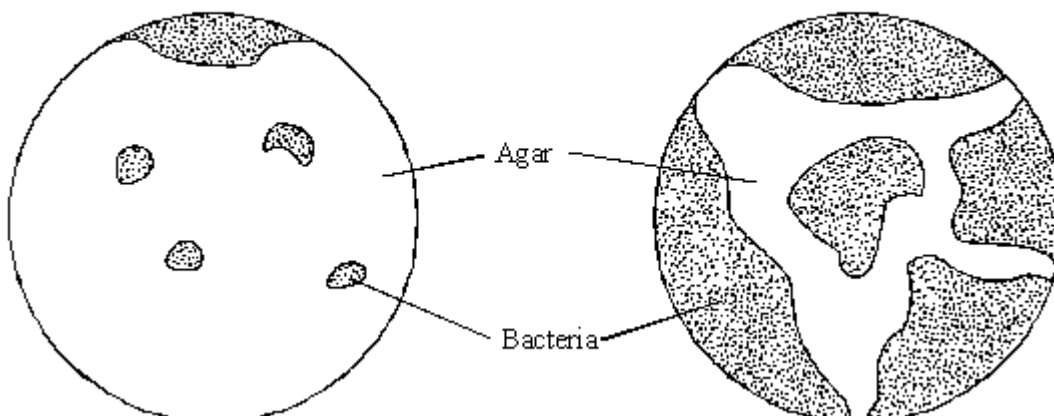
    Area of zone = πr^2 (Use 3.14 as π)

8. Record your results in a suitable table.

Q1.   (a)   In an investigation, two sterile agar plates were inoculated with bacteria from the same culture. Then, using a syringe, 2 cm³ of an antibiotic solution were added to plate **1** and 2 cm³ of sterile water were added to plate **2**. The diagram shows the plates after 24 hours.

(i)   At the start of the investigation, the agar was sterilised. Explain why. **1)**

(ii)   The water was added to plate **2** as a control. Explain why this control was necessary.**1)**

(b)   Explain why some bacteria were able to grow on plate **1**..**(1)**

Q2.   A student was provided with two agar plates. She transferred a culture of bacterium **A** onto one plate and a culture of bacterium **B** to the second plate. She placed paper discs containing antibiotics on the surface of the agar. She then incubated the plates for 24 hours.
The diagram shows the agar plates before and after incubation.
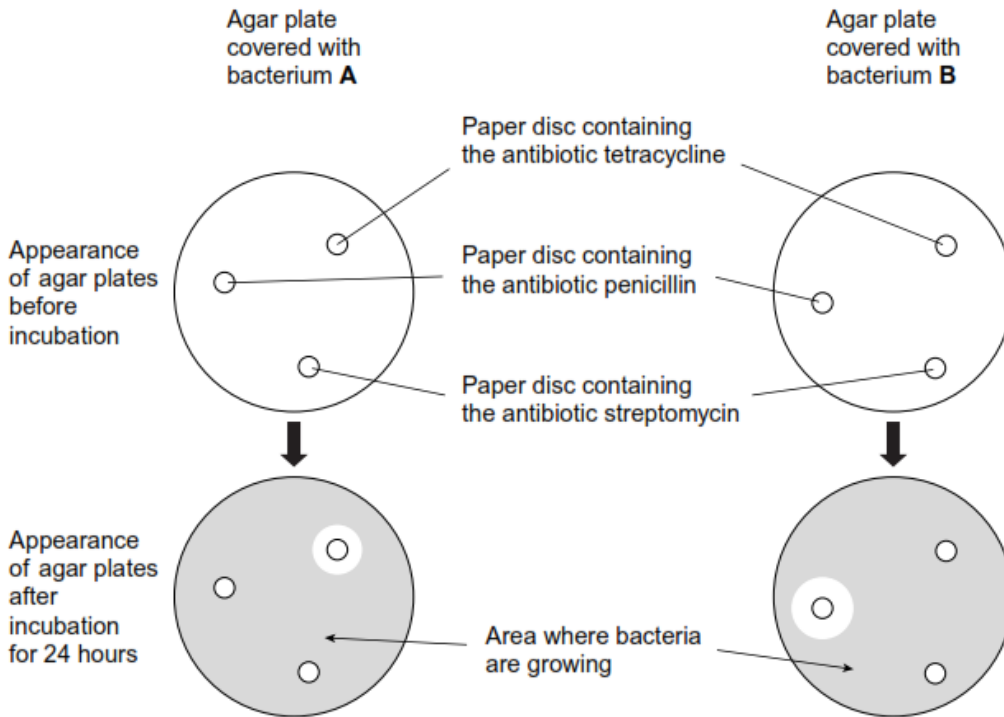
(a)   The student used a pair of forceps to place the paper discs onto the surface of the agar. Explain why she passed the forceps through a Bunsen flame before and after each time she used them. **(2)**

(b)   Explain the appearance of the agar plates after incubation. **(4)**

Q3.   Some strains of the bacterium that causes gonorrhoea are resistant to antibiotics. This makes the disease difficult to treat. One way of testing the effectiveness of antibiotics is to use discs of paper soaked in antibiotic. These are placed in the centre of an agar plate covered by bacteria. A clear zone forms around the disc if the antibiotic is effective.

The table shows some results of an investigation into the effect of four different antibiotics on gonorrhoea bacteria.

Antibiotic	Diameter of clear zone / mm	Minimum diameter of clear zone if antibiotic is effective / mm
A	47	52
B	30	28
C	22	40
D	33	34

(a)   Give **two** reasons why it would be important to use sterile techniques during this investigation. **(2)**

(b)   (i)   The antibiotic reached the bacteria by diffusion. Suggest why an effective antibiotic may produce only a small clear zone.   **(1)**

(ii)   Which antibiotic used in the investigation would be most useful for treating gonorrhoea? Explain your answer.   Antibiotic   Explanation   **(2)**

Q4.   An agar plate was flooded with a culture of a species of bacterium usually found in the mouth. Four sterile paper discs, **A**, **B**, **C** and **D**, each containing a different brand of mouthwash, were then placed on the agar plate. The drawing shows the appearance of the plate after it had been incubated at 37°C for three days.

Bacterial lawn
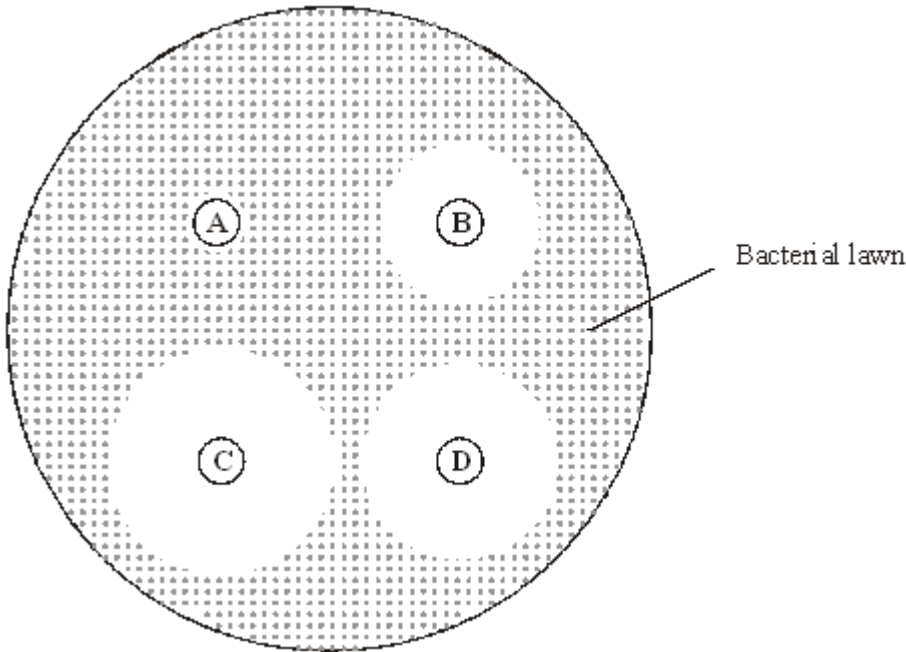
(a)   Describe the aseptic techniques that would be used when flooding the agar plate with bacteria.   **(3)**

(b)   The effectiveness of a mouthwash can be measured by calculating the total area of a paper disc and the clear zone around it. The area of a circle is given by πr^2, where r is the radius of the circle. Calculate how many times more effective mouthwash **C** is than mouthwash **B**. Show your working.

Mouthwash **C** is .... times more effective than mouthwash **B**. **(2)**

S   (c)   Several factors affect the rate at which the antiseptic in the mouthwash from each paper disc diffuses through the agar. Describe the effect of **three** named factors on this rate.
                                                                                          (3)

Q5. In a hospital laboratory, a sterile Petri dish of nutrient agar was inoculated with bacteria from a patient with a throat infection. Four discs, each of which had been soaked in a different antibiotic, were placed on top of the bacteria. The dish was incubated at 37 °C. **Figure 1** shows the appearance of the dish after incubation.
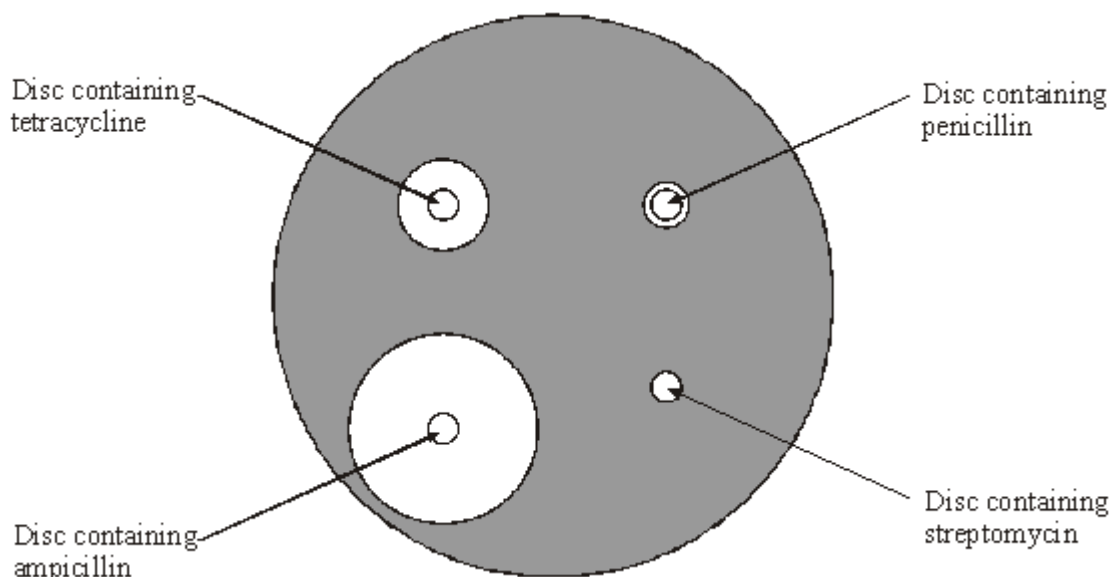

Figure 1

(a) Explain why there are clear zones around some of the discs containing antibiotic. **(2)**

(b) It was suggested that ampicillin might be the best antibiotic to treat the patient's throat infection. Give the evidence from the laboratory test to support this suggestion. **(1)**

(c) Tetracycline binds to bacterial ribosomes. Tetracycline prevents bacterial growth by preventing protein synthesis. Give **two** other ways in which antibiotics can prevent bacterial growth. **(2)**

Markscheme

M1. (a) (i) to ensure that no unwanted bacteria will be present; **1**

(ii) to check that bacteria cells do not die anyway / to show water / solvent has no effect on growth; **1**

(b) some bacteria are resistant / some areas of dish have no antibiotic / antibiotic not spread evenly; **1** **[3]**

M2. (a) To sterilise/kill bacteria;
So that only one kind of bacteria present on agar plate/to prevent contamination (by bacteria); **2**

(b) Clear zone / inhibition zone is where bacteria have not grown/been inhibited/killed;Antibiotic diffuses out of paper disc/into agar;Bacterium **A** inhibited/killed by tetracycline/tetracycline has little effect on bacterium **B**;Bacterium **B** inhibited/killed by penicillin/bacterium **A** resistant to penicillin;Both kinds of bacteria resistant to streptomycin;
Q Ignore references to 'immune' **4 max** **[6]**

M3. (a) To prevent contamination of apparatus with other microorganisms / bacteria;
To prevent personal contact with bacteria;
To prevent release of bacteria into air; **max 2**

(b) (i) Diffuses slowly; **1**

        (ii)   B;
                Produces inhibition zone greater than the minimum diameter;   **2** **[5]**

M4.   (a)   sterilisation of equipment (*once*);
              use of pipette / syringe to transfer culture suspension to plate;
              use of spreader / shake ;
              detail regarding lid, e.g. keeping over plate during transfer / spreading; **3 max**

      (b)   2.25 = 2 marks
              (*general principle ($1.5^2 \div 1^2$) gains 1 mark*) **2**

      (c)   increased temperature increases rate;
              increased concentration increases rate;
              increased molecule size decreases rate;
                        (allow increased distance decreases rate) **3 max** **[8]**

M5.   (a)   antibiotic has diffused / spread / moved into agar;
              killed / inhibited bacteria;   **2**

      (b)   largest clear area / inhibition zone / killed the most bacteria;   **1**

      (c)   disrupts cell wall / prevents cell wall synthesis;
              stops DNA replication;   **2**   **[5]**