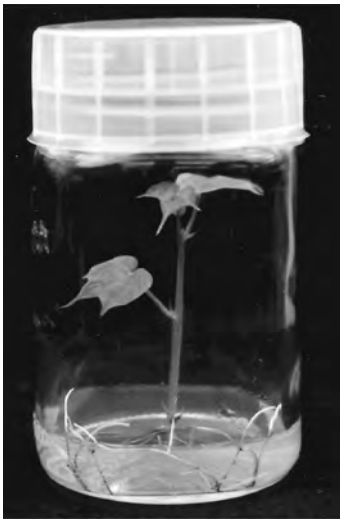
1 Plant tissue culture is a technique that can be used to grow large numbers of genetically identical plants. Scientists have used plant tissue culture techniques to produce large numbers of cotton plants. Cotton plants provide fibres used for clothing.



Cotton plant grown by plant tissue culture

Magnification $\times 1$

(a) Suggest why the sample tube, shown in the photograph, is sealed.

(2)

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(b) Explain why the cotton plants that are produced are genetically identical.

(2)

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(c) The effect of two synthetic plant growth substances, 6-benzylaminopurine (BAP) and 1-naphthalene acetic acid (NAA), on shoot growth was investigated.

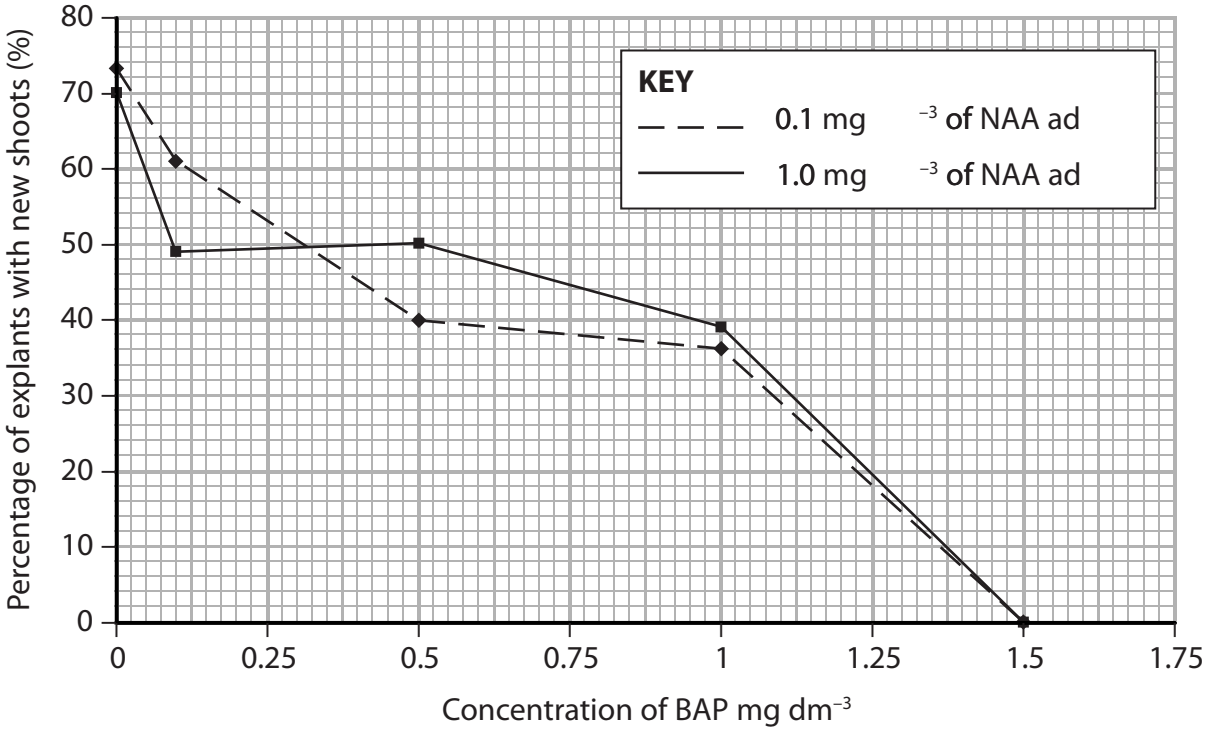
Scientists took samples of tissue, called explants, from cotton plant seedlings that were three days old. These explants were placed on agar in Petri dishes.

The agar contained 0.1 mg dm^{-3} NAA and a range of concentrations of BAP.

The percentages of explants that successfully developed new shoots were recorded.

This was repeated with agar containing 1.0 mg dm^{-3} NAA and the same range of BAP concentrations.

The results are shown in the graph below.



(i) For the explants grown on agar containing 0.1 mg dm^{-3} NAA, describe the effect of increasing BAP concentration on the percentage of explants that developed new shoots.

(3)

(ii) The scientists concluded that NAA had little effect on the percentage of explants that developed new shoots. Discuss the validity of this conclusion.

(3)

(d) Name the property of the cells in the explant tissue which allows them to develop into new shoots.

(1)

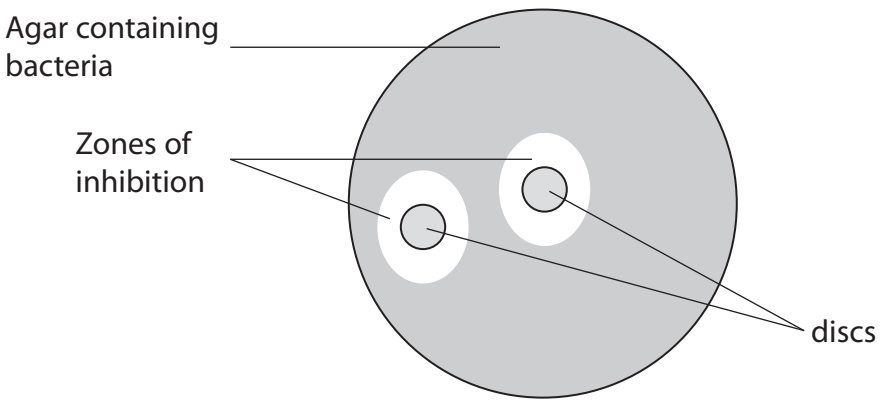
(Total for Question 1 = 11 marks)

2 An investigation was carried out to study the antimicrobial properties of garlic.

A piece of garlic was crushed with 5 cm^3 of sterile water to form a full-strength extract.

Two sterilised paper discs were each soaked in the full-strength extract. Both discs were placed on an agar plate covered in the bacterium *Micrococcus luteus*. This plate was incubated at 25 °C for 24 hours.

After this time, the diameter of the zone of inhibition around each disc was measured and the mean diameter was calculated.



This procedure was repeated using different dilutions of the full-strength extract.

The results of the investigation are shown in the table below.

Concentration of extract as a percentage of the full-strength extract (%)	**Mean diameter of zone of inhibition / mm**
100	18
80	17
60	16
40	12
20	8

(a) Using the information in the table, describe the effect of the concentration of garlic extract on the mean diameter of the zone of inhibition.

(3)

(b) Suggest which concentration of garlic extract has the strongest antimicrobial properties. Give an explanation for your answer.

(3)

(c) Suggest a suitable control for this investigation.

(1)

(d) The discs were sterilised by being placed in alcohol and then left to dry before being soaked in the extract.

Suggest why the discs should be sterilised before being soaked in the extract.

(2)

(e) Suggest how the results in the table might have been different if the discs had not been allowed to dry after being placed in alcohol. Explain your answer.

(2)

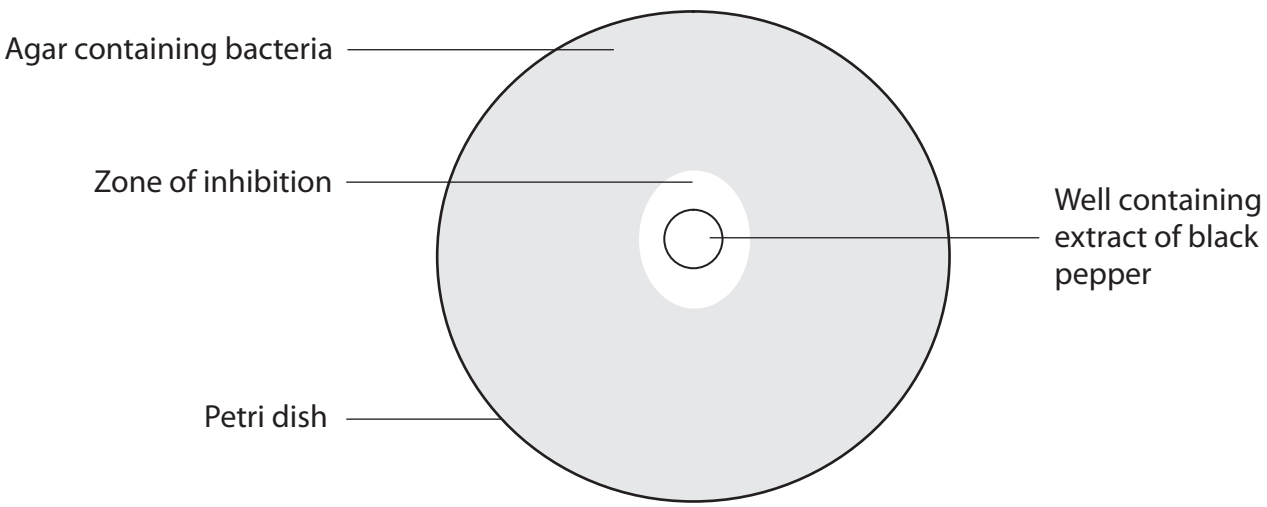
(Total for Question 2 = 11 marks)

3 An investigation was carried out to extract antimicrobial substances from black pepper.

One extraction method used ethanol. The black pepper was crushed and soaked in the ethanol for 24 hours. The crushed pepper was then removed, leaving an ethanol extract.

A Petri dish containing agar and one species of bacterium (B1) had a cylinder of agar removed to produce a well.
The ethanol extract was then placed in the well.

The Petri dish was incubated at 37 °C for 24 hours. After incubation, the diameter of the zone of inhibition around the well was measured. This was repeated using Petri dishes with different species of bacteria (B2, B3, B4 and B5).



The investigation was repeated using an extract prepared with hot water in place of ethanol.

(a) (i) Describe how the bacteria should be added o the Petri dish.

(2)

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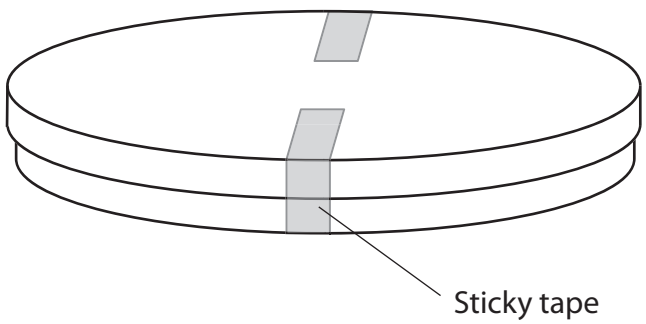
(ii) Before incubation, the lid was secured to the base of the Petri dish as shown in the diagram below.



Explain why the lid was secured in this way.

(2)

(iii) Suggest why an incubation temperature of 37 °C should not be used in a school or college laboratory.

(1)

(b) The results of this investigation are shown in the table below.

Species of bacterium	Mean diameter of zone of inhibition / mm	
	Ethanol extract	Hot water extract
B1	27.4	18.2
B2	26.2	16.8
B3	15.0	29.6
B4	25.0	16.4
B5	15.0	29.8
Mean	21.7	22.2

(i) One student used the data in the table to form the hypothesis that using ethanol was more effective than hot water at extracting antimicrobial substances from crushed black pepper.

Give evidence from the table that supports this hypothesis.

(1)

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(ii) A second student formed the hypothesis that using hot water to extract the antimicrobial substances was more effective than using ethanol.

Give evidence from the table that supports this hypothesis.

(1)

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(c) Another investigation was carried out using cold water to extract the antimicrobial substances. The same method was used but only bacterium species B1 was tested.
The table below shows the mean diameter of the zones of inhibition and the ranges of the data.

Mean diameter of zone of inhibition / mm	
Hot water extract	**Cold water extract**
18.2 ± 1.4	16.4 ± 0.6

(i) A third student stated that some of the results for the hot water extract overlapped with some of the results for the cold water extract.

Suggest what evidence from the table above the student could have used to support this statement.

(2)

(ii) Using the table above, suggest whether the data for the hot or cold water extract were more reliable. Give a reason for your answer.

(2)

(Total for Question 3 = 11 marks)

4 A student investigated the antimicrobial properties of tea tree oil.

She cut three identical discs of blotting paper. She soaked disc 1 in 100% tea tree oil, disc 2 in 50% tea tree oil and 50% vegetable oil and disc 3 in 100% vegetable oil. She then placed all three discs onto a single suitably-prepared Petri dish as shown in diagram 1.

She incubated the Petri dish at 25 °C for 24 hours. The results of the incubation are shown below in diagram 2.

Diagram 1

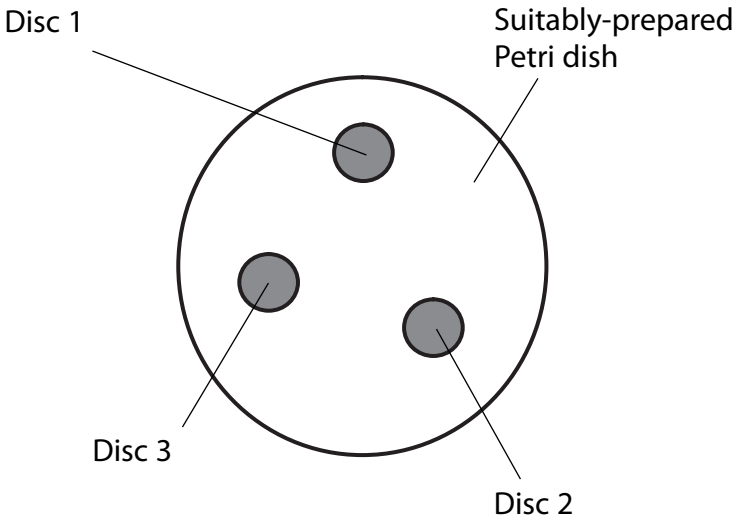
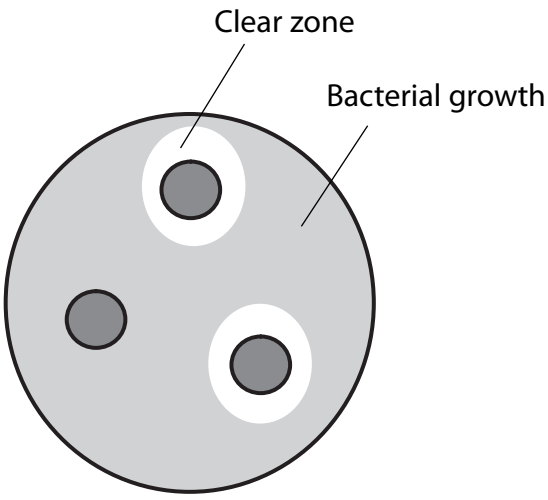


Diagram 2



(a) Suggest what is meant by the phrase **suitably-prepared Petri dish**.

(2)

(b) (i) Describe the function of disc 3.

(1)

(ii) Explain why clear zones are found around disc 1 and disc 2.

(2)

(iii) The clear zone around disc 1 is not a circle. Suggest how you would calculate the mean diameter of this clear zone.

(2)

(c) The mean diameters of the clear zones around disc 1 and disc 2 were found to be the same. This suggests that both strengths of tea tree oil had equally effective antimicrobial properties.

Describe how you would determine the minimum strength of tea tree oil that would be as effective as the 100% tea tree oil.

(3)

(d) Suggest **one** reason why it was good safety practice to incubate the Petri dish at 25 °C rather than at 37 °C.

(2)

(Total for Question 4 = 12 marks)