Surname	Date	Tutor
First name(s)		

BioWelsh

GCE A LEVEL

BW-A2-3.4-W1

TOPIC WORKSHEET 1 – 3.4 MICROBIOLOGY

BIOLOGY – A2 unit 3

Energy, Homeostasis and the Environment

Suggested time: 3 hours 30 minutes

For Tutor's use only

Question	Maximum Mark	Mark Awarded	Question	Maximum Mark	Mark Awarded
1.	11		9.	11	
2.	11		10.	11	
3.	11		11.	11	
4.	10		12.	10	
5.	11		13.	11	
6.	11		14.	10	
7.	10		15.	9	
8.	11		Total	159	

ADDITIONAL MATERIALS

In addition to this worksheet, you will require a calculator and a ruler.

INSTRUCTIONS TO CANDIDATES

Use black ink or black ball-point pen. Do not use gel pen or correction fluid.

You may use a pencil for graphs and diagrams only.

Write your name and the date in the spaces at the top of this page.

Answer **all** questions.

Write your answers in the spaces provided in this booklet. If you are working on screen, write your answers on paper and number each one clearly.

INFORMATION FOR CANDIDATES

The number of marks is given in brackets at the end of each question or part-question. The worksheet is out of **159** marks.

The assessment of the quality of extended response (QER) will take place in question **15**.

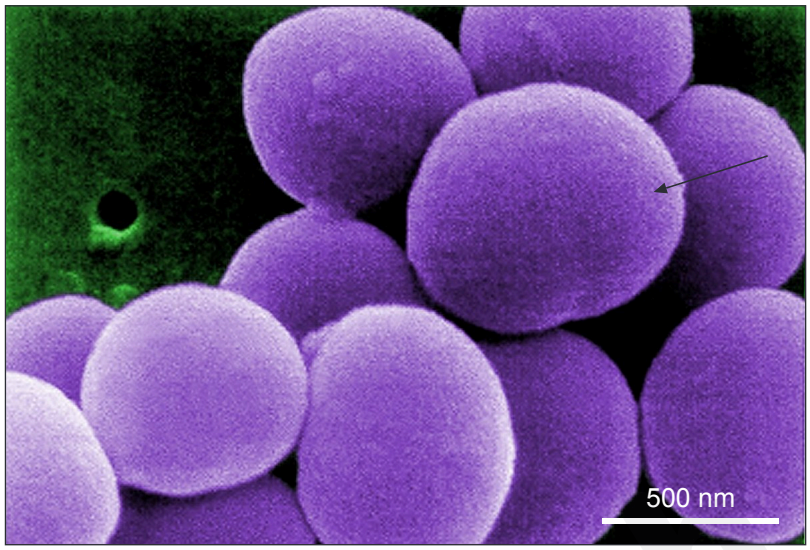
The quality of written communication will affect the awarding of marks.

This worksheet is written by BioWelsh in the style of the WJEC A level Biology examination, following the WJEC specification (topic 3.4). It is not an official WJEC paper.

Tutor only

Answer **all** questions.

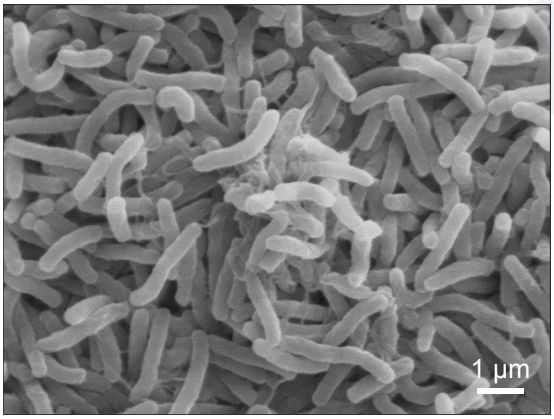
1. **Image 1.1** is a scanning electron micrograph of *Staphylococcus aureus*. The colour was added by computer.



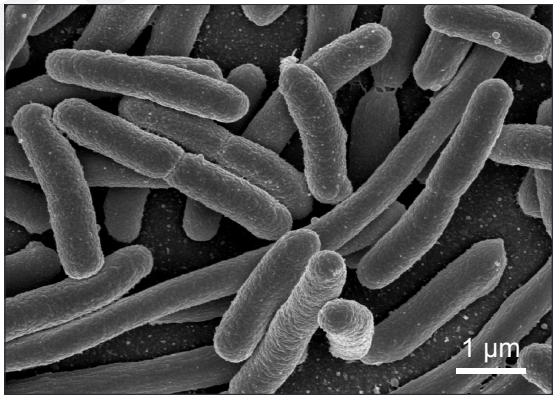
P

Image 1.1

Image 1.2 shows scanning electron micrographs of two other species of bacteria, **B** and **C**.



B



C

Image 1.2

(a) Describe the shape and the arrangement of the cells in **Image 1.1**. [2]

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Tutor only

(b) Name the shape of the cells in **C** and describe how the cells in **B** differ in shape. [2]

C .....

B .....

(c) Use the scale bar to calculate the diameter of cell **P** in **Image 1.1**. [2]

Diameter = ... nm

(d) *S. aureus* reproduces by binary fission. Explain how the arrangement of the cells in **Image 1.1** is produced. [2]

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(e) *E. coli* and *Bacillus subtilis* are both rod shaped. *B. subtilis* is Gram-positive. Suggest how a Gram stain could be used to tell the two species apart. Explain your answer. [2]

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(f) State why the colour in **Image 1.1** must have been added by computer. [1]

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11

Tutor only

2. Bacteria have one of two types of cell wall. **Image 2.1** shows the layers outside the cytoplasm in each type. The drawings are not to scale.

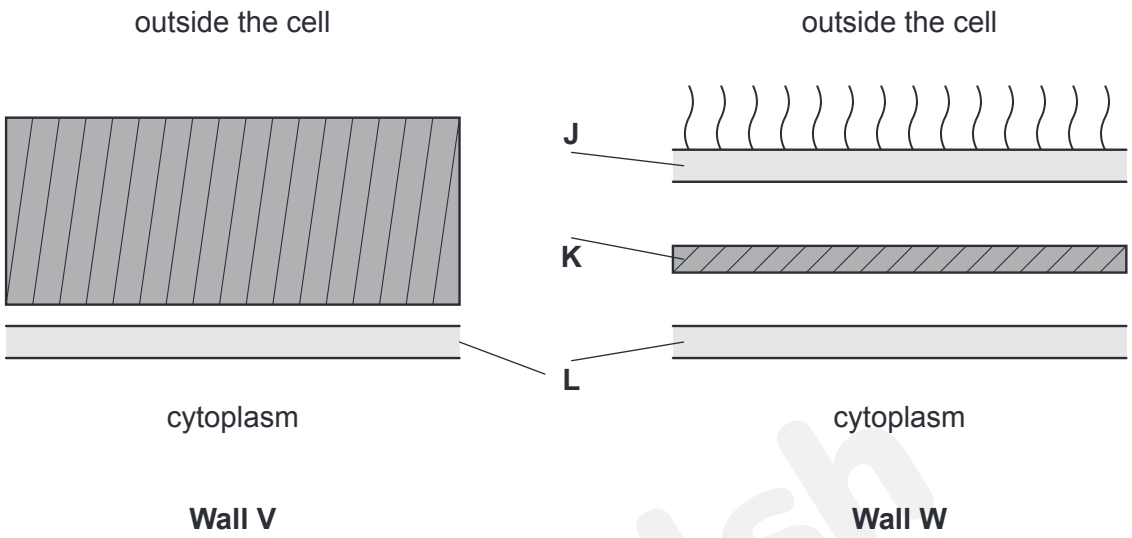


Image 2.1

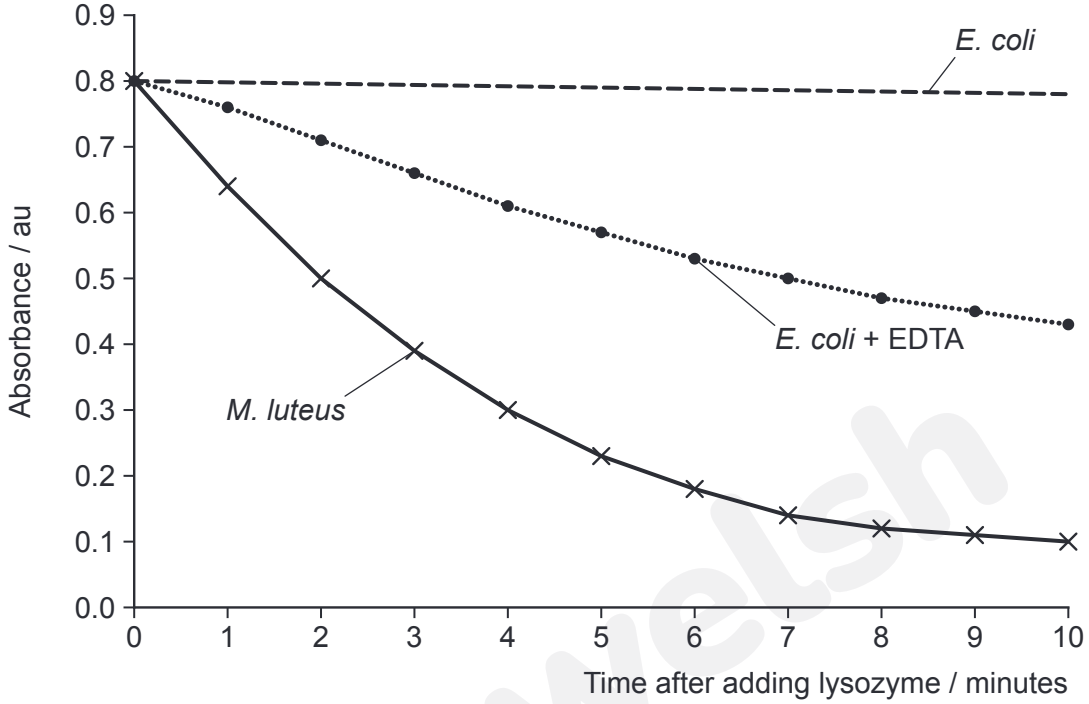
(a) Identify layers **J** and **K**. [2]

J .....

K .....

Tutor only

Lysozyme is an enzyme found in tears and in egg white. It hydrolyses bonds in peptidoglycan. Lysozyme was added to suspensions of two species of bacteria in a buffer solution: *Micrococcus luteus*, which is Gram-positive, and *Escherichia coli*, which is Gram-negative. In a third tube, *E. coli* was first treated with EDTA, a chemical that damages the outer membrane of Gram-negative bacteria. The absorbance of each suspension was measured every minute. The more whole cells in a suspension, the higher its absorbance. **Graph 2.2** shows the results.



Graph 2.2

(b) (i) Explain why the absorbance of the *M. luteus* suspension fell. [2]

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(ii) Calculate the percentage decrease in the absorbance of the *M. luteus* suspension during the first 4 minutes. Give your answer to **1 decimal place**. [2]

Percentage decrease = ... %

Turn over.

Tutor only

(iii) Using **Image 2.1**, explain why lysozyme alone had almost no effect on *E. coli*. [2]

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(iv) Suggest why the absorbance of the *E. coli* suspension treated with EDTA fell more slowly than that of *M. luteus*. [2]

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(c) Suggest a suitable control for this investigation. [1]

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Tutor only

3. A student used the Gram stain to classify two species of bacteria, *Bacillus subtilis* and *Pseudomonas fluorescens*. *B. subtilis* is Gram-positive and *P. fluorescens* is Gram-negative. **Image 3.1** shows the method.

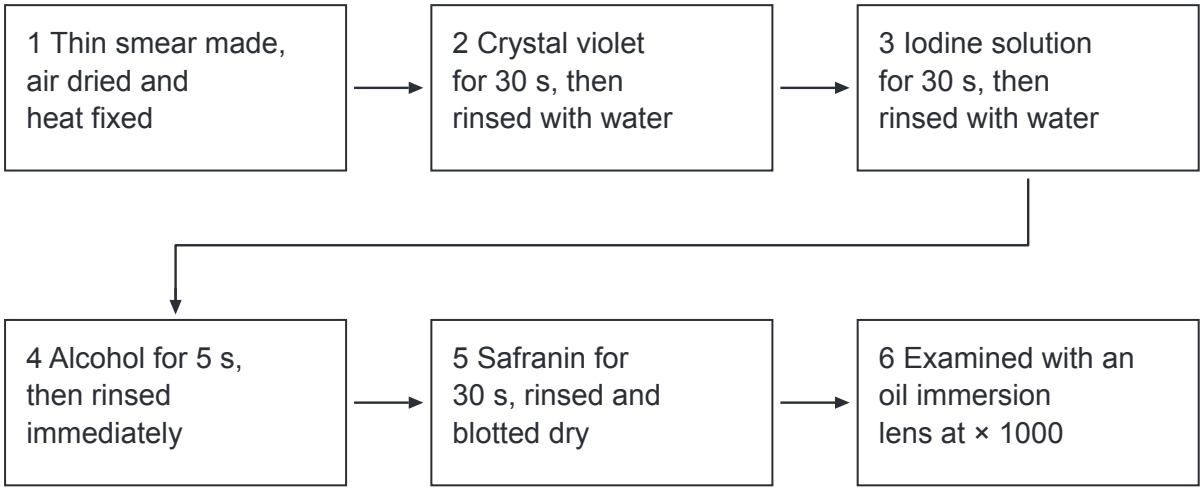

Image 3.1

(a) Explain the purpose of heat fixing the smear in step 1. [2]

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(b) Complete **Table 3.2** to show the colour of each species at the end of steps 2 to 5. [3]

Table 3.2

Step	*B. subtilis*	*P. fluorescens*
2 crystal violet	.....	.....
3 iodine solution	.....	.....
4 alcohol	.....	.....
5 safranin	.....	.....

(c) Explain why *P. fluorescens* lost its colour in step 4 but *B. subtilis* did not. [2]

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Tutor only

(d) Another student left the alcohol on the slide for 2 minutes in step 4. Both species appeared pink. Suggest why. [2]

(e) Suggest why the smear in step 1 must be thin. [1]

(f) Explain why an oil immersion lens at × 1000 is needed to see the shape of bacteria. [1]

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Tutor only

4. **Image 4.1** shows a Gram-stained smear of a mixed culture of two species of bacteria, viewed at × 1000. **X** and **Y** are cells of the two species.

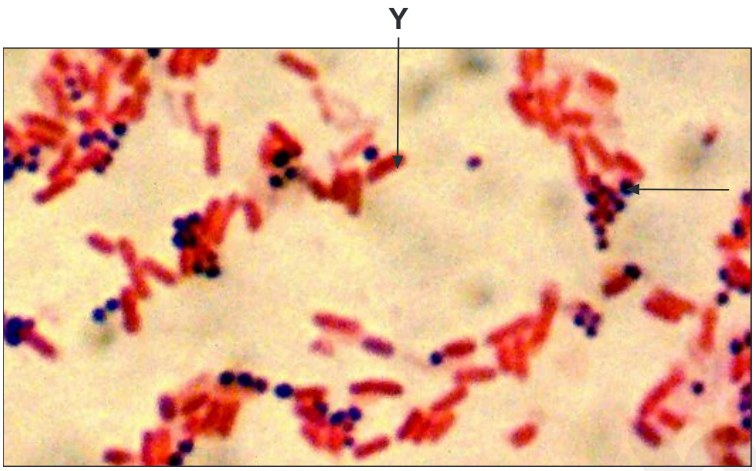

Image 4.1

Image 4.2 shows a Gram-stained smear of *Streptococcus agalactiae*, a bacterium that can cause serious infections in newborn babies.

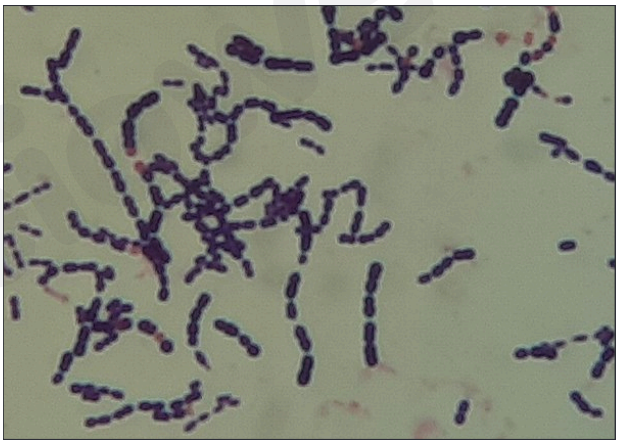

Image 4.2

(a) Complete **Table 4.3**. [3]

Table 4.3

Bacterium	Gram reaction	Shape	Arrangement
X	.....	.....	.....
Y	.....	.....	single cells
S. agalactiae	.....	.....	.....

Tutor only

(b) Explain why cells **X** are purple after Gram staining. [2]

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Penicillin stops cross-links forming in peptidoglycan, so the walls of growing bacteria become weak.

(c) Suggest why penicillin is effective against *S. agalactiae* but much less effective against bacterium **Y**. [2]

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(d) A Gram stain result is ready in about 15 minutes, but growing and identifying bacteria from a sample takes one to two days. Suggest why doctors treating a very ill newborn baby use the Gram stain result. [2]

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(e) Suggest why a Gram stain alone cannot identify the species of bacterium present. [1]

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Tutor only

5. **Table 5.1** shows the ingredients of four culture media, **A** to **D**. Each medium is made up to 1 dm^3 with distilled water and sterilised in an autoclave. Peptone is a digest of protein and yeast extract is a digest of yeast cells.

Table 5.1

Ingredient	Mass in medium A / g	Mass in medium B / g	Mass in medium C / g	Mass in medium D / g
peptone	5	5	0	5
yeast extract	3	3	0	3
glucose	0	0	4	0
ammonium sulfate	0	0	1	0
potassium phosphate	0	0	7	0
sodium chloride	5	5	0	75
agar	0	15	15	15

(a) State which medium is a liquid. Give a reason for your answer. [1]

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(b) Explain which medium is a defined medium. [2]

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(c) Explain why ammonium sulfate is included in medium **C**. [2]

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(d) Gelatine is a protein that sets to a gel. Suggest why agar, rather than gelatine, is used to make culture media solid. [2]

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Tutor only

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(e) Medium **D** is a selective medium. *Staphylococcus aureus* grows well on it but most other bacteria do not. Suggest why. [2]

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(f) Explain why a sample must be spread on a solid medium, rather than grown in a liquid medium, to count the number of living bacteria in it. [2]

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Tutor only

6. A student streaked a sample of a bacterial culture across a nutrient agar plate using a sterile inoculating loop, then incubated the plate. **Image 6.1** shows the plate after incubation.

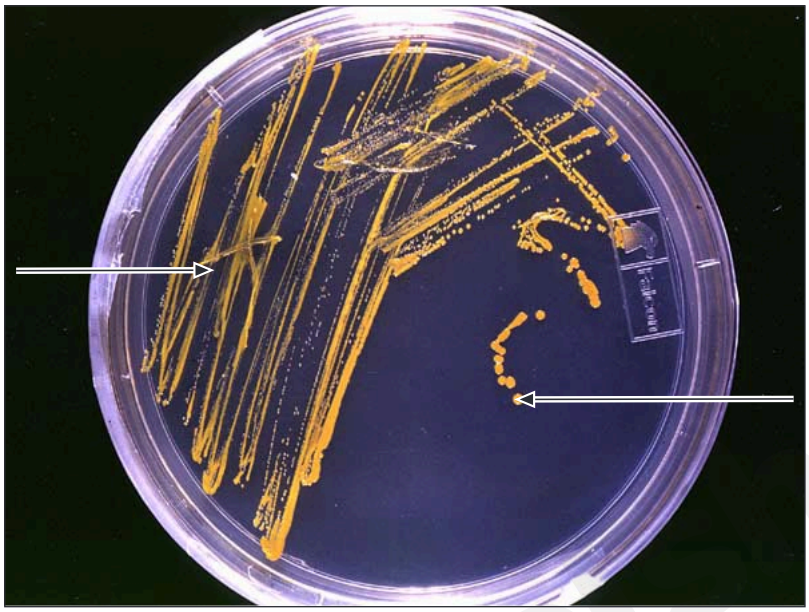

Image 6.1

(a) Explain why the growth at **R** is continuous but separate colonies are seen at **S**. [2]

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(b) State why a colony from area **S** can be used to start a pure culture. [1]

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(c) Describe how the inoculating loop is sterilised, and explain why it must cool before it touches the culture. [2]

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Tutor only

(d) Explain the **two** aims of aseptic technique. [2]

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After inoculation, the lid was held on with two short pieces of tape but not sealed all the way round. The plate was incubated upside down at 25 °C.

(e) Explain the reason for each of these **three** precautions. [3]

Not sealed .....

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Upside down .....

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25 °C .....

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(f) Suggest how the plate should be treated after the colonies have been examined. [1]

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Tutor only

7. Bacteria differ in how they respond to oxygen. Four species of bacteria were each mixed into a tube of melted nutrient agar that had been boiled to remove dissolved oxygen and then cooled. The agar was left to set and the tubes were incubated. Oxygen from the air dissolves at the surface, so its concentration falls with depth. **Image 7.1** shows the colonies that grew in each tube.

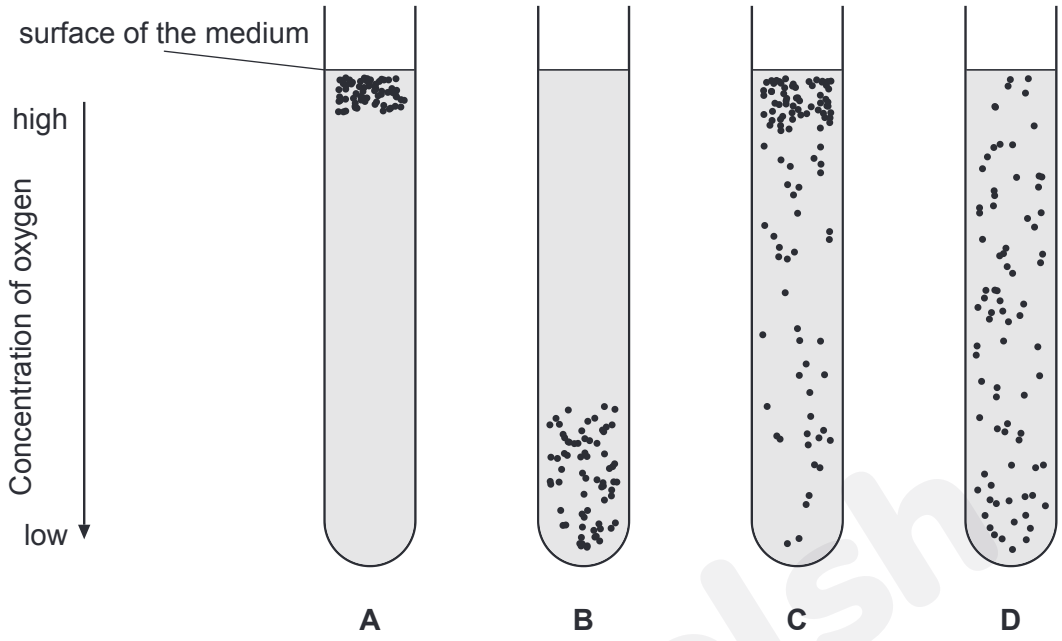

Image 7.1

(a) Identify the tube, **A**, **B**, **C** or **D**, that contains each of the following. [3]

Obligate aerobe .....

Obligate anaerobe .....

Facultative anaerobe .....

(b) Explain the distribution of colonies in tube **C**. [2]

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(c) Explain why the bacteria in tube **A** grow only near the surface. [1]

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Tutor only

(d) The bacterium in tube **D** is a species of *Lactobacillus*. It respires only anaerobically, but oxygen does not harm it. Explain the distribution of its colonies. [2]

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(e) The bacterium in tube **B** is *Clostridium sporogenes*, which is killed by oxygen. Suggest how it could be grown on agar plates. [1]

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(f) *Clostridium botulinum* is related to *C. sporogenes* and makes a deadly toxin. Suggest why it can grow in sealed cans of food that were not heated enough. [1]

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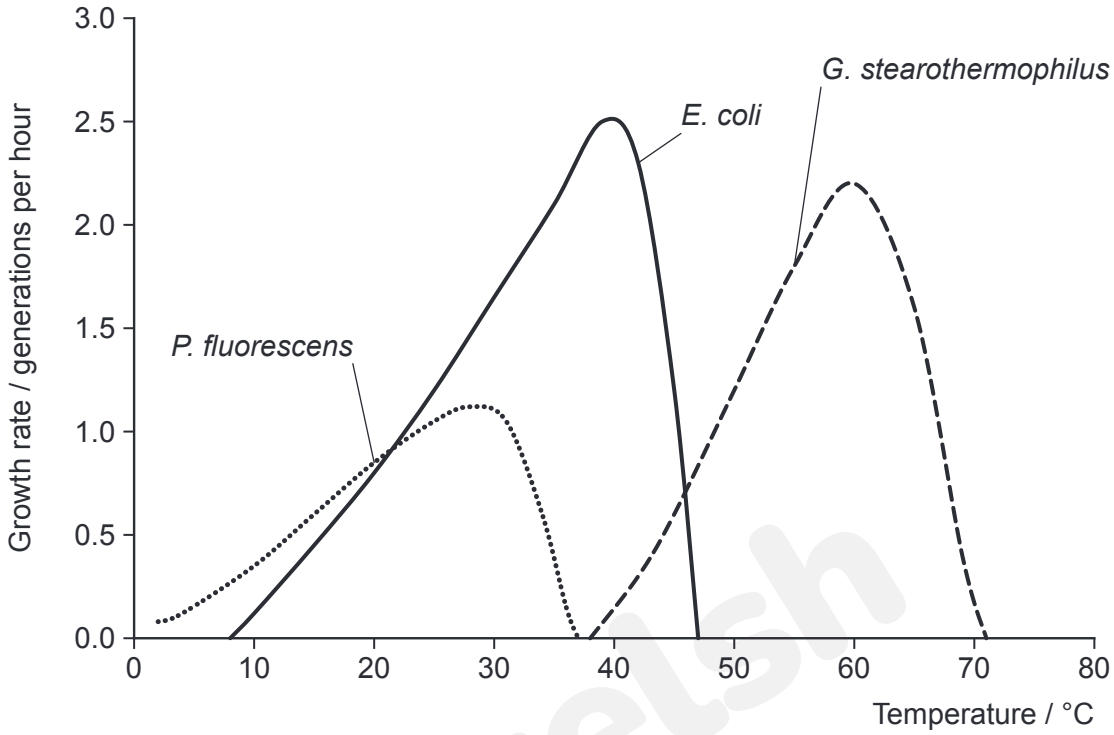
Tutor only

8. **Graph 8.1** shows the growth rate of three species of bacteria at different temperatures. The growth rate is the number of times the population doubles in one hour. *Pseudomonas fluorescens* lives in soil and water and can spoil milk. *Escherichia coli* lives in the human gut. *Geobacillus stearothermophilus* lives in hot springs and compost heaps.



Graph 8.1

(a) State the optimum temperature for the growth of *E. coli*. [1]

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(b) Explain why the growth rate of *E. coli* increases between 10 °C and 35 °C. [2]

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(c) Explain why the growth rate of *E. coli* falls sharply above its optimum temperature. [2]

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Tutor only

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(d) Calculate the time taken for the population of *E. coli* to double at its optimum temperature. Give your answer in minutes. [2]

Doubling time = ... minutes

(e) Milk kept in a refrigerator at 4 °C still goes off after about ten days. Using **Graph 8.1**, suggest why. [2]

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(f) Spores of *G. stearothermophilus* are used to check that an autoclave has sterilised its contents. After the autoclave has run, the spores are incubated at 60 °C. Suggest why this species is used. [2]

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Tutor only

9. A probiotic yoghurt drink contains living *Lactobacillus* bacteria. The label states that each 65 cm^3 bottle contains at least 6.5×10^9 living bacteria. A student carried out a viable count, as shown in **Image 9.1**.

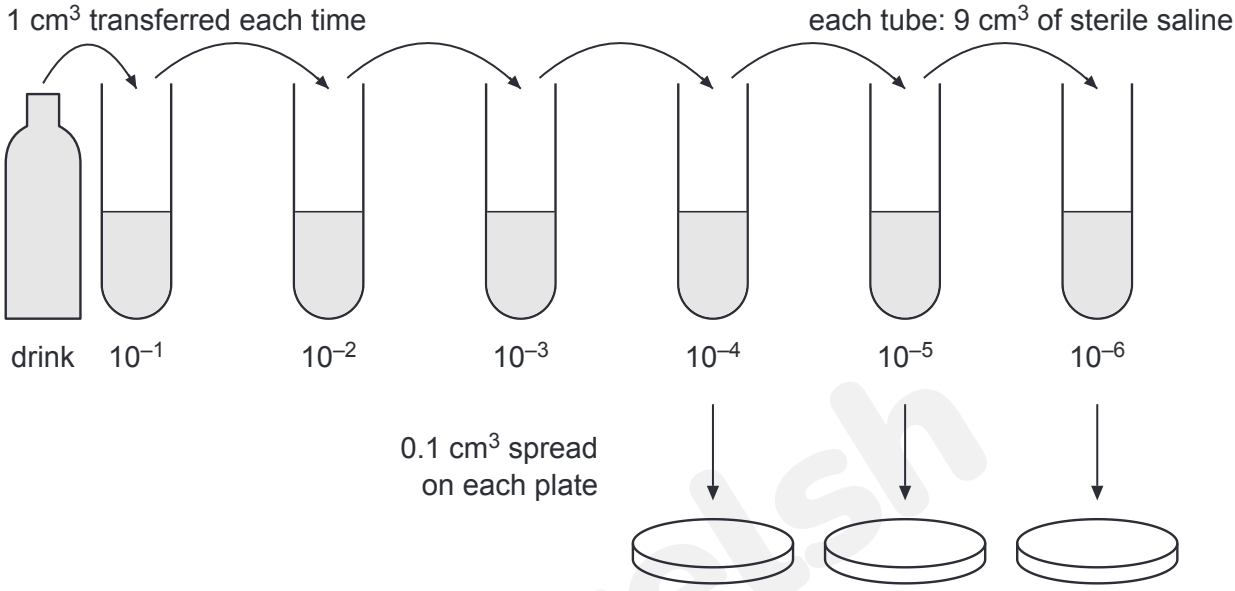

Image 9.1

(a) Explain why the drink had to be diluted before it was spread on the agar. [2]

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The plates were incubated at 37 °C for 72 hours in a container with no oxygen. Two plates were made from each dilution. **Table 9.2** shows the results.

Table 9.2

Dilution plated	Colonies on plate 1	Colonies on plate 2
10^{-4}	too many to count	too many to count
10^{-5}	118	130
10^{-6}	14	9

(b) (i) Explain which dilution should be used to calculate the number of bacteria in the drink. [2]

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Tutor only

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(ii) Calculate the number of living bacteria in 1 cm^3 of the drink. **Give your answer in standard form.** [2]

Number = ... per cm^3

(iii) Use your answer to (ii) to decide whether the result supports the claim on the label. Show your working. [2]

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(c) *Lactobacillus* cells often stay joined in short chains. Suggest how this affects the number calculated in (b)(ii). [2]

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(d) Explain why a new sterile pipette must be used for each transfer in **Image 9.1**. [1]

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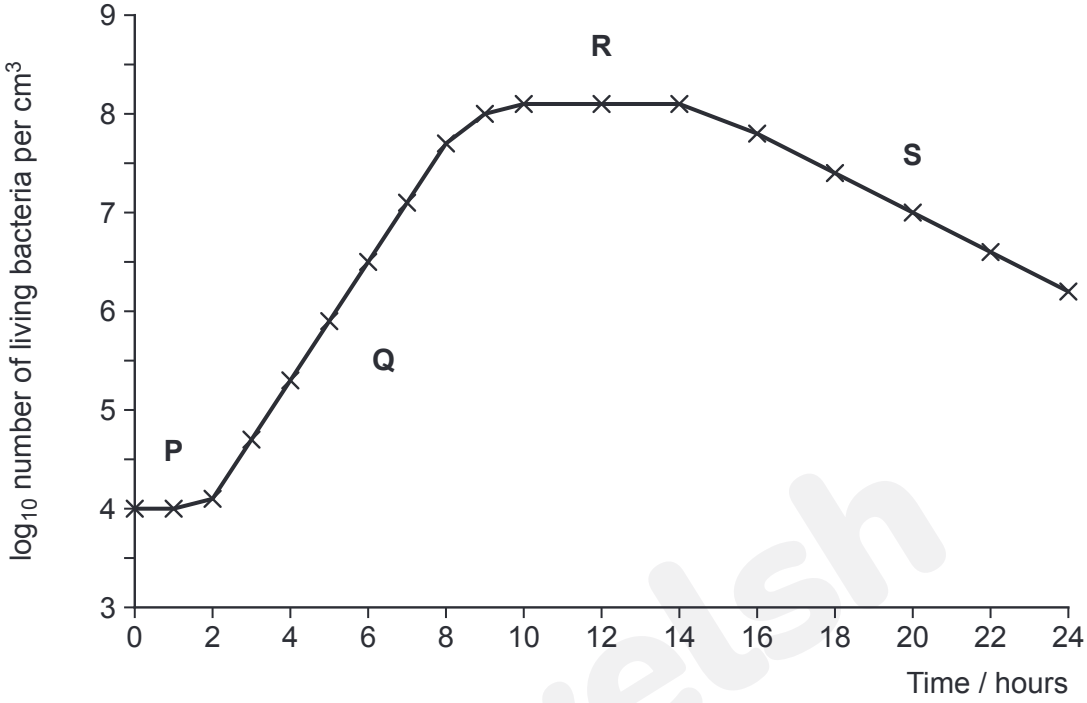
Tutor only

10. A small number of *Bacillus subtilis* cells were added to a flask of sterile nutrient broth and incubated at 30 °C. Samples were removed at intervals and a viable count was carried out. **Graph 10.1** shows the results. **P**, **Q**, **R** and **S** are the four phases of growth.



Graph 10.1

(a) Name phases **P**, **Q**, **R** and **S**. [2]

P .....

Q .....

R .....

S .....

(b) Explain why the number of bacteria hardly increases during phase **P**. [2]

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Turn over.

Tutor only

(c) (i) Use **Graph 10.1** to find the number of living bacteria in 1 cm^3 at 8 hours. **Give your answer in standard form.** [1]

Number = ... per cm^3

The number of generations, n, in a period of time can be calculated using the formula:

$$n = (\log_{10} N_t - \log_{10} N_0) \div 0.301$$

where N_0 is the number of bacteria at the start of the period and N_t is the number at the end.

(ii) Between 2 hours and 8 hours the $\log_{10}$ number of bacteria rose from 4.1 to 7.7. Calculate the mean generation time, in minutes, during this period. [2]

Generation time = ... minutes

(d) Explain why the population stops increasing during phase **R**. [2]

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(e) The number of bacteria was also estimated by measuring the turbidity of the broth. Explain why the turbidity readings would **not** show phase **S**. [2]

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Tutor only

11. *Lactococcus lactis* is used as a starter culture in cheese making. It ferments lactose in milk to lactic acid. A cheese maker grew a culture of *L. lactis* in sterile milk at 30 °C. The total number of cells was estimated from the turbidity of the culture, and the number of living cells was found by a viable count. **Table 11.1** shows the results.

Table 11.1

Time / hours	Total count / 10^8 cells cm^{-3}	Viable count / 10^8 cells cm^{-3}
0	0.2	0.2
4	1.6	1.6
8	9.8	9.5
12	12.0	10.1
16	12.4	6.2
20	12.5	2.5

(a) Explain why the total count is higher than the viable count at 16 hours. [2]

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(b) Calculate the percentage of the cells that were alive at 20 hours. [2]

Percentage = ... %

(c) Suggest why the viable count fell after 12 hours. [2]

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Tutor only

(d) Give **one** advantage to the cheese maker of estimating cell numbers from turbidity rather than by a viable count. [1]

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(e) The cheese maker wants to add as many living cells as possible to the next batch of milk. Using **Table 11.1**, suggest when the culture should be used. Give a reason for your answer. [2]

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(f) Explain why the starter culture must be grown in sterile milk using aseptic technique. [2]

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Tutor only

12. A student investigated the number of bacteria in fresh and stale milk.

1. A new carton of pasteurised milk was opened and a sample was tested at once (fresh milk). The carton was then kept at 20 °C for five days and tested again (stale milk).

2. Each sample was serially diluted with sterile distilled water.

3. 0.1 cm^3 of a chosen dilution was spread over each of three nutrient agar plates with a glass spreader. Before use, the spreader was dipped in ethanol, passed through a Bunsen flame and allowed to cool.

4. The plates were incubated at 25 °C for 48 hours and the colonies were counted.

(a) Suggest why the fresh and stale samples were taken from the same carton. [1]

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(b) Explain why the spreader was dipped in ethanol and flamed, and then allowed to cool before use. [2]

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(c) Describe a control plate that should be set up and explain its purpose. [2]

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Table 12.1 shows the results.

Table 12.1

Sample	Dilution plated	Colonies on plate 1	Colonies on plate 2	Colonies on plate 3
fresh	10^{-2}	34	41	38
stale	10^{-5}	88	95	12

Tutor only

(d) (i) Identify the anomalous result and suggest **one** reason for it. [2]

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(ii) Calculate how many times more bacteria there were in 1 cm^3 of stale milk than in 1 cm^3 of fresh milk. Do **not** use the anomalous result. [2]

Number of times = ...

(e) Suggest **one** safety precaution, other than aseptic technique, for handling the plates after incubation. [1]

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Tutor only

13. A student investigated how the number of bacteria in pasteurised milk changes when it is kept at 20 °C. Each day, a sample was serially diluted and 0.1 cm^3 of one dilution was spread onto nutrient agar. The plates were incubated at 25 °C for 48 hours. **Table 13.1** shows the results.

Table 13.1

Age of milk / days	Dilution plated	Number of colonies	Number of bacteria per cm^3 of milk	$\log_{10}$ number of bacteria per cm^3
0	10^{-2}	43	4.3×10^4	4.63
1	10^{-3}	58	5.8×10^5	5.76
2	10^{-4}	71	.....	.....
3	10^{-5}	62	6.2×10^7	7.79
4	10^{-6}	45	4.5×10^8	.....
5	10^{-6}	92	9.2×10^8	8.96
6	10^{-6}	98	9.8×10^8	8.99

(a) (i) Complete **Table 13.1**. [2]

(ii) Explain why $\log_{10}$ values are used when these results are plotted. [1]

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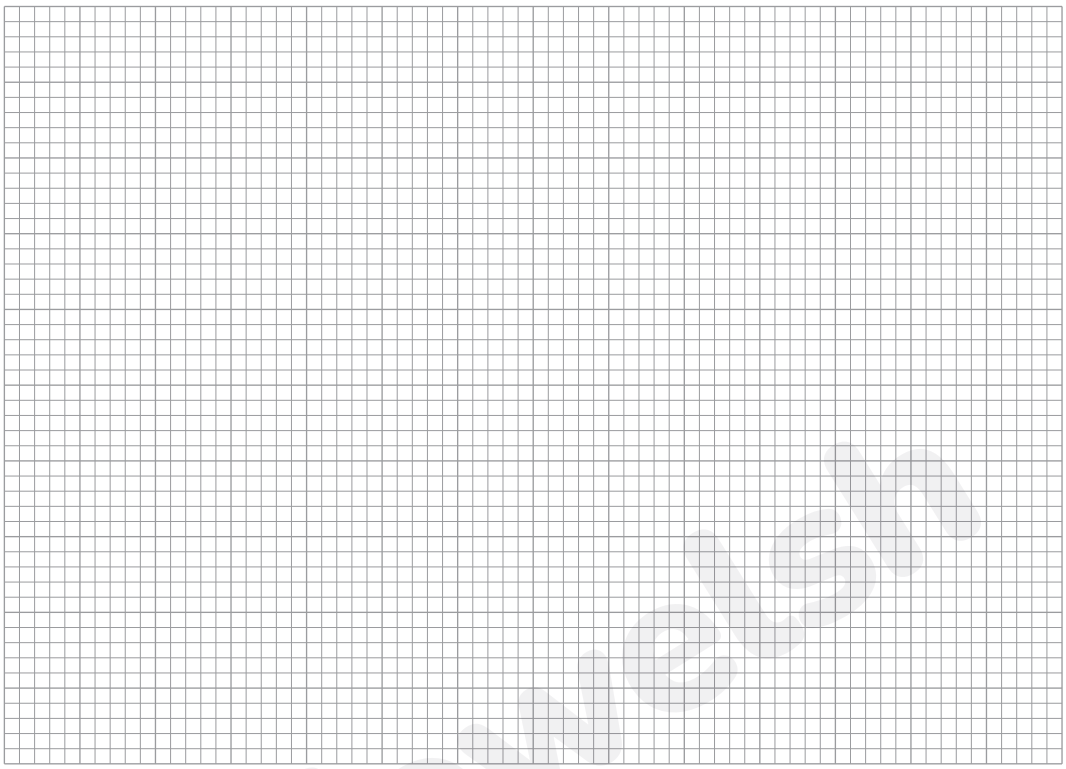
Turn over.

Tutor only

(b) Plot a graph of $\log_{10}$ number of bacteria per cm^3 against age of milk on the grid below. Join the points with straight lines. [4]



(c) Describe and explain the change in the number of bacteria between day 4 and day 6. [2]

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(d) Explain why the milk contained bacteria on day 0 even though it had been pasteurised. [1]

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(e) On day 3 the student also plated the 10^{-4} dilution and recorded 'too many to count'. Explain why this plate could not be used. [1]

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Tutor only

14. Bathing water at beaches is tested for *Escherichia coli*, which lives in the human gut. Its presence shows that the water is contaminated with sewage. Because each cm^3 of seawater contains very few bacteria, membrane filtration is used. A measured volume of seawater is drawn through a sterile membrane filter, as shown in **Image 14.1**. The membrane is then placed on a selective agar medium and incubated at 44 °C for 18 hours. Each *E. coli* cell trapped on the membrane forms a colony.

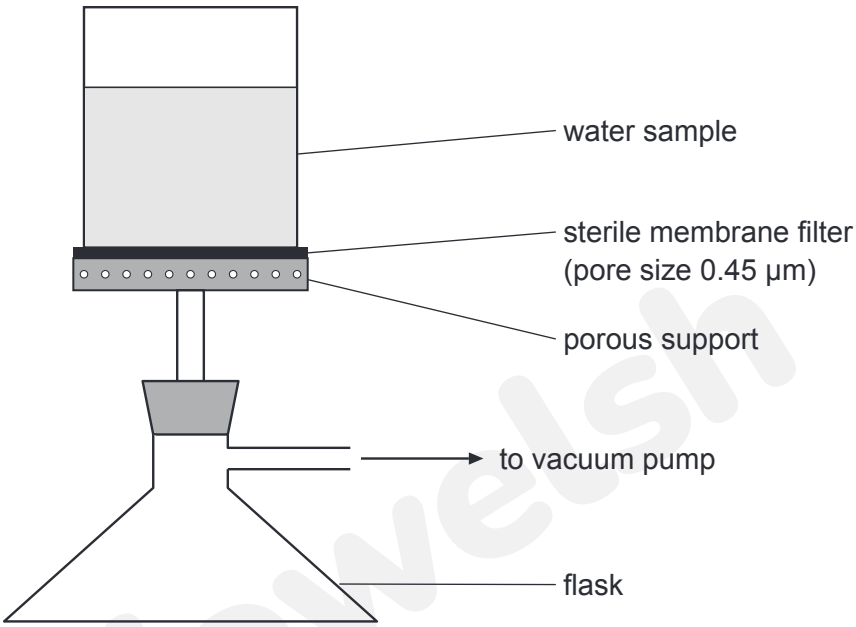

Image 14.1

(a) Explain why bacteria are trapped on the membrane. [1]

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(b) Suggest why membrane filtration is used for bathing water rather than spreading 0.1 cm^3 of the sample on agar. [2]

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Turn over.

Tutor only

Samples from three beaches, **K**, **L** and **M**, were tested. **Table 14.2** shows the results.

Table 14.2

Beach	Volume of seawater filtered / cm^3	Number of colonies	Number of *E. coli* per 100 cm^3
K	100	41	41
L	20	71	.....
M	50	96	.....

(c) (i) Complete **Table 14.2**. [2]

(ii) In this simplified scheme, a beach is classed as excellent if its bathing water contains no more than 250 *E. coli* per 100 cm^3. Identify the beaches that would be classed as excellent. [1]

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(d) Suggest why incubating the membrane at 44 °C helps to make sure that only bacteria from the gut are counted. [2]

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(e) Suggest why results are often reported as 'colony forming units' rather than as numbers of bacteria. [1]

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(f) Explain why the filter holder must be sterilised between samples. [1]

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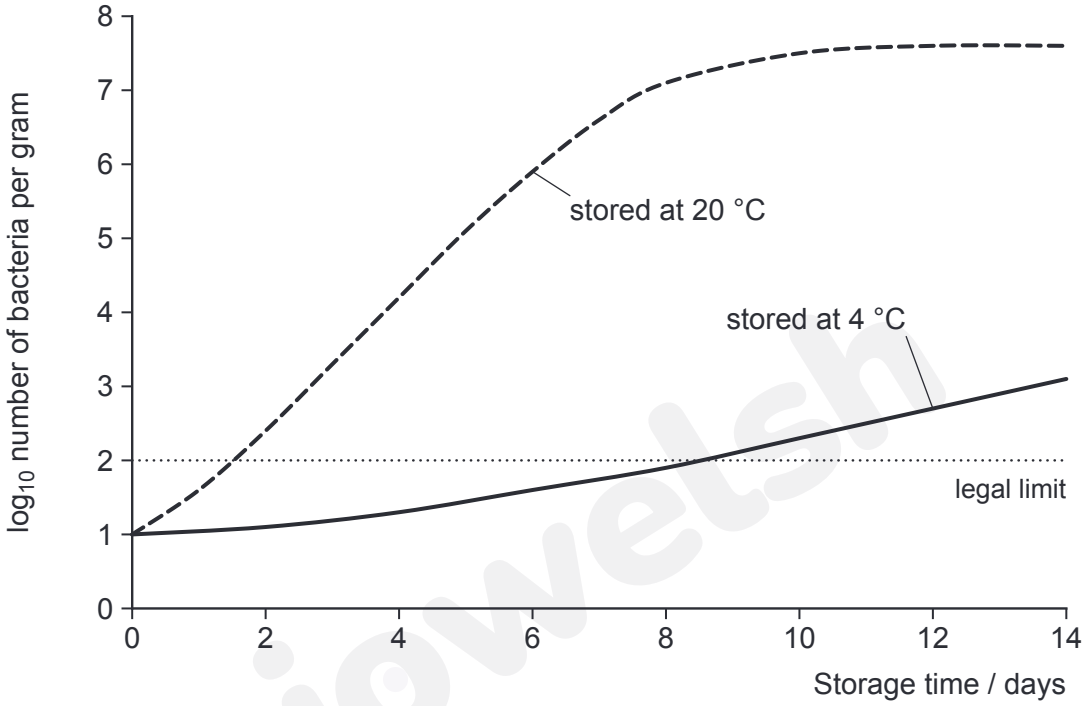
Tutor only

15. *Listeria monocytogenes* is a Gram-positive, rod-shaped bacterium that can cause serious illness, especially in pregnant women. Unlike most food poisoning bacteria, it can grow slowly at refrigerator temperatures.

Samples of a soft cheese were given a small number of *L. monocytogenes* cells and stored at 4 °C or at 20 °C. The number of bacteria per gram was found by a viable count. The legal limit for *L. monocytogenes* in ready-to-eat foods is 100 bacteria per gram. **Graph 15.1** shows the results.



Graph 15.1

Describe how a Gram-stained smear would show that this bacterium is Gram-positive and rod shaped, and explain the result in terms of its cell wall. Describe how a viable count of the bacteria in a sample of cheese could be carried out using aseptic technique. Use **Graph 15.1** to explain why the cheese must be kept at 4 °C and eaten within seven days. [9 QER]

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END OF WORKSHEET

9

A Tutor Meets Tutee project

BioWelsh worksheets are written and checked by Tutor Meets Tutee tutors for WJEC A level Biology students.

Mark this worksheet with the BioWelsh mark scheme **BW-A2-3.4-W1** (MS).

Acknowledgements

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