WJEC EDUQAS A-LEVEL BIOLOGY · COMPONENT 1

BIOLOGY



EXAM QUESTION PACK

1.4 MICROBIOLOGY

SCORE	PERCENTAGE
/99	%

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1. Legionnaires' disease is a serious lung infection caused by *Legionella* bacteria.

(a) In 2015 there were 18 confirmed cases of Legionnaires' disease in Wales. The population of Wales in 2015 was 3 099 100.

Calculate the percentage of the population of Wales that suffered from Legionnaires' disease in 2015. Express your answer in **standard form**. [2]

Answer = ...

(b) *Legionella* is a Gram negative, bacillus bacterium that can live in domestic water supplies. If small droplets of contaminated water are inhaled the bacteria can cause pneumonia-like symptoms.

State what is meant by a Gram negative, bacillus bacterium. [2]

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(c) During an outbreak, a sample of contaminated water was studied to find which antibiotic was most effective. The three antibiotics were erythromycin, ciprofloxacin and azithromycin. A range of concentrations of each antibiotic were used.

A microbiologist carried out the investigation as follows:

- Sterile nutrient agar plates were set up containing different concentrations of erythromycin.
- 0.5 cm^3 of diluted contaminated water was transferred onto each plate.
- The same process was repeated for ciprofloxacin and azithromycin.

The results following incubation are shown in the table.

Concentration of antibiotic / %	Number of colonies on plate		
	erythromycin	ciprofloxacin	azithromycin
0	86	86	86
10	70	43	0
20	47	22	0
30	32	0	0
40	24	0	0

(i) The microbiologist had diluted the original sample of contaminated water by a factor of 10^{-3}. Use the information given to calculate the number of *Legionella* bacteria in 1 cm^3 of the original contaminated sample. Show your working. [2]

Number of *Legionella* bacteria in 1 cm^3 = ...

(ii) Suggest a reason for the following:

I. When the microbiologist diluted the original sample by a factor of 10^{-2} she could not calculate the number of *Legionella* bacteria per cm^3. [1]

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II. When she diluted the original sample by 10^{-6} a lower number of *Legionella* bacteria per cm^3 was calculated. [1]

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(d) The cost of ciprofloxacin and azithromycin are approximately the same per unit mass. Use the results of this experiment to suggest and explain which antibiotic would most likely to be recommended by the Welsh Government to treat this disease. [2]

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2. The Archaea are a domain of single-celled microorganisms. These microbes are prokaryotes. Most Archaea possess a cell wall which is assembled from surface-layer proteins. These form an S-layer which is a rigid array of protein molecules that cover the outside of the cell. This layer provides both chemical and physical protection. Unlike bacteria, most Archaea lack peptidoglycan in their cell walls.

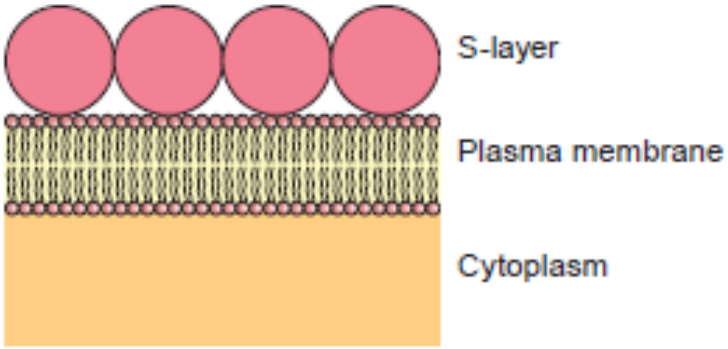
(a) (i) Describe two major features of a eukaryote which would allow you to distinguish it from a prokaryote. [1]

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One type of cell wall found in Archaea is shown below.



(ii) Apart from the lack of peptidoglycan, describe how the cell walls of Gram negative bacteria would differ from those of Archaea. [2]

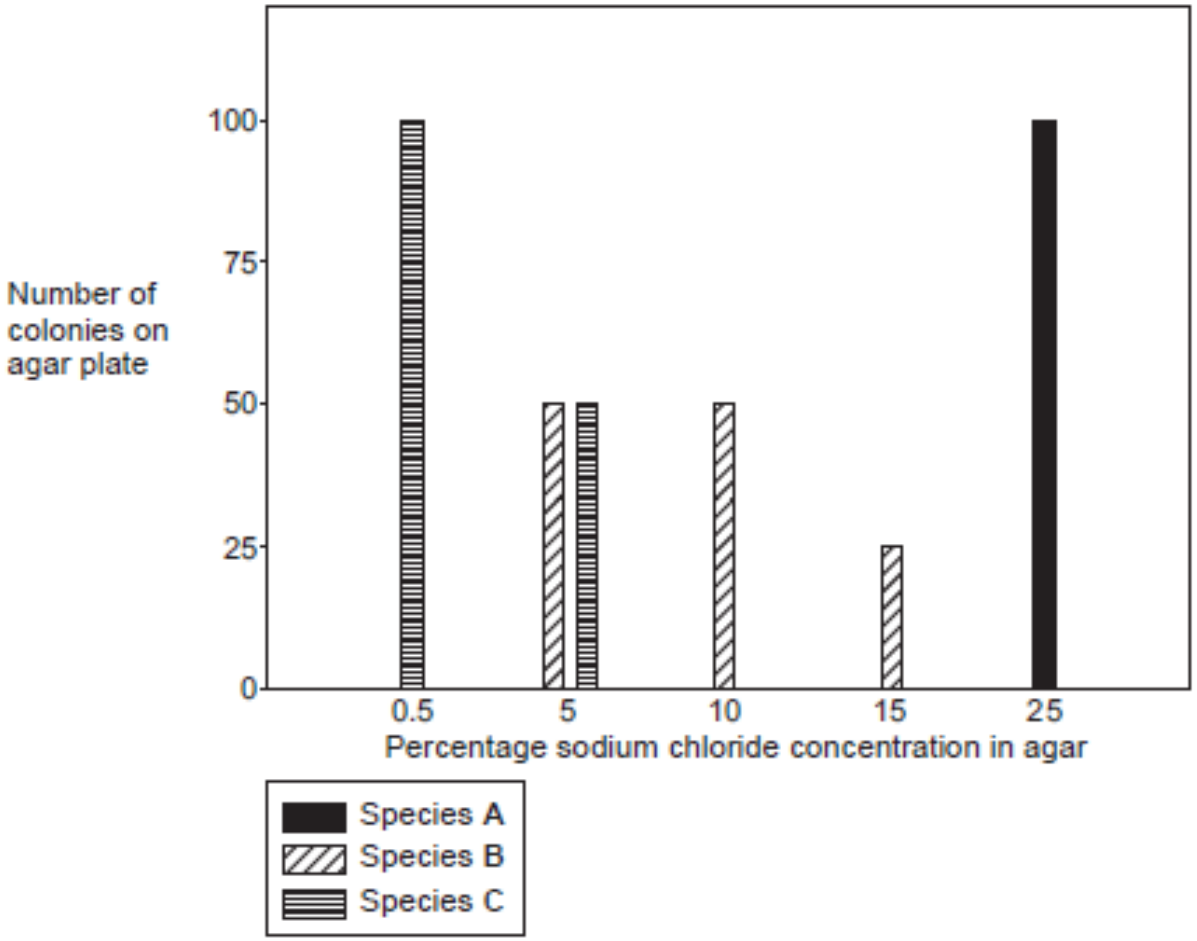
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(b) An experiment was carried out to determine the effect of sodium chloride concentration (salinity) on the growth of different species of prokaryotes. The number of visible colonies of each species was counted on agar plates containing different concentrations of sodium chloride.
A bar chart of the results is shown below.



- *Staphylococcus aureus* is a Gram-positive bacterium that is frequently found on sweaty skin.
- *Halobacterium salinarum* is a marine Gram-negative obligate aerobic archaeon. Despite its name, this microorganism is not a bacterium, but rather a member of the domain Archaea.
- *Escherichia coli* is a Gram-negative, rod-shaped bacterium commonly found in the lower intestine of endothermic (warm-blooded) organisms.

(i) Which agar plate used in this experiment has the lowest water potential? [1]

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(ii) Identify species **A**-C giving reasons for your conclusions. [3]

A .....

.....

B .....

.....

C .....

.....

(iii) *Enterococcus faecalis* is a Gram-positive bacterium which also inhabits the gastro-intestinal tracts of humans and other mammals.
Briefly describe two ways in which you could distinguish between *Enterococcus faecalis* and *Escherichia coli*. [3]

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(c) Some strains of *E. coli* can cause food poisoning. Following an outbreak, samples were taken from suspected food sources and cultured on agar plates. Serial dilutions were carried out to determine the number of viable bacteria in the samples. 0.25 cm^3 was transferred to each agar plate and incubated at 37 °C. The number of colonies on each plate was then counted.

	Number of colonies per plate				
Dilution	Plate 1	Plate 2	Plate 3	Mean	SD
10^{-3}	426	503	521	483	50.5
10^{-5}	444	479	457	460	17.7
10^{-7}	275	293	310	293	
10^{-9}	66	71	78	72	6.0

(i) Calculate the missing standard deviation (SD) using the following formula. [3]

$$s = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$$

s = standard deviation

Σ = sum of

x = each value in the data set

$\bar{x}$ = mean of all values in the data set

n = number of values in the data set

The following table is given to help structure your calculation.

Plate number	Number of colonies per plate	$x - \bar{x}$	$(x - \bar{x})^2$
1	275		
2	293		
3	310		
Mean	293		Σ

Standard deviation = ...

(ii) Suggest why the **mean values** for the first two dilutions were so close despite there being a 100 times difference in the dilutions. [1]

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.....

(iii) State what the standard deviations of the **first two dilutions** tell you about the data. [1]

.....

(iv) Calculate the number of bacteria in **1 cm^3** of the original sample using the 10^{-9} dilution. **Give your answer in standard form.** [3]

Number of bacteria = ...

3. (a) Define the following terms with reference to bacterial growth.

[3]

(i) obligate aerobe

.....

.....

(ii) obligate anaerobe

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.....

(iii) facultative anaerobe

.....

(b) Describe and explain the appearance of Gram positive and Gram negative bacteria following Gram staining.

[3]

(c) Most pathogens in humans are Gram-positive organisms. Six Gram-positive genera are typically pathogenic in humans. Two of these, Streptococcus and Staphylococcus, are cocci. The remaining organisms are bacilli.

What **three dimensional shape** would the cocci and bacilli be?

[1]

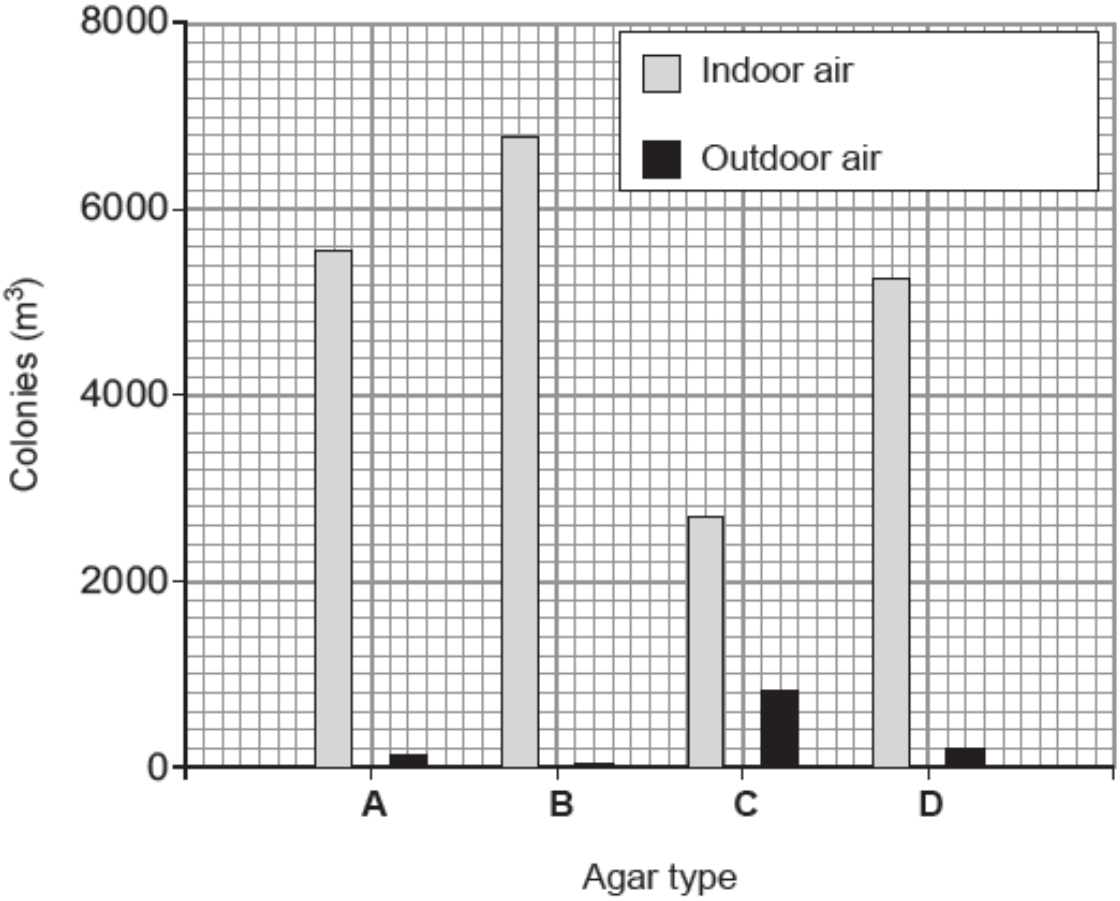
cocci . . . . . .

bacilli . . . . . .

4. 1 m3 of air was filtered from two different environments.

The microorganisms collected were grown, using aseptic technique, on four different types of agar plates (**A**, **B**, **C** and **D**) at the same temperature and for the same length of time.

The number of colonies grown from each sample is shown.



(a) What conclusion can you draw from the graph above about the numbers of microbes in the two air samples?

[1]

(b) The four agar types have resulted in different colony numbers because they contain different nutrients. State **four** ways that the **agar types** could differ in composition.

[2]

i. . . . . .

ii. . . . . .

iii. . . . . .

iv. . . . . .

. . . . . .

5. Complete the sentences below by giving the correct term in each case. [4]

(a) A clone arising from a single bacterium on an agar plate is called a. . . . . .

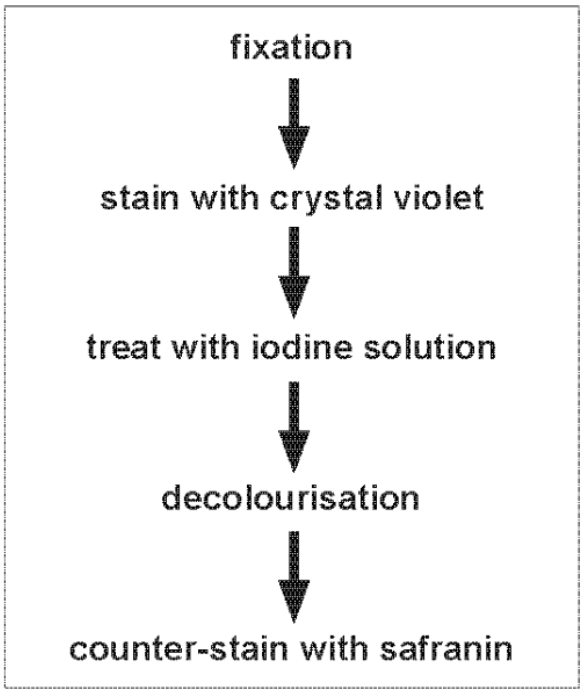
(b) A viable count is a count of . . . . . bacterial cells.

(c) The type of bacterium that lacks lipopolysaccharides in its cell walls is a

. . . . . bacteria.

(d) A corkscrew or helical shaped bacterium is known as a . . . . . .

6. The diagram below shows the process of Gram staining to identify Gram positive (+) and Gram negative (-) bacteria.



(a) State the colour of the bacteria following the application of the counter stain: [1]

Gram positive; .....

Gram negative. .....

(b) Use your knowledge of the structure of the bacterial cell wall to explain the differences in the appearance of the two types of bacteria when stained with the Gram staining technique. [3]

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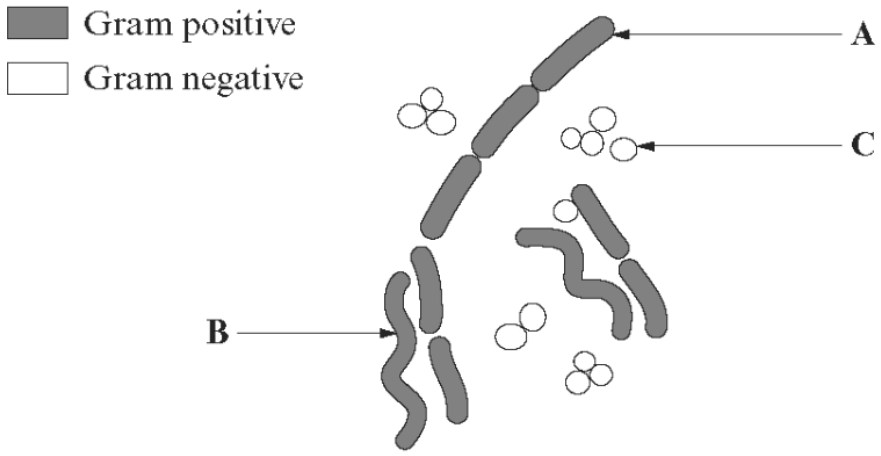
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(c) Following an outbreak of food poisoning in a school, samples were taken from infected patients. The Gram staining technique was used, in conjunction with the shape of bacterial cells, to identify potentially pathogenic bacteria in the samples.

The diagram below shows part of a bacterial smear stained using the Gram staining technique.



(i) State the name given to the shapes of the bacteria labelled **A**, **B** and **C**. [3]

A .....

B .....

C .....

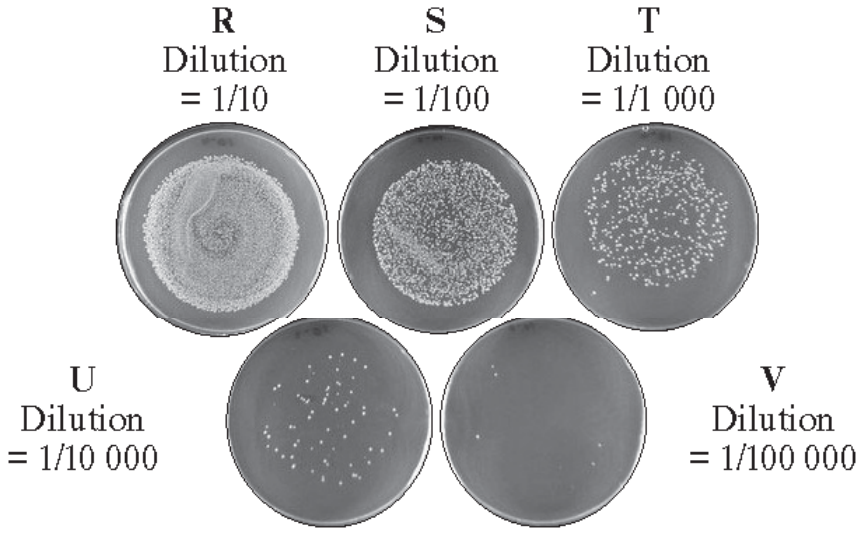
(ii) Suggest why the bacteria labelled **C** in the diagram might be the possible cause of the food poisoning. [1]

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(d) Using the viable count method Environmental Health Officers made an estimate of the number of bacteria in various foods in the school canteen. Dilutions of 1/10, 1/100, 1/1 000, 1/10 000 and 1/100 000 were prepared and 0.5 cm^3 of each dilution were spread evenly over the surface of agar plates. The plates were incubated at 35°C for 24 hours. A photograph of the results for one of the samples is shown below.



They decided to use Plate U to estimate the number of bacteria in the food sample.

(i) With reference to the plates shown above explain why they decided to use Plate U and **not** any of the other plates. [2]

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(ii) On plate U 69 bacterial colonies were counted. Estimate the number of bacteria present in 1 cm^3 of the original food sample. Show your working. [2]

Estimated number of bacteria = ... per cm^3

(iii) Suggest why this number is likely to be an underestimate of the actual number of bacteria present. [1]

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(iv) Suggest why the bacteria were cultured at 35°C and not at 25°C. [1]

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7. Following the Haiti earthquake in 2010 there was an outbreak of cholera. A web site describes the cause as follows:

'**Cholera** is caused by the bacterium *Vibrio cholerae* – a curved bacillus each with one flagellum. The genus *Vibrio* is a member of the family Vibrionaceae, which include Gram-negative, motile, facultative anaerobes that utilize glucose as a source of energy.
The bacteria are typically spread by contaminated drinking water.'

(a) Describe what is meant by the following terms used in the article:

(i) Bacillus, [1]

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(ii) Gram-negative, [2]

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(iii) Facultative anaerobes. [2]

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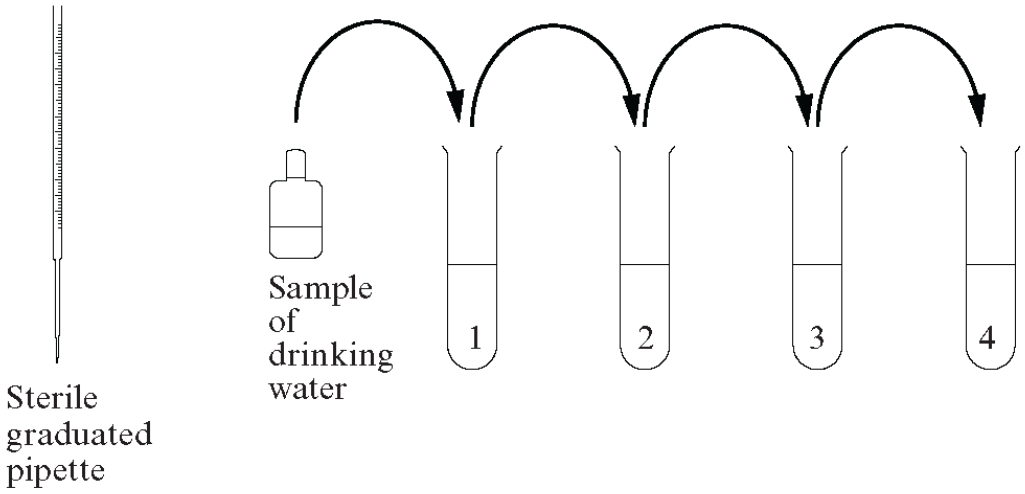
(b) Scientists might be able to determine the extent of contamination by counting the number of bacterial cells in water samples.

(i) What is the difference between a total count and a viable count? [1]

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(ii) Both techniques involve serial dilution.
Annotate the diagram below to explain how you would obtain a series of ten fold dilutions of the drinking water sample. [3]



(iii) If 1 cm^3 of the drinking water sample contained 80 000 living bacterial cells, how many bacterial cells would there be in 0.5 cm^3 of the liquid in tube 4, after this technique had been completed? [1]

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(iv) How would you use the dilutions produced to carry out a viable count? [4]

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(Total 14 marks)

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8. *Staphylococcus aureus* is a Gram positive, facultative anaerobe.
It is the cause of various diseases including blood poisoning and food poisoning.

(a) (i) What shape are the cells of *Staphylococcus aureus?* [1]

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(ii) The bacterium is described as 'Gram positive'. What does this mean in relation to the structure of its cell wall? [2]

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(iii) What is meant by the term 'facultative anaerobe'? [1]

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(b) In order to monitor the population growth of bacteria, a number of different methods may be used.
One method is to use a **viable** count.

(i) What assumption must be made when using this method? [1]

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(ii) State **one** limitation of using this method. [1]

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(c) Another method uses a **total** count. Suggest why this method gives a higher estimate of the population than the viable count. [1]

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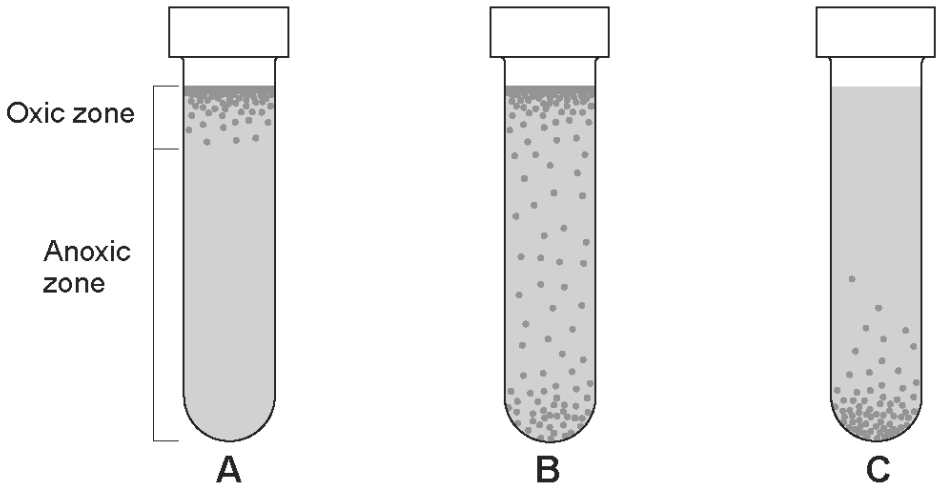
(d) In both the above cases the original culture requires a procedure in order to provide a final number within a countable range. Name this procedure. [1]

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(Total 8 marks)

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9. The diagrams below show the results of a number of experiments investigating bacterial growth with different levels of oxygen.
Bacteria were mixed with agar in bacteriology tubes and the agar was allowed to set. The tubes were incubated for 24 hours at 37°C.



Each dot represents an individual bacterial colony within the agar or on its surface. The surface which is directly exposed to atmospheric oxygen contains oxygen (oxic zone). The oxygen content of the agar decreases with depth until it does not contain oxygen (anoxic zone) towards the bottom of the tube.

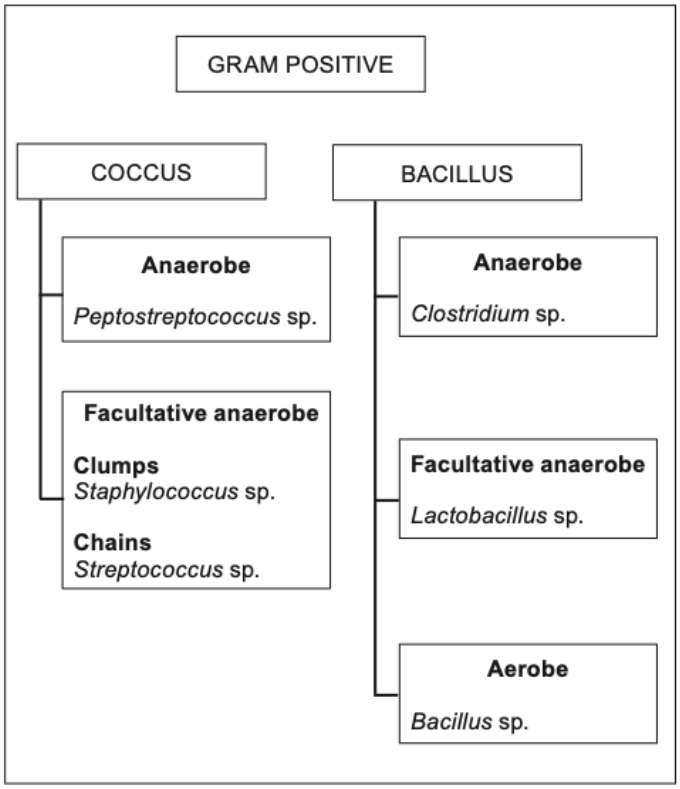
(a) Using the results above, what terms are given to the types of bacteria which grow in tubes **A**, **B** and **C**? [2]

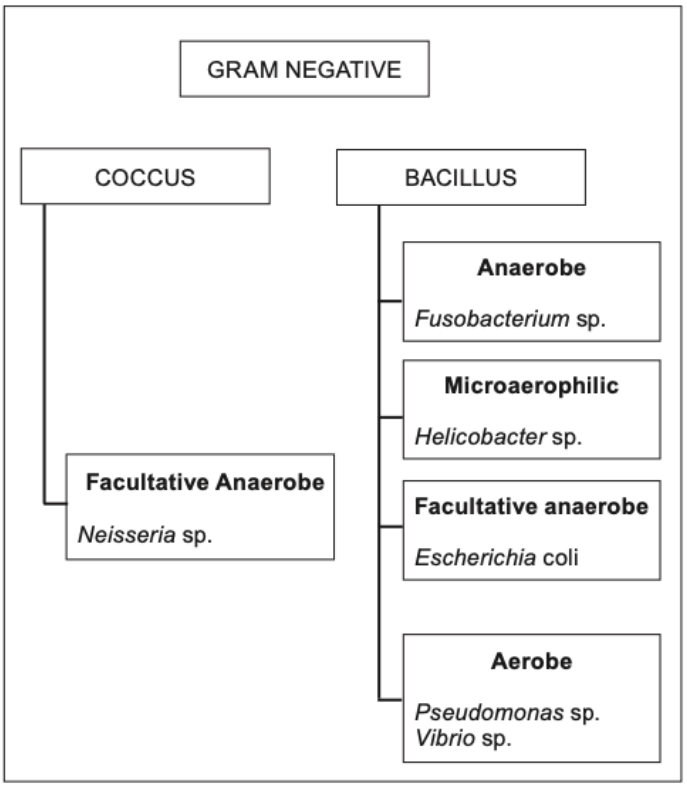
A .....

B .....

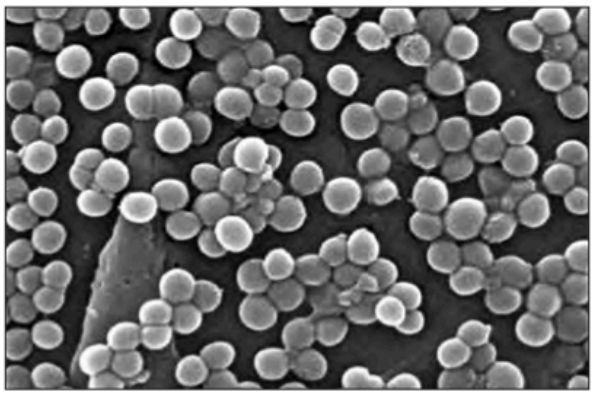
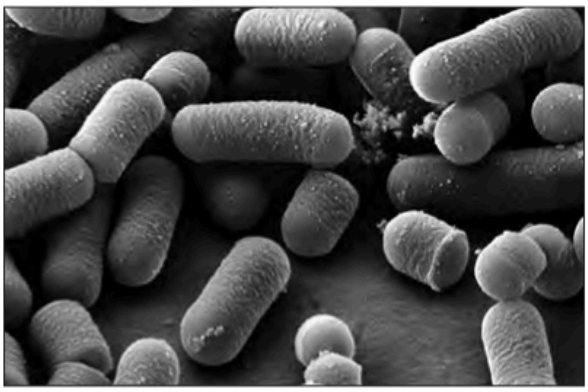
10. 2. Accurate and definitive bacterial identification is essential for disease diagnosis, treatment and the trace-back of outbreaks associated with microbial infections. Bacterial identification is also used in a wide variety of other applications including microbial forensics, criminal investigations, bio-terrorism threats and environmental studies.

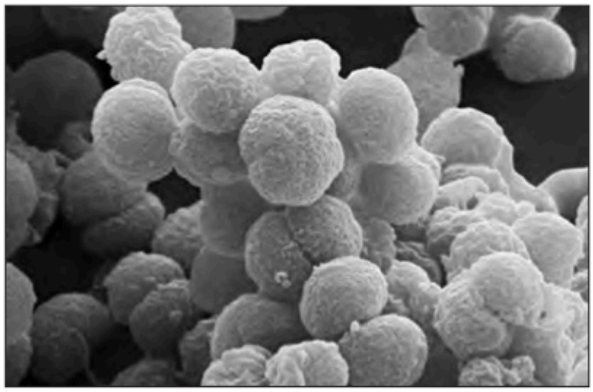
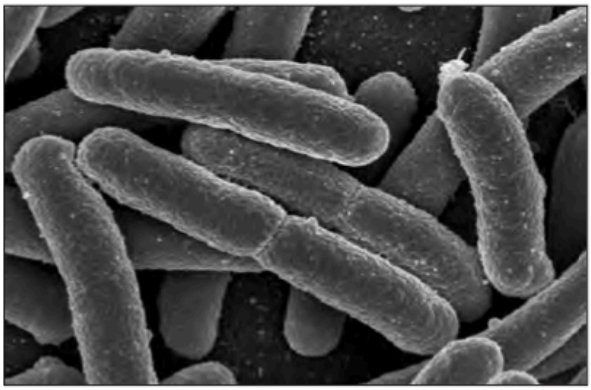
The simplified key shown below can be used to identify bacteria given some of their features.





The images below show four different bacteria together with information about their oxygen requirements and the results of Gram staining.

A	B
	
Facultative anaerobe Gram positive	Facultative anaerobe Gram positive

C	D
	
Facultative anaerobe Gram negative	Facultative anaerobe Gram negative

(a) Using the key provided, identify the **four** bacteria in the images. [4]

A .....

B .....

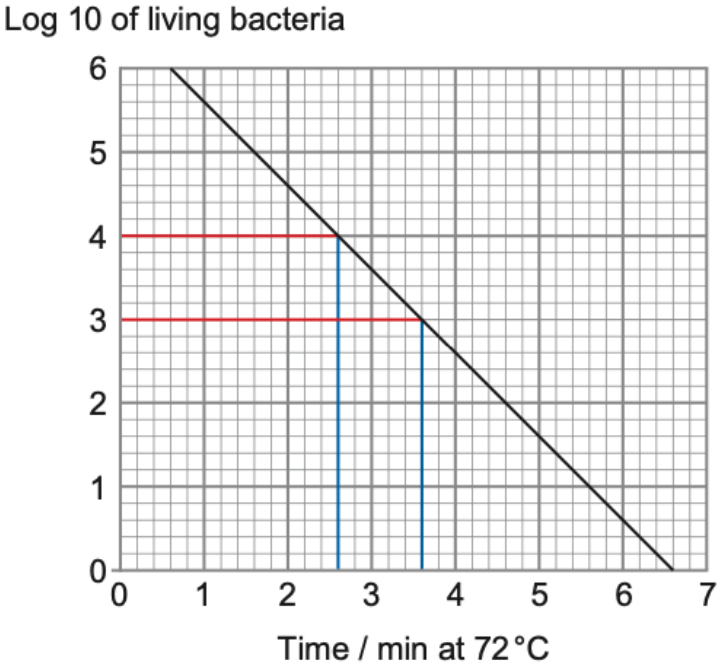
C .....

D .....

Each bacterial species has its own particular heat tolerance. During a process such as pasteurisation, the rate of cell destruction is logarithmic. Bacteria subjected to heat are killed at a rate that is proportional to the number of bacteria present. The process is dependent both on the temperature of exposure and the time required at this temperature to accomplish the desired rate of destruction.

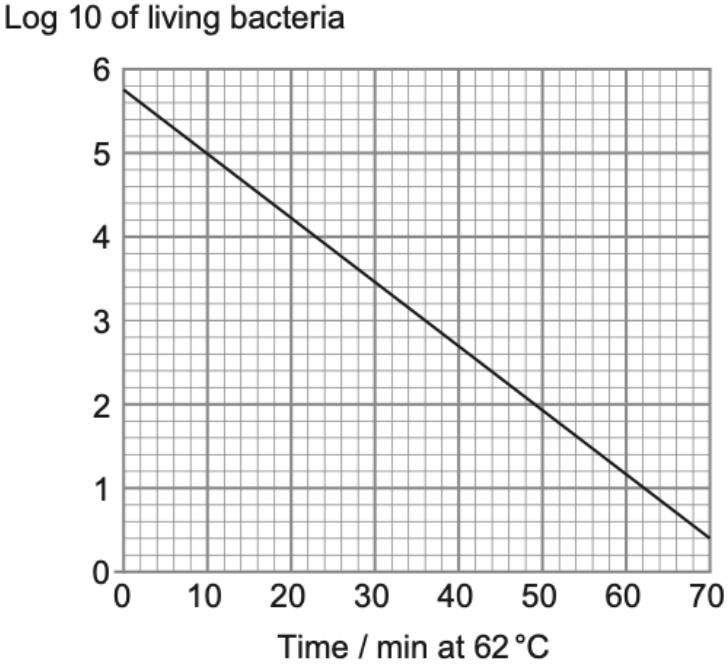
The D value is the time in minutes at a given temperature required to destroy 90% of the bacterial population.

Graph 1



In the example shown above at 72 °C, the D value = 1 minute. This means that for each minute of processing at 72 °C the bacterial population will be reduced by 90%.

Graph 2



(b) Follow the method shown on graph **1** to calculate the D value for 62 °C (graph **2**). [3]

D Value = ...

(c) Describe a method that you could use to determine the number of living bacteria in the original sample prior to heat treatment. No reference to aseptic technique is required. [4]

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(d) Describe the effect of heat in the process of pasteurisation on the proteins in the bacteria. [3]

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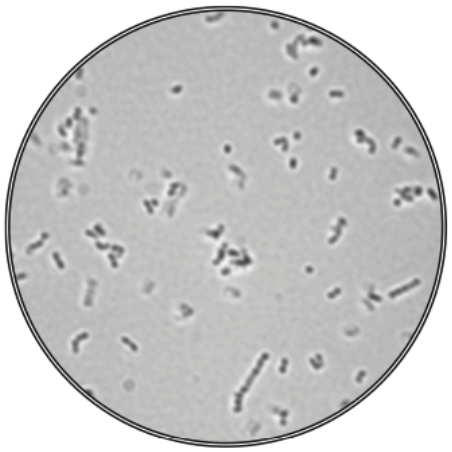
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11.

The Breed method is a fast and simple way of counting the number of bacteria in a suspension.

A known volume of the bacterial suspension is spread uniformly over a glass slide covering a specific area. The bacteria are then heat fixed and stained.

The photograph below shows the results of this method as seen through a light microscope.



(a) The average number of cells in the field of view was found to be 156 and the radius of the field of view was 0.09 mm.

Calculate the number of cells in 100 mm^2.

The area of microscopic field of view = πr^2

where π is 3.14 and r is the radius of the field of view. [3]

Number of cells = ...

(b) A scientist used this method to find the number of bacteria in a sample taken from the back of a patient's throat. There were too many bacteria to be able to count down the microscope.

(i) Describe a method that could be used to produce a range of dilutions from the original sample to a 10^{-5} dilution. [3]

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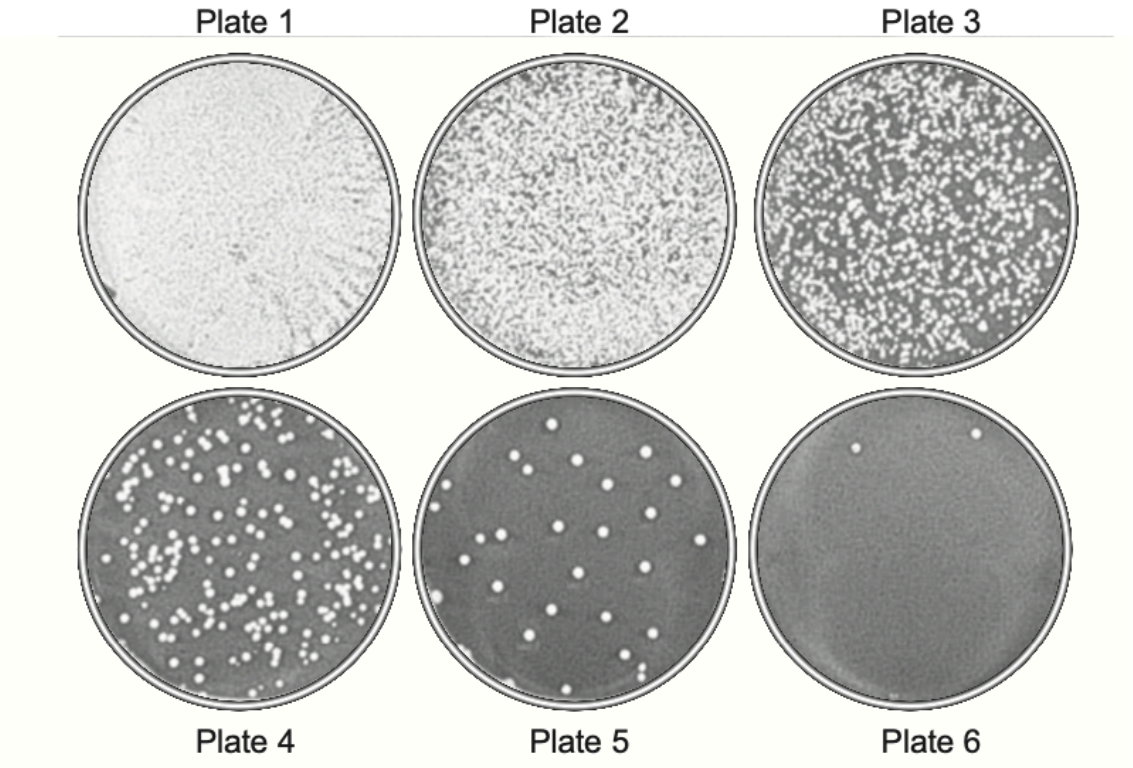
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The scientist obtained the following results for a range of dilutions.



(ii) State which number plate should be used and give reasons why the other plates should not be used. [3]

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The scientist was looking for the pathogenic bacterium *Staphylococcus aureus*.

(iii) Give the reason why the bacteria removed from the patient were incubated at 37 °C not 25 °C. [1]

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(iv) A sample of the *Staphylococcus* was stained purple by the Gram stain technique. Describe what the purple staining indicates about the structure of the bacterial cell wall. [2]

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1.

Question				Marking details	Marks Available					
					AO1	AO2	AO3	Total	Maths	Prac
	(a)	(i)		5.81×10^{-4} or 5.8×10^{-4} = 2 marks (accept any d.ps) If incorrect award 1 mark for 18/3099100 x100 0.00058081 5.80×10^{-4}		2		2	2	
	(b)	(i)		Gram – have capsule/lipopolysaccharide (1) Bacillus rod shaped (1)	2			2		
	(c)	(i)		172000 = 2 marks Accept standard form If incorrect award 1 mark for 86000× 2 86000 (no dilution factor)		2		2	2	
		(ii)	I	there were too many colonies to count/colonies {merged/clumping}			1	1		1
			II	The extra dilution gives additional error/may not have mixed fully/inaccurate representation of whole sample/not valid to count less than 30 colonies/too few to be statistically significant			1	1		1
	(d)			azithromycin (no mark for name only) need to use less antibiotic/use of data e.g. Anti A only needs 10% to kill all bacteria. C needs 30% to kill all bacteria(1) so cheaper for government/less likely to lead to antibictic resistance (1)			2	2		
				Question 1 total	**2**	**4**	**4**	**10**	**4**	**2**

2.

Question				Marking details	Marks Available					
					AO1	AO2	AO3	Total	Maths	Prac
2	(a)	(i)		**Any two for 1 mark from** Have nucleus (1) membrane-bound organelles/ named example(1) 80S ribosomes (1) DNA associated in chromosomes/linear (1)	1			1		
		(ii)		Absence of protein/S layer in Gram negative (1) layer of {lipoproteins/lipopolysaccharides} (1)	1	1		2		
	(b)	(i)		25 (% NaCl)		1		1		
		(ii)		**A** *Halobacterium salinarum* + lives in a marine environment which has high NaCl conc (1) **B** *Staphylococcus aureus* + lives on skin which is often high in salt (from sweat) (1) **C** *Escherichia coli* + in part of gut with low salt (as most has been absorbed)(1)			3	3		
		(iii)		Grams staining/ or description of(1) Accept reference to crystal violet only *Enterococcus faecalis/ Gram positive:* purple {spherical/spheres} (1) NOT round/ circle *Escherichia coli/ Gram negative:* red {rods/bacilli/us} (1) both correct colours = 1 mark both correct shapes for 1 mark Accept correct description of penicillin resistance	1	2		3		3
	(c)	(i)		<table><tr><td></td><td></td><td>$x - \bar{x}$</td><td>$(x-\bar{x})^2$</td></tr><tr><td>1</td><td>275</td><td></td><td>324</td></tr><tr><td>2</td><td>293</td><td></td><td>0</td></tr><tr><td>3</td><td>310</td><td></td><td>289</td></tr><tr><td>Mean</td><td>293</td><td></td><td>$\sum$613</td></tr></table>*$\sum$ = 613 (1)* *Square root of 613/2* *Substitution = (1)* *SD = 17.5 (1) must be to 1dp Correct answer = 3 marks*		3		3	3	
		(ii)		Clumping of bacteria/ Overlapping colonies/ Difficult to count large colony numbers Ignore reference to anomalies/ not enough repeats		1		1		1
		(iii)		data range is very large/10^{-3} is less reliable than 10^{-5}/ both unreliable as they have a large SD/ Data is very variable Reject accuracy/ unreliable unqualified		1		1		1
		(iv)		$72 \times 4 \times 10^9$ (1) 2 880 000 000 000 (2) 2.9×10^{11}/2.88×10^{11} bacteria in $1cm^3$ (3) If do not use x 4 = 7.2×10^{10} = 1 mark		3		3	3	
				Question 2 total	**3**	**12**	**3**	**18**	**6**	**5**

3.

Question		Marking details		Marks Available
(a)	(i)	Obligate aerobe	{microorganisms/bacteria} that {grow/divide/ metabolise} in the presence of <u>oxygen</u>;	1
	(ii)	Obligate anaerobe	{microorganisms/bacteria} that will only {grow/divide/metabolise} in the absence of <u>oxygen</u>;	1
	(iii)	Facultative anaerobe	{microorganism/bacteria} that can {grow/divide/ metabolise} with or without <u>oxygen</u>;	1
				1
(b)		Gram +ve PURPLE Gram –ve RED/PINK (**both** colours correct for one mark); Gram positive retain the {(crystal) violet/ purple} stain because of their {thick/peptidoglycan/murein} cell wall; Gram negative do not retain the stain because of their {thinner cell wall/ less peptidoglycan/ less murein/lipopolysaccharide layer}; Gram positive retain crystal violet because they have a thicker cell wall than the gram negative = 2 marks		3
(c)		**Both for one mark** Cocci sphere/ spherical Bacilli Rod; Accept cylinder		1
			Question 3 Total	**[7]**

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4.

Question		Marking details	Marks Available
1	*(a)*	There more {microorganisms/ bacteria/ fungi} in indoor air than outdoor air samples;	1
	(b)	(Different) pH; (Different) C {source/ concentration}; (Different) N {source/ concentration}; (Different) growth factors: {Different/ different concentration} Vitamins/minerals; NOT nutrients Any 4 1 mark per pair	max 2
		Question 1 total	**[3]**

5.

Question			Marking details	Marks Available
1	*(a)*		colony;	1
	(b)		living;	1
	(c)		Gram positive;	1
	(d)		spirillum;	1
			Question 1 total	**[4]**

6.

Question	Marking details	Marks Available
(a)	Gram positive: purple/ violet NOT crystal violet Gram negative: red / pink [both needed for 1 mark];	1
(b)	<u>Gram +ve</u>: (thick) {murein / peptidoglycan} cell wall (only)/ no lipopolysaccharide layer; {retains/ binds/ absorbs} crystal violet stain / purple colour;	3

{retains/ binds/ absorbs} crystal violet stain / purple colour;

Gram –ve:

{lipoprotein / lipopolysaccharide}{ layer / wall} (external to murein cell wall);

Does not retain {crystal violet stain / purple colour};

Stains {red/pink} with {counter stain / safranin / carbol fuchsin};

max 2 if only discuss one type of bacteria

(c) (i) Ignore references to Gram +ve / Gram –ve.

A bacillus/ bacilli; NOT rod — 1

B spirillum/ spirilli; NOT spiral — 1

C coccus/ cocci; NOT round / staphylococcus — 1

(ii) (lipoprotein / lipopolysaccharide layer)

protects against (some) {antibiotics) / penicillin / antibodies} /

makes them less susceptible to attack by lysozyme/

(lipid component) acts as an (endo)toxin; — 1

(d) (i) *Plate U* *- enough colonies for reliable results/*

- colonies easily countable;

NOT the right number/ we can see them

Plate R/S *- cannot distinguish individual colonies;*

Plate T *- too many colonies to count reliably;*

Plate V *- not enough colonies for reliable estimate*

max 2

(ii) 69 colonies x 10 000 (dilution factor) x 2 (or 1/0.5);

1 380 000/ 1.38 x 10^6 colonies per cm^3; — 2

(iii) does not include {dead / non-viable bacteria}/

cannot be sure that {each colony has grown from a single bacterium/ colonies are not clumped}/ ORA; — 1

(iv) need to count pathogenic bacteria / pathogenic bacteria more likely to grow at temperature close to body temperature/

want bacteria to grow quickly to identify to treat infection as quickly as possible; — 1

NOT want to grow them as quickly as possible without qualification

Question 6 total **[14]**

7.	(a) (i)	rod shaped;	1
	(ii)	retains (red) counter stain/stains red/pink in Gram test/do not retain crystal violet; because of complex nature of cell wall/reference to lipopolysaccharide/does not contain as much murein/peptidoglycan.	1 1
	(iii)	grow best with oxygen; (not: prefer/like oxygen) but can grow with or without oxygen (not: ref. aerobic/anaerobic)	1 1
	(b) (i)	viable only counts living cells/cells which are reproducing/total count includes living and dead cells	1
	(ii)	9 cm^3 of sterile distilled water in each tube; (not: tap water)	
		1 cm^3 of sample transferred to tube 1;	
		mixed well or ref. 1 cm^3 transferred next tube in sequence;	
		any reference to aseptic technique. (Any 3) (labelled diagram=2max e.g. 9cm^3 sterile distilled water + 1 cm^3 sample)	3
	(iii)	4	1
	(iv)	take/prepare 4 sterile nutrient agar plates; (idea of each dilution on a different plate needed) transfer 0.5 cm^3 /other specified volume of each to a separate plate; (allow: 0.1-1 cm^3) spread with sterile spreader; incubate plates at suitable temp./25°C; (allow: 25-40°C) count colonies; 1 colony=1 cell. (any 4 in correct sequence)	4
			Total 14 marks

8. (a) (i) spherical / round / circular [**not:** oval] [1]

(ii) Contains peptidoglycan / murein / glycoprotein

Do not have (or little) outer layer with lipopolysaccharide /

lipoprotein [Reference to dye retention = 0] [1]

(iii) Grow better in the presence of oxygen but can survive in its absence. [1]

(b) (i) each cell produces one colony [1]

(ii) underestimate / doesn't allow for clumping [1]

(c) includes dead bacteria [1]

(d) serial dilution [1]

[Total 8 marks]

9.

Question			Marking details	Marks Available
	(a)		A Obligate aerobe B Facultative anaerobe C Obligate anaerobe All 3 correct for 2 marks 2 correct for 1 mark 0/1 correct = 0 marks **Question 3 Total**	2 **[2]**

10.

Question	Marking details	Marks Available					
		AO1	AO2	AO3	Total	Maths	Prac
(a)	A *Staphylococcus*(1) B *Lactobacillus* (1) C *Neisseria* (1) D *E. coli* (1)			4	4		4
(b)	D value = 13 minutes = 3 marks **If incorrect award 2 marks for** Two correct readings from two consecutive whole numbers e.g. 36-23 **If incorrect award 1 mark for** Two lines shown on graph between two consecutive whole numbers		3		3	3	
(c)	**Any four (× 1) from:** A. 10×/100× series of dilutions/1+9/0.1+9.9cm^3(1) B. Volume 1cm^3 (or less) plated onto each plate (1) C. Incubated at 25-37°C for 24-48 hours (1) D. Number of colonies counted (1) E. Use of dilution factor and calculation of numbers of bacteria (per cm^3 in original sample)/ or description of (1) F. Repeat (×3) and calculate a mean (1)	4			4		
(d)	A. Increased kinetic energy + {breaks hydrogen bonds/ denatures protein} (1) B. Holding together the specific (3-D) shape/ shape of active site changes (1) C. Protein can no longer carry out function/ enzyme- substrate complexes {can no longer form/ reduced} (1)	3			3		
	Question 10 total	**7**	**3**	**4**	**14**	**3**	**4**

11.

Question		Marking Details	Marks Available					
			AO1	AO2	AO3	TOTAL	Maths	Prac
(a)		Accept any answer in the range 613 000 - 624 000 = 3 marks Accept in standard form If incorrect award 2 marks for either 100/(0.09 × 0.09) × 3.14 (π) × 156 100/0.025(43) × 156 If incorrect award 1 mark for area calculation 0.09 × 0.09 × 3.14 (π)		3		3	3	
(b)	i	Serial dilution description (of tenfold/ hundredfold) e.g. 1 cm^3 of sample and 9 cm^3 of {water/ growth medium} mixed (gives 10^{-1}) (1) or equivalent (does not have to give 10^{-5}) Water/ saline/ equipment must be sterile / reference to aseptic technique(1) Repeated four more times/ method to achieve a 10^{-5} dilution (1) Accept annotated diagram	3			3		3
	ii	Plate 5 (1) (Plate 1/2/3/4)- cannot distinguish individual colonies/ too many colonies to count accurately /merged colonies (might provide underestimate) (1) (Plate 6) has too few colonies to provide an accurate estimate (closer to the true value)/ too few to be statistically significant (1) Penalise bacteria once only			3	3		3
	iii	37°C is body temperature which is optimum for these bacteria/ 37°C grows the bacteria found in the patient / 37°C grows the bacteria found in the patient faster (1)		1		1		1
	iv	Thick {murein/peptidoglycan} layer (1) No lipopolysaccharide layer (1)	2			2		
		QUESTION 11 TOTAL	**5**	**4**	**3**	**12**	**3**	**7**