Required Practical 6, Aseptic Technique: AQA A Level Biology Exam Que

18 questions · 39 marks · about 51 minutes. Answer in the spaces, then check the mark schemes at the end.

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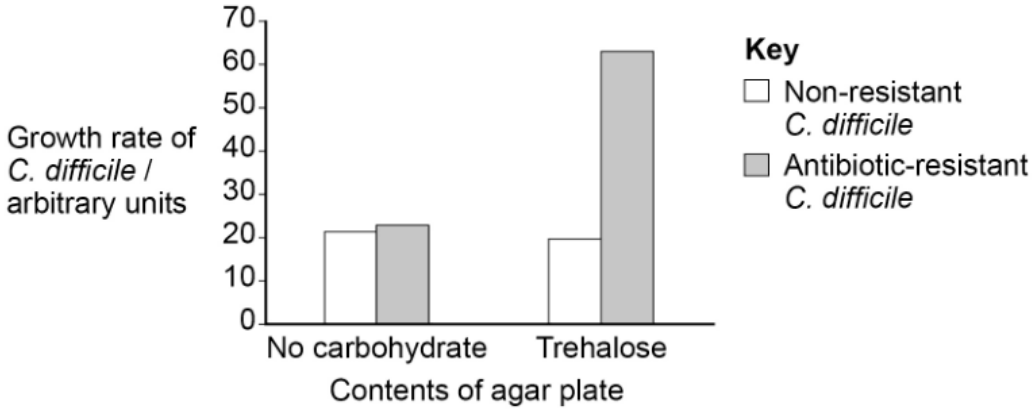
Scientists investigated the effect of trehalose on the growth rate of *C. difficile*. They grew populations of non-resistant and antibiotic-resistant *C. difficile* on separate agar plates with:

- no carbohydrate added
- trehalose added.

They measured the growth rate of the *C. difficile*.

Figure 2 shows the scientists' results.

Figure 2



0 2 . 3 Describe how the scientists could use aseptic techniques to transfer 0.3 cm^3 of *C. difficile* in liquid culture from a bottle onto an agar plate.

[3 marks]

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Question 2 · AS Paper 2 · June 2019 · Q06.1 · 3 marks

0 6 A scientist crossed a strain of the fungus *Neurospora* producing pink spores with a strain of *Neurospora* producing white spores.

To cross these strains, he used aseptic techniques. He moved a small agar cube containing one strain of the fungus onto a new agar plate. Then he placed a second agar cube containing the other strain of fungus next to the first agar cube.

0 6 . 1 Describe and explain **three** ways in which the scientist would ensure he used aseptic techniques to move each cube of agar onto a new agar plate.

[3 marks]

1 _____

2 _____

3 _____

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Question 3 · AS Paper 2 · June 2025 · Q08.2 · 2 marks

One type of infection is pneumonia, caused by the bacterium *Streptococcus pneumoniae*.

A trainee microbiologist cultured *S. pneumoniae* on an agar plate.

The trainee microbiologist:

- disinfected the laboratory bench
- used a sterile pipette to transfer *S. pneumoniae* from a broth bottle to an agar plate
- took a glass spreader and spread the *S. pneumoniae* across the agar plate
- sealed the lid with four strips of tape.

0 8 . 2 Suggest **one** other action the trainee microbiologist took during this method to ensure the technique was aseptic.

Justify why this action was needed.

Do **not** refer to gloves, masks or safety glasses in your answer.

[2 marks]

Action _____

_____

_____

Justification _____

_____

_____

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Question 4 · AS Paper 1 · June 2017 · Q02.1 · 2 marks

0 2 A student investigated the effect of three types of disinfectant on the growth of *Lactobacillus* bacteria.

During the investigation, the student:

- boiled the agar before pouring the agar plates
- transferred 0.5 cm^3 of a diluted liquid culture of *Lactobacillus* onto each agar plate
- left some agar plates as controls
- added to other agar plates different concentrations of the disinfectants as shown in **Table 1** on page 5.

After 2 days, she counted the number of colonies of bacteria on each agar plate.

0 2 . 1 Explain the purpose of:

[2 marks]

boiling the agar _____

_____

_____

_____

transferring the same volume of liquid culture onto each agar plate.

_____

_____

_____

_____

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0 8 A student investigated the effect of two antimicrobial substances, **J** and **K**, on the growth of *E. coli* bacteria.

She transferred *E. coli* cells using a sterilised pipette to make three identical cultures, **1**, **2**, and **3**. She then added:

- no antimicrobial substance to culture **1**
- antimicrobial substance **J** to culture **2**
- antimicrobial substance **K** to culture **3**.

She incubated the cultures for 24 hours, after which she determined the number of cells per mm^3 in each culture.

0 8 . 1 The student used a sterilised pipette to transfer *E. coli* into each culture.

Suggest why the number of *E. coli* cells per mm^3 in each culture after 24 hours might have been lower if the student had **not** used a sterilised pipette. Explain your answer.

[2 marks]

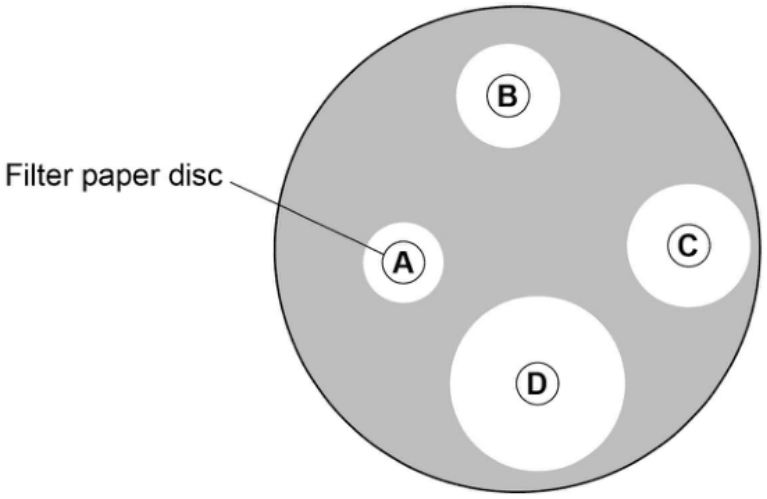
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Question 6 · AS Paper 2 · June 2016 · Q08.1 · 1 mark

0 8 A student investigated the effectiveness of four different concentrations, **A**, **B**, **C** and **D**, of the same disinfectant on the growth of a bacterium. He grew a culture of this bacterium on nutrient agar (a solid growth medium) in a Petri dish. The student then cut out four filter paper discs and soaked each disc in one of the four concentrations. He then placed the discs on the nutrient agar in the Petri dish. He then left the Petri dish at 25 °C for 24 hours.

Figure 8 shows the appearance of the Petri dish after 24 hours.

Figure 8



0 8 . 1 Explain why there is a clear zone around each paper disc.

[1 mark]

Question 7 · AS Paper 2 · June 2016 · Q08.2 · 1 mark

0 8 . 2 The student researched information on this disinfectant prior to carrying out his investigation. On the basis of this research, the student used a maximum concentration of disinfectant of 40%.

Use **Figure 8** to explain why.

[1 mark]

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Question 8 · AS Paper 2 · June 2016 · Q08.3 · 2 marks

0 8 . 3 Suggest **two** variables the student should control in using the filter paper discs in this investigation.

[2 marks]

1 _____

_____

2 _____

_____

Question 9 · AS Paper 2 · June 2016 · Q08.4 · 2 marks

0 8 . 4 Use the areas of the clear zones in **Figure 8** to determine how many times more effective concentration **D** is than concentration **B**. Show your working.

[2 marks]

Answer = _____

Question 10 · information from earlier in the question

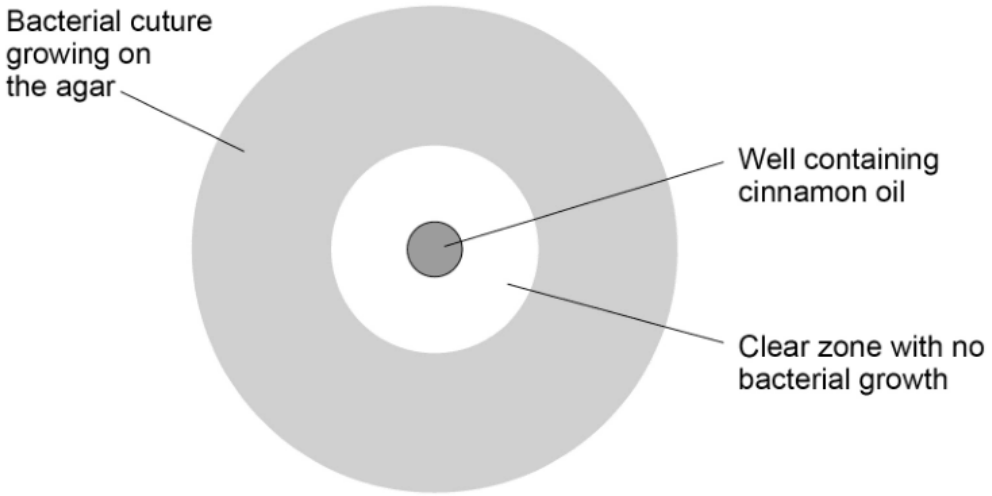
0 5 A student investigated the use of cinnamon oil as an antimicrobial substance. She investigated the effect of cinnamon oil on the growth of five different bacterial cultures grown on agar plates.

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The student kept the plates at 25 °C for 24 hours.

Figure 5 shows what one of her plates looked like after 24 hours.

Figure 5



The student measured the diameter of the clear zone with no bacterial growth around each well. She made these measurements to the nearest whole mm

Table 1 shows her results.

Table 1

Bacterial culture	**Diameter of clear zone / mm**		
	Cinnamon oil	Positive control	Negative control
Bacillus spp.	15	14	0
Staphylococcus aureus	20	17	0
Listeria monocytogenes	18	12	0
Escherichia coli	16	12	0
Klebsiella spp.	14	12	0
Median for all cultures			0
Mean for all cultures			0
Standard deviation for all cultures	2.4	2.2	0

0 5 . 3 Suggest exactly what the student added to the wells to get the positive control **and** negative control results.

[2 marks]

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Question 11 · information from earlier in the question

0 2 A student investigated the effect of three types of disinfectant on the growth of *Lactobacillus* bacteria.

During the investigation, the student:

- boiled the agar before pouring the agar plates
- transferred 0.5 cm^3 of a diluted liquid culture of *Lactobacillus* onto each agar plate
- left some agar plates as controls
- added to other agar plates different concentrations of the disinfectants as shown in **Table 1** on page 5.

After 2 days, she counted the number of colonies of bacteria on each agar plate.

The three disinfectants used by the student were Lysol, propan-2-ol and ammonia.

Table 1 shows the student's results.

Table 1

Concentration of disinfectant / arbitrary units	Number of colonies of bacteria		
	Lysol	Propan-2-ol	Ammonia
0	300	300	300
5	0	290	300
10	0	195	295
15	0	0	275
20	0	0	240

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Question 11 · AS Paper 1 · June 2017 · Q02.3 · 2 marks

0 2 . 3 The student concluded that the minimum concentration of propan-2-ol needed to stop the growth of *Lactobacillus* was 15 units. This conclusion is incorrect.

Describe how you could obtain a more accurate estimate of the minimum concentration of propan-2-ol needed to stop the growth of this species of bacterium.

[2 marks]

Question 12 · information from earlier in the question

0 6 . 1 Disinfectants are used to kill microorganisms on non-living surfaces. A student investigated the effect of different concentrations of disinfectant **X** on the growth of *Bacillus subtilis*.

The student:

- added 5 cm^3 of a different concentration of disinfectant **X** to 5 different test tubes
- added 5 cm^3 of distilled water to another test tube
- added 2 cm^3 of a culture of *B. subtilis* to all 6 test tubes
- incubated the test tubes at 25 °C for 24 hours
- used a colorimeter to record the percentage of light absorbed by the contents of each tube.

Table 2 shows the student's results.

Table 2

Percentage concentration of disinfectant X	0	20	40	60	80	100
Percentage light absorbance	100	100	87	52	10	10

The student prepared the different concentrations of disinfectant **X**.

Describe how the student made 5 cm^3 of the 60% concentration using distilled water and undiluted disinfectant.

[1 mark]

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Question 12 · AS Paper 2 · June 2024 · Q06.3 · 4 marks

0 6 . 3 Use **Table 2** to evaluate the effectiveness of disinfectant **X** at killing microorganisms on non-living surfaces.

[4 marks]

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Question 13 · information from earlier in the question

A student investigated the effect of **two** antibiotics on the growth of the bacterium *Micrococcus luteus*.

During the investigation, the student:

- transferred 9 cm^3 of a liquid culture of *M. luteus* into each of **three** bottles
- added the antibiotic chloramphenicol to the first bottle
- added the antibiotic novobiocin to the second bottle
- added no antibiotic to the third bottle.

After 24 hours, he diluted the contents of each bottle by 1 in 100 000 (10^{-5}). He then transferred 0.25 cm^3 samples from the first bottle onto each of 3 separate agar plates. He repeated this with 0.25 cm^3 samples from the second bottle and the third bottle, resulting in 9 agar plates in total.

He incubated the plates for 48 hours.

Table 1 shows the number of colonies of bacteria he counted on each plate after 48 hours' incubation.

Table 1

Plate	Number of colonies of bacteria		
	with chloramphenicol	with novobiocin	with no antibiotic
1	2	238	276
2	4	263	258
3	6	261	324

Questions, mark schemes and examiners' report extracts © AQA.

Question 13 · A level Paper 3 · June 2023 · Q03.4 · 2 marks

0 3 . 4 *M. luteus* is **not** resistant to chloramphenicol.

Suggest **two** reasons why the bacteria were able to grow in the culture containing chloramphenicol.

[2 marks]

1 _____

_____

_____

2 _____

_____

_____

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Question 14 · AS Paper 2 · June 2018 · Q08.1 · 3 marks

0 8 . 1 A student used a dilution series to investigate the number of cells present in a liquid culture of bacteria.

Describe how he made a 1 in 10 dilution and then used **this** to make a 1 in 1000 dilution of the original liquid culture of bacteria.

[3 marks]

Question 15 · AS Paper 2 · June 2018 · Q08.2 · 2 marks

0 8 . 2 Using an optical microscope, the student determined there were 15 cells in 0.004 mm^3 of the 1 in 1000 dilution of the culture.

Calculate the number of cells in 1cm^3 of undiluted liquid culture.

[2 marks]

Answer = _____ Number of cells

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Question 16 · AS Paper 2 · June 2018 · Q08.3 · 2 marks

0 8 . 3 The student looked at cells in the 1 in 10 dilution during his preliminary work. He decided **not** to use this dilution to determine the number of cells in the undiluted liquid culture.

Suggest an explanation for the student's decision.

[2 marks]

Question 17 · information from earlier in the question

0 2 A student investigated the effect of three types of disinfectant on the growth of *Lactobacillus* bacteria.

During the investigation, the student:

- boiled the agar before pouring the agar plates
- transferred 0.5 cm^3 of a diluted liquid culture of *Lactobacillus* onto each agar plate
- left some agar plates as controls
- added to other agar plates different concentrations of the disinfectants as shown in **Table 1** on page 5.

After 2 days, she counted the number of colonies of bacteria on each agar plate.

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The three disinfectants used by the student were Lysol, propan-2-ol and ammonia.

Table 1 shows the student's results.

Table 1

Concentration of disinfectant / arbitrary units	Number of colonies of bacteria		
	Lysol	Propan-2-ol	Ammonia
0	300	300	300
5	0	290	300
10	0	195	295
15	0	0	275
20	0	0	240

0 2 . 2 The liquid culture the student transferred was diluted by 1 in 10 000 (10^{-4}).

Use information in this question to calculate how many bacteria were present in 1 cm^3 of undiluted liquid culture.

[2 marks]

Answer = _____

Questions, mark schemes and examiners' report extracts © AQA.

A student investigated the effect of **two** antibiotics on the growth of the bacterium *Micrococcus luteus*.

During the investigation, the student:

- transferred 9 cm^3 of a liquid culture of *M. luteus* into each of **three** bottles
- added the antibiotic chloramphenicol to the first bottle
- added the antibiotic novobiocin to the second bottle
- added no antibiotic to the third bottle.

After 24 hours, he diluted the contents of each bottle by 1 in 100 000 (10^{-5}). He then transferred 0.25 cm^3 samples from the first bottle onto each of 3 separate agar plates. He repeated this with 0.25 cm^3 samples from the second bottle and the third bottle, resulting in 9 agar plates in total.

He incubated the plates for 48 hours.

Table 1 shows the number of colonies of bacteria he counted on each plate after 48 hours' incubation.

Table 1

Plate	Number of colonies of bacteria		
	with chloramphenicol	with novobiocin	with no antibiotic
1	2	238	276
2	4	263	258
3	6	261	324

0 3 . 2 Calculate the mean number of bacteria in the **undiluted** bottle of liquid culture containing novobiocin.

Give your answer in standard form. Show your working.

[2 marks]

Answer _____ bacteria

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Mark schemes

Questions, mark schemes and examiners' report extracts © AQA.

Question 1 · A level Paper 1 · June 2022 · Q02.3 · 3 marks

02.3	1. Wash hands with soap **OR** Disinfect surfaces; 2. Use sterile pipette/syringe (to transfer bacteria); 3. (Remove bottle lid and) flame neck of bottle; 4. Lift lid of (agar) plate at an angle; 5. Work close to upward air movement; 6. Use sterile spreader; 7. Place pipette/spreader into disinfectant (immediately after use);	3 max (3 x AO1)

1. Ignore sterilise hands OR surfaces

1. Accept sanitise for disinfect

1. Accept antiseptic /antimicrobial/alcohol (wipes)

1. and 7 Accept a named type of disinfectant

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Question 2 · AS Paper 2 · June 2019 · Q06.1 · 3 marks

06.1	1. Wash hands **to** remove/kill microbes **OR** Wear gloves/apron **to** prevent contamination; 2. Burning Bunsen close by **to** create upward current of air; 3. Disinfect bench/work on disinfected cloth **to** kill microbes/prevent contamination; 4. Flame instrument/equipment **to** sterilise/kill microbes/prevent contamination; 5. Lift lid slightly **to** prevent entry of microbes;	3 max

Each **must** include an explanation

3. Accept use sterile/ antiseptic/bleach for 'disinfect'

4. Accept autoclave/ use alcohol/steam/boil for flame

4. Accept scalpel/ inoculating loop/wire loop/loop/forceps/ spreader for instrument

1. 3. 4. & 5. Accept microorganisms/ spores/bacteria/fungi/ contaminants for 'microbes'

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08.2	**Mark as pairs 1 and 2 or 3 and 4 or 5 and 6 or 7 and 8. Justification must match action.** 1. Lift lid of (agar) plate at an angle; 2. To prevent contaminating the dish **OR** Prevent spread of bacteria outside the dish; 3. Flame the spreader **OR** Use a sterile spreader **OR** Flame the neck of the container of the culture; 4. To maintain a pure culture **OR** To prevent contaminating the dish **OR** Prevent spread of bacteria outside the dish; 5. Work close to a Bunsen burner/flame; 6. To create a convection current to carry other contaminants away/prevent them landing on the dish **OR** To create upward air movement to carry other contaminants away/prevent them landing on the dish; 7. Place used pipette/spreader into (container of) disinfectant; 8. To ensure no transfer of bacteria to bench;	2 max (2 x AO3)

1. Accept lift lid slightly **OR** keep lid

Question 4 · AS Paper 1 · June 2017 · Q02.1 · 2 marks

02.1	1. So no contamination/ other bacteria; 2. So same number of bacteria transferred **to** allow comparison;	2	1. Accept sterilisation / kills all (bacteria) 2. Allow amount / concentration for number

Question 5 · AS Paper 1 · June 2022 · Q08.1 · 2 marks

08.1	1. Unknown/new/different microorganisms/pathogens/microbes/bacteria (introduced); 2. (these bacteria) use food/space **OR** (these bacteria) produce toxins;	2 (2 x AO2)

1. Ignore chemical contaminant 2. Accept description of competition for other resources

Question 6 · AS Paper 2 · June 2016 · Q08.1 · 1 mark

08.1	Bacteria killed;	1	Ignore: no growth or growth of bacteria prevented. Accept: bacteria destroyed. Accept: no living bacteria.

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Question 7 · AS Paper 2 · June 2016 · Q08.2 · 1 mark

08.2	1. Clear zone would be too large **OR** Clear zones would overlap/merge **OR** Could kill all bacteria (on the plate);	1	1. Must convey idea of too large.

Question 8 · AS Paper 2 · June 2016 · Q08.3 · 2 marks

08.3	1. (Same) size; 2. (Same) material/absorbency; 3. In solution for same time period;	2 max	1. Accept: any measure of size e.g. thickness, area, diameter. 1. Ignore: 'same shape' as shape shown on Figure 8. Ignore: reference to volume of disinfectant.

Question 9 · AS Paper 2 · June 2016 · Q08.4 · 2 marks

08.4	Any number between 2.5 to 3.2 = **two** marks;;	2	Allow **one** mark for an incorrect answer but shows method of calculating how many times more effective D is than B e.g. 22 divided by 13/14 or 11 divided by 6.5/7 or 1.57/1.6/1.69/1.7.

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Question 10 · A level Paper 1 · June 2023 · Q05.3 · 2 marks

05.3	(Positive control) 1. Antimicrobial/ antibacterial (solution) **OR** Antibiotic **OR** Antiseptic/disinfectant; (Negative control) 2. (Sterile) water **OR** Oil (without cinnamon);	2 (2 x AO3)

If not specified, accept the first answer as being for the positive control

1. Accept named antimicrobial/antibacterial/ antibiotic/antiseptic/disinfectant

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02.3	1. (Several) values between 10 and 15 (units); 2. Repetitions of each;	2

1. Accept descriptions of this

Ignore repeat the investigation / repeat at 10 and 15 units.

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06.3	1. Equally effective at 80% as at 100%; 2. Not effective at 20% **OR** Need higher than 20% concentration to be effective; 3. Light absorbance may not indicate number of living bacteria/cells **OR** Dead cells/bacteria absorb light **OR** Don't know if cells alive/dead at 80/100 %; 4. 100% not totally effective as still 10%/some absorbance; 5. Only (tested on) one species; **OR** Only tested on *B. subtilis*; 6. Only tested in laboratory/test tube **OR** May not be as effective on other surfaces; 7. Only (tested) at 25°C;

4 max (4 x AO3)	6. Accept any description which indicates not tested

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03.4	1. The concentration (of chloramphenicol) was too low/dilute (to kill all) **OR** There were too many *M. luteus*/bacteria for chloramphenicol/antibiotic to kill; 2. (Contamination with) other/resistant bacteria; 3. Mutation (occurred);	2 max (2 x AO3)

Accept as an additional mark point

4. Chloramphenicol/ antibiotic not (evenly) mixed in the liquid culture/bottle

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08.1	1. Add 1 part (bacteria) culture to 9 parts (sterile) liquid (to make 10^{-1} dilution); 2. Mix (well); 3. Repeat using 9 parts fresh (sterile) liquid and 1 part of 10^{-1} and 10^{-2} dilutions to make 10^{-3} dilution; OR Add 1 part 10^{-1} (suspension) to 99 parts (sterile) liquid (to make 10^{-3} dilution);	3

1. and 3. Accept water / nutrient / broth for liquid
2. Accept stir
3. Reject 1 part (undiluted) culture added to 999 parts liquid

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Question 15 · AS Paper 2 · June 2018 · Q08.2 · 2 marks

08.2	3.75×10^9 / 3 750 000 000;;	2	Accept for 1 mark: 3750 000 / 3.75×10^6 (cells per mm^3) OR 3.75×10^{12} (wrong volume conversion) OR 3750 (cells per mm^3 of diluted culture) OR Evidence of using correct dilution conversion and correct volume conversion, i.e., x1000 and x 1000

Question 16 · AS Paper 2 · June 2018 · Q08.3 · 2 marks

08.3	1. **Count** unlikely to be accurate / repeatable / reproducible / reliable;	2
	2. Because too many cells; OR Because cells overlapping / not spread out;	

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Question 17 · AS Paper 1 · June 2017 · Q02.2 · 2 marks

02.2	6 000 000 OR 6×10^6;;	2	1 mark for 3 000 000 OR 3×10^6 Allow 1 mark for 600 (in $1cm^3$ of diluted culture)

Question 18 · A level Paper 3 · June 2023 · Q03.2 · 2 marks

03.2	A correct answer of $9(.144) \times 10^8$ **= 2 marks**;; Answer not in standard form (914 400 000) **= 1 mark** **OR** Answer in incorrect standard form **= 1 mark** **OR** 1.016×10^8 (everything else correct except failed to multiply by 9) **= 1 mark** **OR** 2.286×10^8 (everything else correct but failed to take the plating of 0.25 cm^3 into account) **= 1 mark** **OR** 9.432×10^8 (discounted 238 as anomalous, all other calculations correct) **= 1 mark**	2 (2 x AO2)

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