PAG 7 Microbiological Techniques: OCR A Level Biology Exam Question

15 questions · 29 marks · about 35 minutes. Answer in the spaces, then check the mark schemes at the end.

Question 1 · A level Paper 2 · June 2022 · Q17(a)(i) · 1 mark

17 Some students investigated the effect of time on the growth of bacterial populations.

The students prepared a large flask of bacterial culture.

They divided this large culture into a number of smaller flasks each containing 50 cm^3 of bacterial culture.

They then incubated the smaller flasks at 20 °C for up to 48 h.

Every 4 h the students removed one of the flasks and counted the bacteria.

The students recorded the total number of bacteria and the number of viable bacteria in each flask.

(a) When growing bacteria in culture, it is important that aseptic techniques are used.

(i) State why it is important that the technique used for culturing microorganisms be aseptic.

.....

..... **[1]**

Question 2 · A level Paper 3 · June 2017 · Q2(b)(ii) · 2 marks

(ii) Describe **two** practical considerations to ensure the *S. cerevisiae* population grows successfully when the initial culture is established.

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..... **[2]**

Turn over

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Question 3 · information from earlier in the question

19 Two students investigated the growth of bacteria at different temperatures.

Three flasks containing identical solutions of nutrient broth were used.

- Flask 1: inoculated with 1 cm^3 of broth containing the bacterium *Bacillus subtilis* and incubated at 20 °C.
- Flask 2: inoculated with 1 cm^3 of broth containing *B. subtilis* and incubated at 30 °C.
- Flask 3: inoculated with 1 cm^3 of broth containing no bacteria and incubated at 30 °C.

Aseptic techniques were used throughout.

At set times over the next 3 days the students removed samples from each flask and measured the number of viable bacteria present.

(a) State one further variable the students should have controlled in their investigation in order to produce **valid** results.

..... **[1]**

Question 3 · A level Paper 2 · June 2018 · Q19(c)(ii) · 2 marks

(ii) No bacteria were detected at any time in the flask that was inoculated with nutrient broth that did not contain bacteria.

Explain the purpose of this flask.

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..... **[2]**

Question 4 · A level Paper 2 · June 2018 · Q19(c)(iii) · 1 mark

(iii) The teacher told the students they should not investigate the growth of bacteria at 35 °C.

Suggest why the teacher told them not to grow bacteria at 35 °C.

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..... **[1]**

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Question 5 · information from earlier in the question

17 Some students investigated the effect of time on the growth of bacterial populations.

The students prepared a large flask of bacterial culture.

They divided this large culture into a number of smaller flasks each containing 50 cm^3 of bacterial culture.

They then incubated the smaller flasks at 20 °C for up to 48 h.

Every 4 h the students removed one of the flasks and counted the bacteria.

The students recorded the total number of bacteria and the number of viable bacteria in each flask.

(a) When growing bacteria in culture, it is important that aseptic techniques are used.

(i) State why it is important that the technique used for culturing microorganisms be aseptic.

.....

..... **[1]**

Question 5 · A level Paper 2 · June 2022 · Q17(a)(ii) · 2 marks

(ii) The students prepared the culture by adding a suspension of bacteria to a flask containing nutrient broth.

List **two** precautions that should be taken when preparing a bacterial culture in order to ensure that the procedure is aseptic.

1 .....

2 .....

[2]

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Question 6 · A level Paper 2 · June 2022 · Q17(b)(i) · 1 mark

(b) When counting the number of bacteria, the students performed serial dilutions on samples removed from each small flask. In each serial dilution, the students removed 0.1 cm^3 and added it to 9.9 cm^3 of water.

To estimate the total number of bacteria, the students used a light microscope to count the number of bacterial cells in a 0.01 cm^3 sample of the final serial dilution.

To estimate the number of viable bacteria, the students spread 0.1 cm^3 of the final serial dilution on an agar plate and counted the number of colonies that had grown after 24 h.

(i) The students shook each flask before they removed the samples for counting.

Suggest why the students shook the flasks.

.....

..... **[1]**

Question 7 · A level Paper 2 · June 2018 · Q19(a) · 1 mark

19 Two students investigated the growth of bacteria at different temperatures.

Three flasks containing identical solutions of nutrient broth were used.

- Flask 1: inoculated with 1 cm^3 of broth containing the bacterium *Bacillus subtilis* and incubated at 20 °C.
- Flask 2: inoculated with 1 cm^3 of broth containing *B. subtilis* and incubated at 30 °C.
- Flask 3: inoculated with 1 cm^3 of broth containing no bacteria and incubated at 30 °C.

Aseptic techniques were used throughout.

At set times over the next 3 days the students removed samples from each flask and measured the number of viable bacteria present.

(a) State one further variable the students should have controlled in their investigation in order to produce **valid** results.

..... **[1]**

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(b) The students used the following procedure to determine the number of viable bacteria in each flask at a given time.

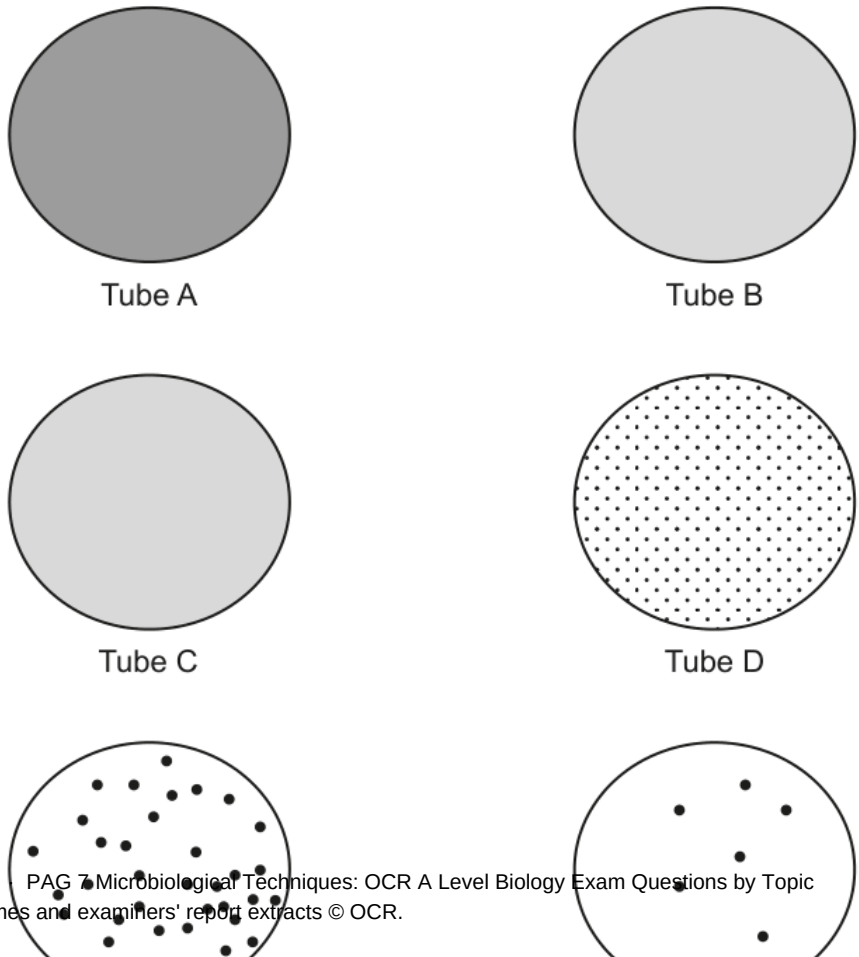
From each flask, 0.1 cm^3 was removed and mixed with 9.9 cm^3 of sterile water in a test tube. This was labelled **Tube A**. A serial dilution then proceeded, as shown in Table 19.1.

Tube	Contents	
B	1 cm^3 of Tube A mixture	9 cm^3 of sterile water
C	1 cm^3 of Tube B mixture	9 cm^3 of sterile water
D	1 cm^3 of Tube C mixture	9 cm^3 of sterile water
E	1 cm^3 of Tube D mixture	9 cm^3 of sterile water
F	1 cm^3 of Tube E mixture	9 cm^3 of sterile water

Table 19.1

From each tube, A–F, 0.1 cm^3 of mixture was cultured on nutrient agar for 24 hours at 30 °C.

The results from Flask 2 after 7 hours of incubation are shown in Fig. 19.



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Question 9 · A level Paper 2 · June 2018 · Q19(b)(ii) · 3 marks

(ii) The students disagreed about which tube's result to use as a starting point for their calculation.

Discuss whether the petri dish resulting from Tube F was the most appropriate for them to use.

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..... **[3]**

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Question 10 · A level Paper 3 · June 2024 · Q1(b)(i) · 2 marks

(b) A student carries out a serial dilution of an *E. coli* culture.

This is the method the student uses:

- Transfer 10 cm^3 of *E. coli* culture to a sterilised test tube from an original culture that has a volume of 50 cm^3.
- Carry out four 10-fold serial dilutions. Each dilution involves transferring 1 cm^3 of culture from one test tube to another test tube containing 9 cm^3 of distilled water.
- Transfer 1 cm^3 of the final 10 cm^3 diluted culture to an agar plate. Evenly spread 1 cm^3 of liquid across the plate using a sterilised spreader.
- Use a micropipette for each transfer.
- Incubate the agar plates for 24 h.
- Count the number of colonies that develop on the plate. Each colony is assumed to develop from a single bacterium.
- Estimate the *E. coli* population in the original 50 cm^3 culture.

3

(i) Describe **two** improvements to the student's method, **other than** using different equipment, that could improve the accuracy of their population estimate.

1 .....

.....

2 .....

.....

[2]

Question 11 · A level Paper 3 · June 2024 · Q1(b)(ii) · 3 marks

(ii) The student counts 22 colonies on the agar plate.

Calculate the total number of *E. coli* cells in the original 50 cm^3 culture.

Give your answer in standard form.

Total number of *E. coli* cells in 50 cm^3 = ... **[3]**

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Question 12 · information from earlier in the question

17 Some students investigated the effect of time on the growth of bacterial populations.

The students prepared a large flask of bacterial culture.

They divided this large culture into a number of smaller flasks each containing 50 cm^3 of bacterial culture.

They then incubated the smaller flasks at 20 °C for up to 48 h.

Every 4 h the students removed one of the flasks and counted the bacteria.

The students recorded the total number of bacteria and the number of viable bacteria in each flask.

(a) When growing bacteria in culture, it is important that aseptic techniques are used.

(i) State why it is important that the technique used for culturing microorganisms be aseptic.

.....

..... **[1]**

(b) When counting the number of bacteria, the students performed serial dilutions on samples removed from each small flask. In each serial dilution, the students removed 0.1 cm^3 and added it to 9.9 cm^3 of water.

To estimate the total number of bacteria, the students used a light microscope to count the number of bacterial cells in a 0.01 cm^3 sample of the final serial dilution.

To estimate the number of viable bacteria, the students spread 0.1 cm^3 of the final serial dilution on an agar plate and counted the number of colonies that had grown after 24 h.

(i) The students shook each flask before they removed the samples for counting.

Suggest why the students shook the flasks.

.....

..... **[1]**

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Question 12 · A level Paper 2 · June 2022 · Q17(b)(iii) · 3 marks

(iii) In one 0.01 cm^3 sample the students counted 52 bacterial cells under the microscope.

Describe the calculation steps the students would then need to make to estimate the total number of bacteria in the small flask.

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..... **[3]**

Turn over

Question 13 · A level Paper 3 · November 2021 · Q2(d)(iii) · 2 marks

(iii) Describe a laboratory procedure that the scientists might have used to estimate the bacterial population.

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..... **[2]**

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Question 14 · A level Paper 3 · June 2019 · Q6(b)(i) · 3 marks

(b) (i) Serial dilutions can be used to estimate the size of a bacterial population in a culture.

A scientist used 20 cm^3 of a bacterial culture that contained 1.0×10^6 bacterial cells.

- 5% of the 20 cm^3 culture was transferred to a new test tube and made up to 10 cm^3 with water.
- An additional ten-fold dilution was carried out, which produced a final 10 cm^3 solution.
- 0.1 cm^3 of the final 10 cm^3 solution was transferred to an agar plate.

Each colony that developed on the agar plate was assumed to represent a single bacterial cell in the bacterial culture.

Estimate the number of colonies that you would expect to develop on the agar plate.

number of colonies = ... **[3]**

Question 15 · A level Paper 3 · June 2019 · Q6(b)(ii) · 1 mark

(ii) A student carried out a different serial dilution to estimate the size of another bacterial population.

The serial dilution resulted in four colonies developing on an agar plate.

Explain why the student's estimation of this bacterial population is likely to be inaccurate.

.....

.....

..... **[1]**

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Mark schemes

Question 1 · A level Paper 2 · June 2022 · Q17(a)(i) · 1 mark

17	**(a)**	**(i)**	prevent contamination (by unwanted microorganisms) ✓ to prevent , entry / growth , of unwanted microorganisms ✓

1 max	1.2	**IGNORE** kill

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Question 2 · A level Paper 3 · June 2017 · Q2(b)(ii) · 2 marks

ii	(use) aseptic techniques / avoid contamination ✓ provide (sources of) nutrients / respiratory substrates ✓ (incubate at) suitable temperature ✓ use (pH) buffer ✓ agitation / stirring / shaking ✓

2 max	**Mark first two suggestions given** **ACCEPT** a description of an aseptic technique **ACCEPT** sterile techniques **ACCEPT** a specific example of a nutrient **ACCEPT** optimum temperature / right temperature /a specific, appropriate temperature (15- 35°C) **IGNORE** keep temperature constant / low temperature/ monitor temperature / control temperature **ACCEPT** maintain optimum pH / right pH /a specific, appropriate pH (4-7) **IGNORE** keep pH constant / monitor pH / control pH **ACCEPT** mixing **IGNORE** ref to aeration / oxygen supply / sparging

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

Question 3 · A level Paper 2 · June 2018 · Q19(c)(ii) · 2 marks

(ii)	<u>control</u> ✓ *idea of* checking for contamination ✓	**2**

DO NOT CREDIT control , group / variable / condition

ALLOW shows growth due only to *B. subtilis*
ALLOW e.g. to ensure conditions were aseptic / if the flask had bacterial growth the results would be invalid

Question 4 · A level Paper 2 · June 2018 · Q19(c)(iii) · 1 mark

(iii)	*idea that* it could encourage the growth of human pathogens ✓

1	**ALLOW** harmful microbes could grow **DO NOT CREDIT** refs to denaturation **IGNORE** bacteria will grow rapidly which could be dangerous

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Question 5 · A level Paper 2 · June 2022 · Q17(a)(ii) · 2 marks

(ii)		use , sterile / autoclaved , flask / pipette / equipment / broth ✓ stopper flask (to prevent contamination) ✓ disinfect / sterilise , surfaces ✓ (nearby) Bunsen flame (to create upward air flow) ✓
2 max	1.2 3.3	*Mark first two answers only or first answer on each prompt line, which ever gives the candidate most benefit.* **ALLOW** 'pasteurise' as AW for 'autoclave' **DO NOT CREDIT** if airtight seal is implied **ALLOW** flame neck (of flask) / remove stopper for minimal time / do not put stopper on bench **ALLOW** wash hands / wear gloves

Question 6 · A level Paper 2 · June 2022 · Q17(b)(i) · 1 mark

(b)	**(i)**	*idea of* so bacterial cells are evenly distributed ✓	**1**	3.4

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Question 7 · A level Paper 2 · June 2018 · Q19(a) · 1 mark

19	(a)	1 volume of broth (in flask) ✓ 2 pH (of broth) ✓ 3 oxygen (concentration in flask) ✓ 4 number / concentration , of bacteria in , broth at beginning / AW ✓ 5 volume removed (from each flask) ✓ 6 (standard) stirring / mixing , before withdrawal of samples ✓	**1 max**

IGNORE 'amount' throughout

4 ALLOW batch of broth / starting population of bacteria
4 IGNORE volume / mass

Question 8 · A level Paper 2 · June 2018 · Q19(b)(i) · 2 marks

(b)	(i)	6.0 / 6 , x 10^7 ✓✓	2	*Max 1 if answer not given as standard form* **ALLOW** 1 mark for 6 x 10^6 / 6 x 10^8

Question 9 · A level Paper 2 · June 2018 · Q19(b)(ii) · 3 marks

(ii)	1 should have used E ✓ 2 (has) most / more , (countable) colonies ✓ 3 *idea that* anomalies will have smaller effect ✓ 4 more representative / larger , sample ✓ 5 (fewer serial dilutions) decreases chance of error ✓ 6 F (is appropriate) because , colonies / AW , are countable ✓	**3 max**

1 Other points can be awarded in the context of plates other than E

2-5 ora for F

4 ALLOW estimate will be more accurate
4 IGNORE valid / reliable / repeatable

6 ALLOW bacteria as AW for colonies for this marking point only

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Question 10 · A level Paper 3 · June 2024 · Q1(b)(i) · 2 marks

1	(b)	(i)	mix (each dilution) ✓ replace (micro)pipette tips between each transfer ✓ repeat the plating (at least three times) and calculate a mean ✓ take photo of final plate so colonies can be counted ✓ reduce the number of, dilutions / transfers (to reduce random error) ✓

2 max	**mark as prose** **ALLOW** stir / shake (test tube before plating) **ALLOW** replace pipettes between each transfer / sterilise pipette between dilutions **IGNORE** 'repeat the experiment' (*as this suggests starting with a new population so does not improve accuracy)* **ALLOW** 'use more than one agar plate and calculate the mean' **IGNORE** 'average' **IGNORE** general ref to aseptic technique e.g. use sterile, water /agar

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Question 11 · A level Paper 3 · June 2024 · Q1(b)(ii) · 3 marks

1	(b)	(ii)	**FIRST CHECK THE ANSWER ON ANSWER LINE** **If answer = 1.1 x 10^7 award three marks** (22 x 10^5 =) 2,200,000 ✓ x 5 (= 11,000,000) ✓ (standard form =) 1.1 x 10^7 ✓	**3**

*If answer is incorrect, **ALLOW ECF** within working for max 2 marks*
If answer = 11,000,000 award 2 marks

ECF from step 1 (e.g. 220,000 x 5 = 1,100,000)

ECF from steps 1 and 2 (e.g. 1,100,000 in standard form = 1.1 x 10^6)
(The third marking point is awarded for the correct use of standard form based on their calculation)

DO NOT ALLOW incorrect use of standard form e.g. 11000000x10^1

ALLOW alternative calculation with same **ECF** from step1 or steps 1 and 2

(22 x 10^4 =) 220,000 ✓
x 50 (cm^3) (= 11,000,000) ✓
(standard form =) 1.1 x 10^7 ✓

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Question 12 · A level Paper 2 · June 2022 · Q17(b)(iii) · 3 marks

(b)	**(iii)**	*Calculate the number in 10 cm^3* **1** multiply , 52 / number of bacteria in sample , by 1000 ✓ **2** *Correct treatment of serial dilutions* multiply by , 100^n (where n is the number of serial dilutions) ✓ **3** *Calculate the total in 50 cm^3* multiply (answer to **1**) by 5 ✓

3	2.8	*Credit steps in any order* **1 ALLOW** if 52 000 seen as part of a calculation **1 ALLOW** 52 x 100 if working out number in $1cm^3$ **3** 52 x 5 000 = 2 marks (**1** and **3**) *If mp1 has not been awarded* **ALLOW** 1 mark for 260 000 **ALLOW** answer written as single formula, e.g., • 52 x 1000 x 100^n x 5 = 3 marks • 52 000 x 100^n x 5 = 3 marks • 52 x 100 x 100^n x 50 = 3 marks (if working out no. in $1cm^3$ first) • 100^n x 260 000 = 2 marks (steps not clearly described)

Question 13 · A level Paper 3 · November 2021 · Q2(d)(iii) · 2 marks

iii	serial dilution ✓ *idea of* grow colonies (on agar plate) and count number of colonies ✓ *idea of* scale up /multiply up, count (to estimate population size) ✓

2 max	2.7	**ALLOW** *idea of* flow cytometry / described

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Question 14 · A level Paper 3 · June 2019 · Q6(b)(i) · 3 marks

b	**i**	50 ✓✓✓	**3**	If the answer is incorrect, award one mark for (1,000,000 / 20 =) 50000 / 5 x10^4
				and one mark for (50,000 / 10 =) 5000 / 5 x 10^3

Question 15 · A level Paper 3 · June 2019 · Q6(b)(ii) · 1 mark

ii	*idea of* (with low colony numbers) small (random) differences (in plating) produce large errors when estimating / scaling ✓	**1**

DO NOT ALLOW assumes bacteria are equally distributed when removing the sample

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