Antiseptics, antibiotics and bacterial growth

Answers, marking points and revision notes | AQA GCSE Biology 8461 only (not Combined Science)

GCSE science required practicals

This practical online

Answers

1 Name the independent variable and the dependent variable in Zainab's investigation. (2 marks)

The independent variable is the type of antiseptic. The dependent variable is the size (diameter or area) of the clear zone around the disc.

Marking points

- independent: type of antiseptic
- dependent: size / diameter / area of the clear zone

2 Zainab cut all the paper discs with the same hole punch. Explain why the discs needed to be the same size. (2 marks)

A bigger disc would hold more antiseptic and cover more agar, so it could give a bigger clear zone even if the antiseptic was no stronger. Keeping the discs the same size means any difference in the clear zone is caused only by the type of antiseptic.

Marking points

- a bigger disc holds more antiseptic / covers more agar so would give a bigger zone
- so the only thing affecting the zone size is the type of antiseptic (fair test)

3 Why did Zainab include a disc soaked in sterile water? (1 mark)

It is a control, to show that the paper disc on its own does not stop bacteria growing, so any clear zone is caused by the antiseptic.

Marking points

- control to show the disc / water alone does not stop growth, so clear zones are due to the antiseptic

4 Describe **three** steps Zainab should take to stop other microbes getting onto her agar plate. (3 marks)

She should pass the forceps through a Bunsen flame and let them cool before using them. She should lift the lid of the Petri dish only a little and for as short a time as possible. She should work close to a lit Bunsen burner so that the rising air carries microbes up and away from the plate.

Marking points

- sterilise equipment, e.g. flame the forceps or use sterile pipettes and spreaders
- open the lid only slightly / for as short a time as possible
- work near a Bunsen flame / disinfect the bench first

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5 After placing the discs, Zainab taped the lid on with two short strips of tape and incubated the plate at 25 °C.

a. Why must the lid not be sealed all the way round?

b. Why is the plate incubated at 25 °C rather than 37 °C? (3 marks)

a. So that oxygen can still get into the dish. Without oxygen, anaerobic bacteria, some of which are harmful to humans, could grow.

b. 37 °C is body temperature, so microbes that cause disease in humans would grow well. At 25 °C they are much less likely to grow.

Marking points

- (a) lets oxygen in
- (a) stops harmful anaerobic bacteria growing
- (b) 37 °C would encourage pathogens that grow at human body temperature

6 Look at the results for Antiseptic B.

a. Which value is anomalous?

b. What should Zainab do with this value when working out the mean? (2 marks)

a. 9 mm on plate 3.

b. Leave it out and work out the mean from the other two values.

Marking points

- (a) 9 mm (plate 3)
- (b) ignore it / leave it out of the mean

7 Calculate the mean diameter of the clear zone for Antiseptic B, leaving out the anomalous result. (2 marks)

(22 + 21) ÷ 2 = 21.5 mm

mean = (22 + 21) ÷ 2

mean = 43 ÷ 2

mean diameter = 21.5 mm

Marking points

- adds 22 and 21 and divides by 2
- 21.5 mm

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8 Use your answer to the last question to calculate the area of the clear zone for Antiseptic B.

Use area = πr^2. Give your answer to 3 significant figures. Show your working. (3 marks)

Radius = 21.5 ÷ 2 = 10.75 mm. Area = π × 10.75^2 = 363.05... = 363 mm^2.

radius = diameter ÷ 2

radius = 21.5 mm ÷ 2 = 10.75 mm

area = πr^2

area = π × 10.75^2

area = π × 115.5625

area = 363.05... mm^2

area = 363 mm^2 (3 significant figures)

Marking points

- radius = 21.5 ÷ 2 = 10.75 mm
- area = π × 10.75^2 = 363.05...
- 363 mm^2 to 3 significant figures

9 The mean diameter of the clear zone for Antiseptic A is 14 mm. Calculate the area of this clear zone to 3 significant figures. (2 marks)

Radius = 7 mm. Area = π × 7^2 = 153.9... = 154 mm^2.

mean diameter = (14 + 15 + 13) ÷ 3 = 14 mm

radius = 14 mm ÷ 2 = 7 mm

area = π × 7^2 = π × 49

area = 153.9... mm^2

area = 154 mm^2 (3 significant figures)

Marking points

- radius 7 mm and uses π × 7^2
- 154 mm^2

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10 **Higher tier**

On another plate, a new antiseptic gave a circular clear zone with an area of 254 mm^2.

Calculate the diameter of this clear zone. Give your answer to 3 significant figures. Show your working.

(3 marks)

$r^2 = 254 \div \pi = 80.85...$, so $r = \sqrt{80.85...} = 8.99$ mm, and the diameter $= 2 \times 8.99... = 18.0$ mm.

area $= \pi r^2$, so $r^2 =$ area $\div \pi$

$r^2 = 254 \div \pi$

$r^2 = 80.85...\ mm^2$

$r = \sqrt{80.85...}$

$r = 8.991...$ mm

diameter $= 2 \times 8.991...$

diameter $= 17.98... = 18.0$ mm (3 significant figures)

Marking points

- rearranges to r^2 = area $\div \pi$ (= 80.85...)
- square root gives r = 8.99 mm
- diameter = 18.0 mm

11 **Higher tier**

A broth starts with 500 bacteria. Under the conditions used, the bacteria divide once every 25 minutes.

Calculate how many bacteria there will be after 5 hours. Give your answer in standard form to 2 significant figures. Show your working.

(3 marks)

5 hours = 300 minutes, so there are $300 \div 25 = 12$ divisions. Number $= 500 \times 2^{12} = 500 \times 4096 = 2\ 048\ 000 = 2.0 \times 10^6$ bacteria.

time $= 5 \times 60 = 300$ minutes

number of divisions $= 300 \div 25 = 12$

number of bacteria $= 500 \times 2^{12}$

$2^{12} = 4096$

number of bacteria $= 500 \times 4096 = 2\ 048\ 000$

number of bacteria $= 2.0 \times 10^6$ (2 significant figures)

Marking points

- number of divisions $= 300 \div 25 = 12$
- $500 \times 2^{12} = 2\ 048\ 000$
- 2.0×10^6

12 Zainab concluded that Antiseptic B is the best at stopping bacterial growth. Use the results to explain whether she is right.

(2 marks)

Yes. Leaving out the anomaly, Antiseptic B has the largest mean clear zone (21.5 mm) compared with 18 mm for D, 14 mm for A and about 8.7 mm for C, so it stopped bacteria growing over the largest area.

Marking points

- B has the largest (mean) clear zone
- supported by figures, e.g. 21.5 mm compared with 18 mm for D

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13 Some of the clear zones were not perfect circles, and the edges were fuzzy.

a. Suggest how Zainab could get a more accurate measurement of a zone that is not a perfect circle.

b. The three results for Antiseptic D were 18 mm, 17 mm and 19 mm. What does this tell you about the precision of these results?

(3 marks)

a. Measure the diameter in two directions at right angles to each other and take the mean of the two, so a zone that is wider one way is not over- or under-estimated.

b. The values are close together, within 2 mm of each other, so the results are precise and repeatable.

Marking points

- (a) measure the diameter across two (or more) directions at right angles
- (a) and use the mean of the measurements
- (b) the values are close together so they are precise / repeatable

14 Plan an investigation to find how the concentration of one antiseptic affects the growth of bacteria on agar plates.

(6 marks)

Make up the antiseptic at several concentrations, for example 100%, 75%, 50%, 25% and 0% (sterile water), by mixing measured volumes of antiseptic and sterile water with a pipette. Wipe the bench with disinfectant and work near a lit Bunsen burner. Lifting the lid only slightly, spread the same volume of the same bacterial culture evenly over a nutrient agar plate with a sterile spreader. Using flamed and cooled forceps, dip same-sized paper discs into each concentration for the same time and place them well apart on the agar. Tape the lid with two strips, not all the way round, label the base and incubate upside down at 25 °C for 48 hours. Without opening the plate, measure the diameter of each clear zone in two directions at right angles and take a mean. Set up three plates in total and calculate a mean diameter for each concentration, leaving out any anomalies, then work out the area using πr^2. Keep the type of bacterium, the agar, the disc size, the temperature and the incubation time the same. Plot mean area against concentration to see whether a higher concentration gives a bigger clear zone. Wash hands at the end and give the plates to the technician to be sterilised.

Marking points (any 6, with the method in a logical order)

- makes a range of at least four concentrations by diluting with sterile water, including a 0% control
- spreads the same volume of bacteria evenly on each agar plate
- aseptic technique: disinfect bench, flame forceps, lid lifted only slightly, near a Bunsen flame
- same-sized discs soaked in each concentration placed on the agar
- lid taped but not sealed, incubated at 25 °C for a set time (e.g. 48 hours)
- measures diameter of each clear zone (across two directions) without opening the plate
- calculates the area with πr^2
- repeats with more plates and calculates means
- controls: same bacteria, agar, disc size, temperature, time

How it is marked

- 5-6 marks: a clear plan that would work, with a range of concentrations made up by dilution, aseptic technique explained, safe incubation, measurement of clear zones, repeats with means and the main control variables.
- 3-4 marks: the main steps are there (different concentrations, discs on a plate of bacteria, measuring the clear zone) but some detail is missing, such as how the dilutions are made, safe incubation or repeats.
- 1-2 marks: a few relevant ideas, such as putting antiseptic on a plate of bacteria and looking at what grows, with little order or no way of measuring the effect.

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Revision notes: the method

Aim: To compare how well different antiseptics stop the growth of bacteria by measuring the clear zones they produce on an agar plate.

Equipment

- sterile Petri dishes of nutrient agar
- culture of a harmless bacterium in nutrient broth
- sterile pipette or sterile spreader
- sterile paper discs of the same size
- antiseptic solutions to test and sterile water
- forceps
- Bunsen burner and heat-proof mat
- disinfectant for the bench
- clear adhesive tape
- marker pen
- incubator set to 25 °C
- ruler marked in mm

Method

1. Wipe the bench with disinfectant and work near a lit Bunsen burner, whose rising air carries microbes away from the plate.
2. Label the base of an agar plate around the edge with your name, the date and a letter for each disc position.
3. Lift the lid only slightly and use a sterile pipette to add a few drops of the bacterial culture, then spread it evenly over the whole agar surface with a sterile spreader.
4. Using forceps passed through the flame and allowed to cool, dip one paper disc in each antiseptic, and one disc in sterile water as a control.
5. Place the discs on the agar, spaced well apart and away from the edge, and close the lid straight away.
6. Secure the lid with two short strips of tape, but do not seal it all the way round, so that oxygen can still get in.
7. Turn the plate upside down and incubate it at 25 °C for about 48 hours.
8. Without opening the plate, measure the diameter of the clear zone around each disc with a ruler, measuring across two directions at right angles and taking a mean.
9. Repeat with two more plates and calculate a mean diameter for each antiseptic, then work out the area of each clear zone.

Variables

- **Independent:** The type of antiseptic on the paper disc
- **Dependent:** The size of the clear zone around the disc where bacteria have not grown (diameter measured with a ruler, used to work out the area)
- **Control:** the same type and volume of bacterial culture on each plate; the same size of paper disc and the same volume of each antiseptic; the same incubation temperature and time; the same type and depth of agar

Hazards

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Hazard	What could happen	How to reduce the risk
Bacteria from the culture, or harmful microbes from the air that may grow on the plate	infection if microbes are breathed in, swallowed or get into a cut	use aseptic technique, never open the plate after incubation, incubate at 25 °C (not 37 °C) so human pathogens are unlikely to grow, and wash hands afterwards
Bunsen burner flame	burns, or setting fire to hair or alcohol used to clean equipment	tie hair back, keep flammable liquids away from the flame and use the safety flame when not in use
Antiseptic solutions	can irritate the eyes and skin	wear eye protection and handle the discs only with forceps

Key points

- A bigger clear zone means the antiseptic killed or stopped more bacteria
- Area of a clear zone = πr^2, so halve the diameter to get the radius first
- Incubate at 25 °C in school, never 37 °C, which would suit microbes that infect humans
- Tape the lid with a few strips but do not seal it, so oxygen gets in and harmful anaerobic bacteria are not encouraged
- Sterilise equipment and keep the lid lifted only slightly, so no other microbes contaminate the plate
- The disc soaked in sterile water is a control: it shows that the paper disc alone does not stop growth

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