

Antiseptics, antibiotics and plant extracts

Investigate the effects of antiseptics, antibiotics or plant extracts on microbial cultures

For Edexcel GCSE Biology 1B10 and Combined Science 1SC0. **Triple only** This practical is not in Combined Science. Teacher answers start on a new page.

Name

Date

Aim

To find out which antiseptic, antibiotic or plant extract is most effective at stopping bacteria growing, by measuring the clear zone around discs on a bacterial lawn.

Background

Bacteria can be grown in the laboratory on nutrient agar jelly in a petri dish. The agar and dish are sterilised first (in an autoclave) so that only the bacteria you want to study grow. **Aseptic technique** stops other microorganisms getting in and stops the bacteria you are growing escaping.

Antibiotics are medicines that kill bacteria or stop them growing, without harming the host's cells.

Antiseptics are chemicals used on living tissue, such as skin, to kill bacteria. Some plants make chemicals that also stop bacteria growing, so plant extracts can be tested in the same way.

If a substance stops bacteria growing, the bacteria close to it die or cannot divide. This leaves a **clear zone** (an inhibition zone) in the lawn around the disc. The bigger the clear zone, the more effective the substance. The clear zone is roughly circular, so its area is found using πr^2 , where r is the radius.

Hypothesis

The antibiotic will produce the largest clear zone, because it is designed to kill bacteria, and the water disc will produce none, because water has no effect on bacterial growth.

Variables

Independent	The type of antiseptic, antibiotic or plant extract on the disc
Dependent	The size (diameter, then area) of the clear zone around each disc
Control	• Species and volume of bacterial culture spread on the plate • Type, volume and depth of agar in the dish • Size of paper disc and volume of liquid soaked into it • Incubation temperature (25 °C) and incubation time (48 hours) • Distance between discs on the plate

Equipment

- Sterile nutrient agar plates (petri dishes)
- Broth culture of a harmless bacterium (for example *Escherichia coli* K12 or *Micrococcus luteus*) from a reputable supplier
- Sterile pipette and sterile spreader
- Sterile filter paper discs (about 6 mm across)
- Sterile forceps
- Test substances: an antibiotic solution, an antiseptic solution, one or two plant extracts (for example crushed garlic or mint leaves in a little water) and sterile distilled water
- Disinfectant, adhesive tape and a marker pen

- Incubator or warm cupboard set at 25 °C
- Transparent ruler with a millimetre scale
- Eye protection (disposable gloves if your teacher asks)

Risk assessment

Hazard	Risk	Precaution
Microorganisms (bacteria on the plates)	Infection if the bacteria get onto skin, into a cut or into the mouth; harmful microorganisms could grow if the dish is not kept closed.	Use only harmless strains from a supplier. Use aseptic technique. Wash hands before and after. Keep the lid on and taped, do not open the dish after incubation, and sterilise used dishes in an autoclave.
Antiseptic and antibiotic solutions	Skin or eye irritation; some people are allergic to antibiotics.	Wear eye protection. Wash off any splashes. Tell your teacher if you know you are allergic to antibiotics.
Plant extracts	Some people have allergic reactions to plants.	Use only plants provided by your teacher and tell them about any known allergies. Wash hands after handling.
Ethanol or a Bunsen flame (if used to sterilise equipment)	Fire or burns.	Keep ethanol away from the flame. Tie back long hair. Never leave a lit Bunsen burner unattended.

Method

1. Wash your hands and wipe the bench with disinfectant. Put on eye protection. If your teacher tells you to, work close to a lit Bunsen burner, whose rising hot air carries microorganisms away from the plate.
2. Turn a sterile agar plate over and use a marker pen to divide the base into five sections. Write a code for each substance on the base, near the edge. Do not write on the lid.
3. Lift the lid just enough to work. Using a sterile pipette, transfer a small volume (about 0.1–0.2 cm³, as your teacher instructs) of the bacterial broth onto the agar.
4. Spread the broth evenly over the whole surface with a sterile spreader to make a bacterial lawn. Close the lid straight away.
5. Soak a sterile paper disc in each test substance. Use a separate disc for each one and let the extra liquid drip off.
6. Lift the lid slightly and use sterile forceps to place each disc in its labelled section, well spaced from the others and from the edge. Press each disc gently onto the agar.
7. Place a disc soaked in sterile distilled water in the fifth section as a control.
8. Fasten the lid with two short strips of tape. Do not seal it all the way round, so that oxygen can get in.
9. Turn the dish upside down and incubate it at 25 °C for 48 hours.
10. After incubation, do not open the dish. Look at it from underneath. Measure the diameter of each clear zone, including the disc, to the nearest millimetre. Measure twice at right angles and calculate the mean.
11. Halve each mean diameter to find the radius and calculate the area of each clear zone using $\text{area} = \pi r^2$.

L2 . Record your results in the table. Return the dish to your teacher to be sterilised, clean the bench with disinfectant and wash your hands.

Results

Clear zones around each disc			
Substance on disc	Diameter of clear zone, including disc (mm)	Radius (mm)	Area of clear zone, πr^2 (mm ²)
Antibiotic			
Antiseptic			
Plant extract 1			
Plant extract 2			
Water (control)			

Graph

Draw a bar chart. Put the substance on the x-axis (categoric data, so bars with gaps between them) and the area of the clear zone (mm²) on the y-axis. Label both axes and use a sensible scale.



Exam questions 9 questions · 25 marks

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Name the independent variable in this investigation.

[1 mark]

2 A disc soaked in sterile water was placed on each plate. Explain why. **[2 marks]**

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3 The dishes were incubated at 25 °C and not at 37 °C. Explain why. **[2 marks]**

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4 The lid of each dish was taped on but not sealed all the way round. Give two reasons for this. **[2 marks]**

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5 A clear zone around a disc had a diameter of 16 mm. Calculate the area of the clear zone. **[3 marks]**
Use the formula $\text{area} = \pi r^2$ and $\pi = 3.14$. Give your answer to the nearest whole number.

Answer =

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- 6** A student tested three substances, P, Q and R, on a bacterial lawn. A disc soaked in water was used as a control. The table shows the area of the clear zone around each disc. State which substance was most effective and which had no effect. Give a reason for each answer. **[4 marks]**

Substance	Area of clear zone (mm ²)
P	113
Q	254
R	28
Water	28

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- 7** Suggest two ways the student could make the results more reliable. **[2 marks]**

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- 8** The student concluded that the antibiotic would be a good antiseptic to use on skin. Evaluate this conclusion. **[3 marks]**

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9 Describe how you would use aseptic technique to set up a petri dish to test the effect of three plant extracts on bacterial growth. **[6 marks]**

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Teacher answers

Core practical 5: Antiseptics, antibiotics and plant extracts. The example results are practice data, not real measurements.

Example results

Example results (practice data). Areas calculated with $\pi = 3.14$ and rounded to the nearest whole number.

Substance on disc	Diameter of clear zone, including disc (mm)	Radius (mm)	Area of clear zone, πr^2 (mm ²)
Antibiotic	20	10	314
Antiseptic	14	7	154
Plant extract 1 (garlic)	10	5	79
Plant extract 2 (mint)	6	3	28
Water (control)	6	3	28

Conclusion

The antibiotic gave the largest clear zone (314 mm²), so it was the most effective at stopping the bacteria growing. The antiseptic (154 mm²) and the garlic extract (79 mm²) also stopped some bacterial growth. The mint extract gave the same area as the water control (28 mm²), which is just the area of the 6 mm disc itself, so it had no effect. The control shows that the disc and the water do not stop bacteria growing, so the clear zones are caused by the substances tested. Clear zones appear because the substance spreads out from the disc into the agar and kills the bacteria or stops them dividing.

Errors and improvements

Error	Effect on the results	Improvement
The clear zone is not a perfect circle, so one diameter measurement may not represent the whole zone.	The calculated area could be too large or too small (random error).	Measure the diameter in two directions at right angles and use the mean.
Different discs may hold different volumes of liquid, or the substances may have different concentrations.	A larger clear zone could be due to more substance, not a more effective substance (an unfair test).	Use the same volume on every disc, for example with a micropipette, and the same concentration where possible.
Only one plate was used and there is only one disc for each substance.	An anomalous result cannot be spotted, so the conclusion is less reliable.	Use several discs of each substance or several plates and calculate a mean.
Discs placed too close together, so that clear zones overlap or the substance spreads from one disc to another.	Clear zones are hard to measure and may be wrongly attributed (systematic error).	Space the discs well apart and away from the edge of the dish.
The lawn is uneven or the plate was contaminated by other microorganisms.	Growth varies across the plate, so clear zones are not comparable.	Spread the bacteria evenly with a sterile spreader and use strict aseptic technique.

Mark scheme

One mark for each point unless a maximum is given. Words in brackets are not needed for the mark.

1. [1]

- the type of antibiotic / antiseptic / plant extract (on the disc) (1) ignore 'the disc' alone

2. [2]

- it is a control (1) ignore 'for comparison' alone
- to show that the disc / water alone does not stop the bacteria growing, so any clear zone is caused by the substance being tested (1)

3. [2]

- harmful (human) pathogens grow best at body temperature / 37 °C (1)
- so growing at a lower temperature reduces the risk that pathogens grow (1) do not accept 'bacteria are killed at 37 °C'

4. [2]

- the tape stops the lid coming off / stops microorganisms getting in or out (1)
- a gap lets oxygen in, so harmful anaerobic bacteria do not grow (1)

5. [3]

- radius = $16 \div 2 = 8$ (mm) (1)
- area = 3.14×8^2 / $3.14 \times 8 \times 8$ (1)
- 201 mm² (1) allow 200.96
- Correct answer with no working gains 3 marks
- Allow 1 mark for 804 (diameter used as radius)

6. [4]

- Q is the most effective (1)
- because it has the largest clear zone / largest area where bacteria did not grow (1)
- R had no effect (1)
- because its area is the same as the water control / the area is just the area of the disc (1)

7. [2]

- use more than one disc or plate for each substance and calculate a mean (1)
- measure each clear zone in two directions and calculate a mean (1)
- use the same volume and concentration of each substance (1)
- Max 2

8. [3]

- the large clear zone shows the antibiotic stops these bacteria growing (1)
- but antibiotics are medicines taken into the body, while antiseptics are used on skin / surfaces / overuse of antibiotics could lead to resistant bacteria (1)
- only one species of bacteria was tested / the effect on human skin cells was not tested (1)

9. [6]

Level 3 5-6	A detailed, logically ordered method that could be followed to set up a valid test, with aseptic steps linked to their reasons (such as preventing contamination) and a control included.
Level 2 3-4	A method with most of the main steps and some aseptic technique, but with some steps missing or few reasons given.
Level 1 1-2	A few relevant steps, with little order or little aseptic technique.

Indicative content

- wash hands / disinfect the bench
- use a sterile (autoclaved) agar plate and sterile pipette to spread the bacteria into a lawn
- lift the lid only slightly and for a short time to stop microorganisms getting in
- use sterile forceps to place discs soaked in each plant extract on the agar, well apart
- include a control disc soaked in sterile water
- tape the lid (not sealed all round) and label the base
- incubate upside down at 25 °C for 48 hours
- measure the clear zones after incubation without opening the dish

Exam tips

- Always say the clear zone is where the bacteria have been killed or stopped growing. The bigger the zone, the more effective the substance.
- For a control disc, say that it shows the disc or water alone has no effect. Do not just write 'for comparison'.
- Show every step of a πr^2 calculation: halve the diameter first, then square the radius. Many marks are lost by using the diameter as r .
- Know the safety reasons: 25 °C so human pathogens do not thrive, dish taped but not sealed, never open dishes after incubation.
- Read area questions carefully. Usually you measure the diameter of the whole clear zone, including the disc. If a question asks for the area of clear agar only, subtract the area of the disc.