

Antiseptics and antibiotics on bacterial growth

Investigate the effect of antiseptics or antibiotics on bacterial growth using agar plates and measuring zones of inhibition.

For AQA GCSE Biology 8461 and Combined Science: Trilogy 8464. **Triple only** This practical is not in Combined Science. Teacher answers start on a new page.

Name

Date

Aim

To compare how well different antiseptics stop bacteria growing by measuring the zones of inhibition on an agar plate.

Background

Bacteria can be grown in a nutrient broth or as colonies on an agar gel plate. To grow an uncontaminated culture you use aseptic technique. The Petri dishes and the agar must be sterilised before use, and any equipment used to transfer bacteria must be sterilised. This stops unwanted microorganisms getting in and the culture escaping.

Antiseptics are chemicals that kill or stop the growth of microorganisms on the skin or on surfaces.

Antibiotics are medicines that kill bacteria inside the body. If a chemical is effective, it diffuses out of a paper disc into the agar and stops the bacteria growing near the disc. This leaves a clear area called the zone of inhibition.

The larger the zone of inhibition, the more effective the chemical is against that type of bacteria. The cross-sectional area of the zone is calculated using $\text{area} = \pi r^2$, where r is half of the diameter.

The lid of the Petri dish is held on with adhesive tape and the dish is stored upside down so that condensation does not drip on to the agar. In school laboratories the dishes are incubated at no more than 25 °C. At this temperature dangerous pathogens that grow best at body temperature (37 °C) are unlikely to grow.

Hypothesis

The antiseptic with the strongest effect on bacterial growth will have the largest zone of inhibition, because more of the bacteria near the disc are killed or stopped from reproducing.

Variables

Independent	The type of antiseptic (or antibiotic) on the disc
Dependent	The size of the zone of inhibition (diameter, then area in mm ²)
Control	• Type and volume of bacterial culture spread on the plate • Type, volume and depth of agar • Size and type of paper disc • Volume of solution and concentration soaked into each disc • Incubation temperature and time

Equipment

- 3 sterile nutrient agar plates (Petri dishes)
- Broth culture of a non-pathogenic bacterium, supplied by the teacher
- Sterile dropping pipette (or sterile syringe) and sterile spreader (or sterile cotton bud)
- Sterile filter paper discs, about 6 mm across
- Antiseptic A, antiseptic B and antiseptic C (solutions supplied by the teacher), and sterile water as the control
- Sterile forceps
- Marker pen (permanent) and adhesive tape
- Ruler (mm) for measuring the zones
- Disinfectant solution and a container for used materials
- Eye protection

Risk assessment

Hazard	Risk	Precaution
Bacteria in the culture and on the plates	Harmful microorganisms could infect you or contaminate the lab, especially if the plate is opened after incubation.	Use aseptic technique. Tape the lid on, do not open the plate after incubation, wash your hands, and give the plates to the teacher to be sterilised at the end.
Antiseptic solutions	They can irritate the skin and eyes.	Wear eye protection and wash any splashes off with plenty of water.
Spills of the bacterial culture	Spreading bacteria on the bench or skin.	Wipe the bench with disinfectant before and after the practical. Put spills and used materials into disinfectant.
Incubating the plates	Growth of dangerous bacteria at body temperature.	Incubate at no more than 25 °C only.

Method

1. Wipe the bench with disinfectant and wash your hands. Keep the lids on the Petri dishes as much as possible.
2. Label the base of the Petri dish (not the lid) with your name, the date and the bacteria used. Divide the base into four sections with a marker pen and label them A, B, C and control.
3. Use a sterile dropping pipette to put a few drops of the bacterial broth culture on to the agar. Lift the lid just enough to do this.
4. Use a sterile spreader (or a sterile cotton bud) to spread the culture evenly over the whole surface of the agar to make a bacterial 'lawn'. Close the lid.
5. Using sterile forceps, dip a sterile paper disc into antiseptic A, let the extra liquid drip off and place the disc in section A, pressing it gently on to the agar.
6. Use a new sterile disc and clean sterile forceps for antiseptic B and antiseptic C. Place each disc in its section, at least 2 cm apart.
7. Soak a fourth disc in sterile water and place it in the control section.
8. Secure the lid with two or three small strips of adhesive tape. Do not seal it fully, so that oxygen can still get in.
9. Set up two more plates in the same way (steps 2 to 8), so that there will be three results for each antiseptic.

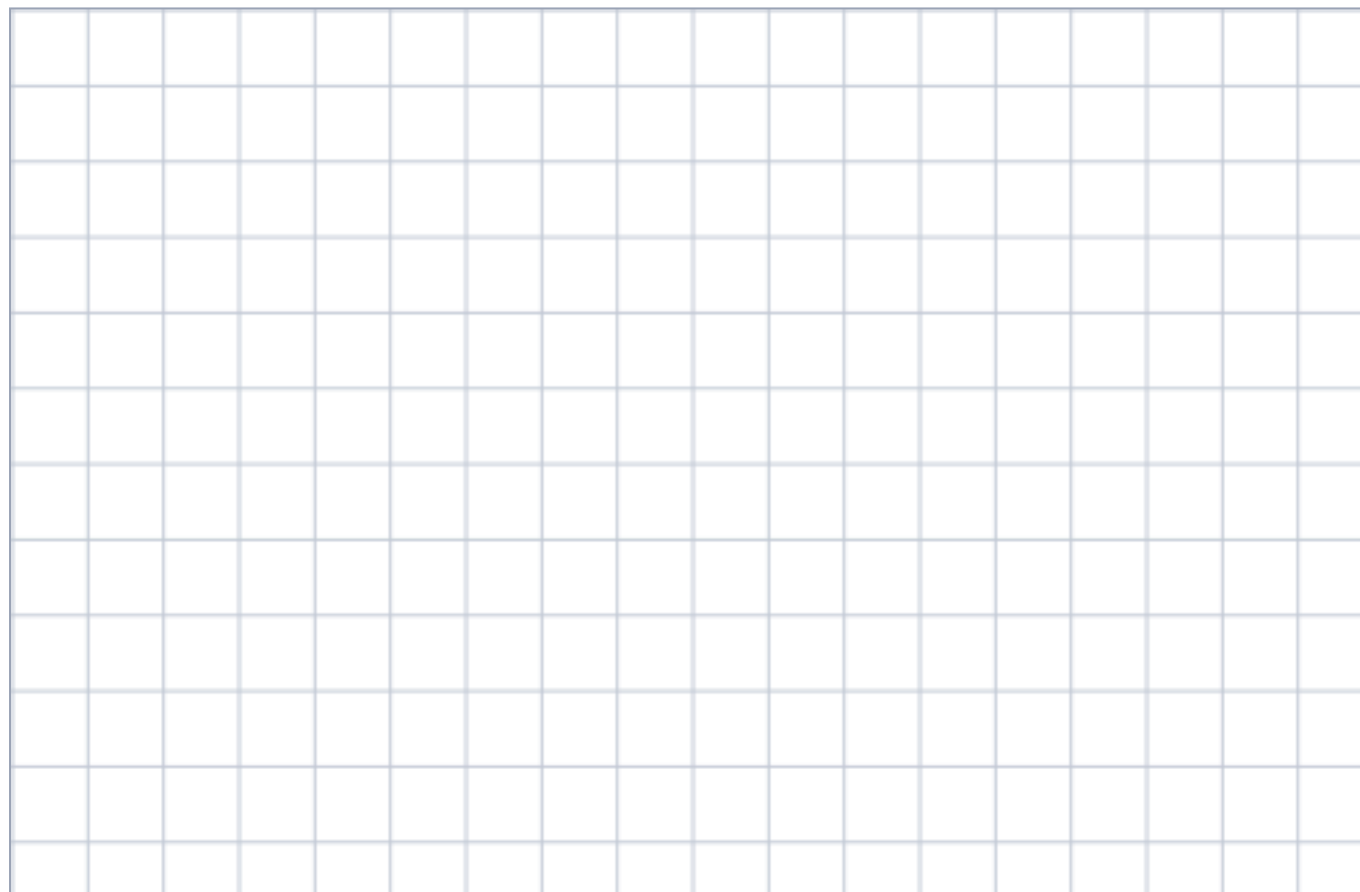
- L0 . Turn the dishes upside down and incubate them at 25 °C for 48 hours.
- L1 . After incubation, do not open the plates. Use a ruler to measure the diameter of the clear zone around each disc in mm, across the middle of the zone, through the base of the dish. Record 0 if there is no clear zone.
- L2 . Calculate the mean diameter for each antiseptic, then work out the area of the zone using πr^2 , where r is half the mean diameter.
- L3 . Give the plates to the teacher to be sterilised and wash your hands.

Results

Diameter of the zones of inhibition					
Substance on disc	Diameter of zone, plate 1 (mm)	Diameter of zone, plate 2 (mm)	Diameter of zone, plate 3 (mm)	Mean diameter (mm)	Area of zone (mm ²)
Antiseptic A					
Antiseptic B					
Antiseptic C					
Control (sterile water)					

Graph

Draw a bar chart. Put the substance on the disc (antiseptic A, B, C and control) on the x-axis and the mean area of the zone of inhibition (mm²) on the y-axis. Leave gaps between the bars because the x-axis variable is not numerical.



Exam questions 10 questions · 28 marks

1 A disc soaked in sterile water is placed on the plate as a control. Explain why this control is needed. **[2 marks]**

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2 Explain why the Petri dishes and agar must be sterilised before the bacteria are added. **[2 marks]**

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3 In a school laboratory the plates are incubated at 25 °C, not 37 °C. Explain why. **[2 marks]**

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4 The lid of the Petri dish is taped on and the dish is stored upside down. Give one reason for each. **[2 marks]**

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5 The zone of inhibition around one disc has a diameter of 18 mm. Calculate the area of the zone. Use the equation: $\text{area} = \pi r^2$. Give your answer in mm^2 to the nearest whole number. **[3 marks]**

Answer =

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- 6** A student measured the mean diameter of the zones of inhibition for three antiseptics. **[2 marks]**
Which antiseptic is the most effective? Give a reason for your answer.

Zone of inhibition for three antiseptics	
Antiseptic	Mean diameter of zone (mm)
X	11
Y	19
Z	6

- 7** Name the independent variable and the dependent variable in this investigation, and give **[3 marks]**
one variable that should be controlled.

- 8** After incubation, a student finds a few colonies of bacteria growing inside the zone of **[2 marks]**
inhibition around an antibiotic disc. Suggest an explanation.

- 9** A student wants to improve the investigation. Describe two changes the student could **[4 marks]**
make and explain why each would help.

10 Describe how you would use agar plates to compare the effectiveness of three antiseptics against a species of bacteria. Include how you would keep the investigation safe. **[6 marks]**

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Teacher answers

Required practical 2: Antiseptics and antibiotics on bacterial growth. The example results are practice data, not real measurements.

Example results

Example results (practice data)					
Substance on disc	Diameter of zone, plate 1 (mm)	Diameter of zone, plate 2 (mm)	Diameter of zone, plate 3 (mm)	Mean diameter (mm)	Area of zone (mm ²)
Antiseptic A	22	24	23	23	415
Antiseptic B	14	13	15	14	154
Antiseptic C	8	10	9	9	64
Control (sterile water)	0	0	0	0	0

Conclusion

Antiseptic A had the largest zone of inhibition (area 415 mm²), then B (154 mm²), then C (64 mm²). The control had no clear zone. This shows that antiseptic A was the most effective at stopping the growth of this bacterium, because it killed or stopped the reproduction of bacteria over the largest area. The control shows that sterile water does not affect the bacteria, so the clear zones were caused by the antiseptics. The results are repeatable, because the three plates gave similar values for each antiseptic.

Errors and improvements

Error	Effect on the results	Improvement
Bacteria from the air or hands contaminate the plate (random error).	Other colonies grow and may form clear zones or hide the edge of a zone, so the measured diameter is wrong.	Use good aseptic technique: keep the lid on, work near a flame or on a disinfected bench, and use sterile equipment.
The discs hold different volumes of solution.	Different amounts of chemical diffuse into the agar, so the zones are not comparable (this is a failure to control a variable).	Dip each disc for the same time and let the same amount of liquid drip off, or use a micropipette to put the same volume on each disc.
The zone edge is hard to see and the diameter is measured to the nearest mm with a ruler.	Measurements are not very precise, so areas calculated from them are uncertain.	Measure the diameter in two directions at right angles and calculate a mean, or measure the plate over a dark background.
Only one plate is used for each antiseptic.	A single result could be an anomaly and there is no way to check it.	Use at least three plates for each antiseptic and calculate a mean.

Mark scheme

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

1. [2]

- to show that the water / the disc itself does not stop the bacteria growing (1)
- so any zone around the other discs must be caused by the antiseptic (1)

2. [2]

- to kill any other microorganisms (1)
- so that only the bacteria being investigated grow / the culture is not contaminated (1)

3. [2]

- 37 °C is the temperature at which human pathogens grow best / 25 °C is too low for most harmful bacteria to grow well (1)
- so there is less risk of growing pathogens that could harm people (1)

4. [2]

- lid taped on to prevent microorganisms getting in or out / stop the lid being opened (1)
- stored upside down so that condensation does not fall on to the agar / the culture (1)

5. [3]

- radius = $18 \div 2 = 9$ mm (1)
- area = $\pi \times 9^2$ (1)
- 254 mm² (1) allow 255; allow 254.5 rounded

6. [2]

- Y (1)
- it has the largest zone of inhibition / killed bacteria over the largest area (1)

7. [3]

- independent variable: type of antiseptic / antibiotic (1)
- dependent variable: size (diameter or area) of the zone of inhibition (1)
- control: any one from the volume of culture, type of agar, size of disc, volume of antiseptic on the disc, temperature, incubation time (1)

8. [2]

- these bacteria were resistant to the antibiotic (1)
- so they survived and reproduced / formed colonies (1)

9. [4]

- repeat the experiment on several plates (1) so a mean can be calculated / anomalies can be spotted (1)
- measure the diameter in two directions at right angles and take a mean (1) to reduce the effect of an irregular zone / reading error (1)
- use the same volume of antiseptic on each disc, for example with a micropipette (1) so the amount of chemical is the same / it is a fair test (1)
- Max 4: 1 mark for each change and 1 mark for its linked explanation

10. [6]

Level 3 5-6	A clear, logical method that covers making the lawn, using control and test discs, incubation and measurement, with at least two correct safety or aseptic points. Variables are controlled.
Level 2 3-4	A method with most of the main steps (lawn, discs, incubation, measuring zones) and at least one safety or aseptic point. There may be gaps in detail or control.
Level 1 1-2	A few relevant points, for example putting discs on bacteria or measuring clear zones, with little detail or order.

Indicative content

- use sterile agar plates and sterile equipment (aseptic technique)
- spread the same volume of bacterial culture evenly over the agar to make a lawn
- place sterile discs soaked in each antiseptic on the plate, with a control disc soaked in sterile water
- keep discs apart and use the same volume of solution on each disc
- tape the lid, turn the dish upside down and incubate at 25 °C for about 2 days
- do not open the plate after incubation
- measure the diameter of the clear zone around each disc, calculate the area using πr^2
- repeat to get a mean; the largest zone means the most effective antiseptic

Exam tips

- Call it the zone of inhibition, and say it is where bacteria did not grow. A larger zone means a more effective antiseptic or antibiotic.
- State the incubation conditions exactly: 25 °C in school. Explain that a higher temperature could grow pathogens harmful to people.
- To get the area, halve the diameter first. Many students forget this and put the diameter in πr^2 .
- Control discs are soaked in sterile water. Explain that they show the disc and water alone do not stop bacterial growth.
- Do not say that antibiotics kill viruses. Antibiotics kill bacteria; antiseptics are used on skin and surfaces, not inside the body.