Edexcel IAL Biology A Level

Core Practical 14

Investigate the effect of different antibiotics on bacteria.

The effectiveness of different antibiotics on a specific bacteria can be investigated in the following practical. Antibiotics **kill bacteria** or **slow their growth** and their effectiveness and this will be shown by the **clear area** produced around the sample of each antibiotic.

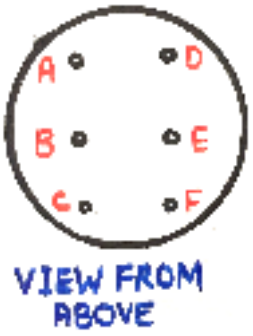
Independent variable: Type of antibiotic used
Dependent variable: Area of inhibition zone

Equipment list

- 5 different types of antibiotics
- Agar plate
- Filter paper cut into 6 equally sized discs
- Sterile forceps
- Disinfectant
- Known bacterial culture
- Sterile pipette
- Sterile water
- Sterile plastic spreader
- Bunsen burner
- Ruler
- Tape

Method

1. Before starting the experiment disinfect the workbench, this helps to **prevent contamination** when preparing the bacteria culture.
2. Mark on the agar plate the **date, your initials and the bacteria being grown.** Also mark evenly across the plate 6 letters A-F to show where to place the different antibiotic discs.



3. Use a bunsen burner to flame the bottle of the neck of the bacterial flask causing the air to rise and carry away undesired airborne microorganisms and then use the sterile pipette to transfer 2 cm^3 of the liquid broth to the agar plate.
4. Use the sterile plastic spreader to evenly distribute the bacteria across the agar jelly.
5. Take the first filter paper disc and use the forceps to **completely submerge** it in the first antibiotic for 10 seconds. Slightly lift the lid of the agar plate at a angle allowing you to quickly transfer the disc to the agar jelly.

6. Repeat step 5 for the other 4 antibiotics as well as a control disc soaked in sterile water, sterilising the forceps in between each antibiotic by holding them in the bunsen burner flame.
7. Tape the lid on at 4 points making it secure enough but also **allowing oxygen to enter** so the **bacteria may respire aerobically.** Then incubate the dish at 25 °C for 48 hours.
8. Disinfect the work surface again and wash your hands.
9. After the 48 hours have passed use a ruler to measure the diameter of the **inhibition zone (clear zone)** created around each disc.

Tips to minimise contamination

- Have the lid removed from the agar plate for **as little time as possible.**
- Work near the bunsen burner flame as this **helps to sterilise the air** by heating and raising air containing potential contaminants such as other microorganisms.

Risk assessment

Risk	Hazard	Precaution
Bacteria	If not handled safely and appropriately the users could become infected	Store bacterially safely, minimise the time spent with the lid off and incubate at temperatures below 30°C Don't eat or drink during the course of the experiment
Disinfectant, agar jelly	Could cause skin irritation	Avoid skin contact, wear gloves if necessary

Results table

Antibiotic used	**Diameter of clear zone (mm)**	**Area of clear zone (Radius² x π) (mm²)**
A		
B		
C		
D		
E		
Control		

Conclusion

The effectiveness of each antibiotic can be compared by **looking at the area of the inhibition zones created.** The one with the largest inhibition zone has killed the most bacteria and is therefore the most effective. The disc dipped in water acts as a control to show that the antibiotics alone are causing the death of bacteria and not any other factor.