

Edexcel A Biology A-Level

Core Practical 15

Investigate the effect of different antibiotics on bacteria.





Aseptic technique is used to **avoid contamination** of the sample from outside substances, such as microorganisms. This is important to get **reliable** and **repeatable** data. Inoculating an antibiotic agar plate (to provide nutrients), growing bacteria on it and then determining **zone of inhibition** can be used to measure the **effectiveness** of different antibiotics against particular species of bacteria.

Equipment

- Agar plate seeded with bacteria
- Pipette
- Bunsen burner
- Disinfectant
- Soap
- Paper towels
- Forceps
- Antibiotic impregnated paper discs
- Sellotape
- Incubator

Aseptic Technique

- Wipe down surfaces with **antibacterial cleaner** both **before and after** experiment.
- Use a **Bunsen burner** in the work space so that **convection currents** draw microbes away from the culture.
- **Flame the wire hoop** before using to transfer bacteria.
- **Flame the neck of any bottles** before use to prevent any bacteria entering the vessel (air moves out so unwanted organisms don't move in).
- Keep all vessels containing bacteria **open for the minimum amount of time**.
- **Close windows and doors** to limit air currents.

Method

1. Carry out the whole experiment using **aseptic technique**.
2. **Flame** the forceps and pick up a paper disc.
3. Slightly lift the lid of the petri dish and **place the paper disc onto the agar**.
4. **Tape the dish** with two pieces of sellotape (don't tape all the way around to **avoid conditions becoming anoxic**).
5. Wash your hands and disinfect the bench.
6. **Incubate** for **24 hours** at approximately **30 degrees**.



7. Measure the **radius of the clear zone** on the agar plate. Calculate the area

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Disinfectant	Flammable	Keep away from naked flame	Put out fire; seek assistance	Low
Naked flame	Fire hazard; burns	Keep away from flammable materials; tie up long hair, keep away from edge of desk	Put out fire; seek assistance; run burns under cold water immediately	Low
Biohazard	Contamination	Use aseptic technique; wash hands; wear eye protection	Seek assistance	Medium
Broken glass	Cuts from sharp object	Take care when handling glass objects; keep away from edge of desk	Elevate cuts; apply pressure; do not remove glass from wound; seek medical assistance	Low

Graph

- Plot a bar graph of **type of antibiotics** against **area of clear zone**.

Conclusion

- The area of the **zone of inhibition** / 'clear zone' will be more effective when the antibiotics are more effective against the type of bacteria being used.
- How effective an antibiotic is against a certain type of bacteria is dependent on whether the bacteria are **gram-positive or gram-negative** and what **type of antibiotics** are used.

