

Edexcel A Biology A-Level

Core Practical 9

Investigate the antimicrobial properties of plants, including aseptic techniques for the safe handling of 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.

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.

Equipment

- Agar plate seeded with bacteria
- Sample of garlic
- Sample of mint
- Mortar and pestle
- Methylated spirit
- Pipette
- Paper discs
- Petri dish
- Forceps
- Tape

Method

1. Carry out **aseptic techniques** detailed above.
2. **Crush 3g** of the garlic and mint (separately) with methylated spirit. Shake occasionally.
3. Use a **sterile pipette** to transfer plant extract to paper disc.
4. Leave paper discs to dry for **10 minutes**.
5. Use **sterile forceps** to place the paper disc onto a **petri dish**.
6. Lightly tape a lid on, **invert** and **incubate** at 25°C for 24 hours. DO NOT tape around the entire dish as this **prevents oxygen entering** and so promotes the growth of more harmful **anaerobic** bacteria.



7. **Sterilise equipment** used to handle bacteria and **disinfect work surfaces**.
8. Measure the **diameter** of the **inhibition zone** (clear circle) for each plant. DO NOT remove lid from agar plate.
9. Work out the **area** of the inhibition zone using the formula:

$$A = \frac{\pi d}{4}$$

where d is the diameter.

NB: Bacteria sample is incubated at **25°C** as incubating at 37°C (human body temperature) could enable pathogens to grow that are **harmful to humans**.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Disinfectant	Flammable	Keep away from naked flame	Put out fire; seek assistance	Low
Biohazard	Contamination; infection	Use disinfectant; wash hands with soap after dissection; do not incubate at human body temperature; do not open agar plate post incubation	Seek assistance	Low/medium (depends on likelihood of bacteria sample used to cause infection)
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
Methylated Spirit	Flammable and toxic	Keep away from naked flame. Do not ingest. Wear gloves and goggles.	Seek assistance	Medium





Graph

- Plot a **bar chart** of the **area of the inhibition zone against plant**.
- Graph could include **range bars** to show the **uncertainty** from the ruler in measuring the diameter.

Conclusion

- If there is a **larger inhibition zone** around the plant, it has **killed more bacteria**. Therefore, the larger the inhibition zone, the better the antimicrobial properties of the plant.

