# Edexcel IAL Biology A Level

## Core Practical 9

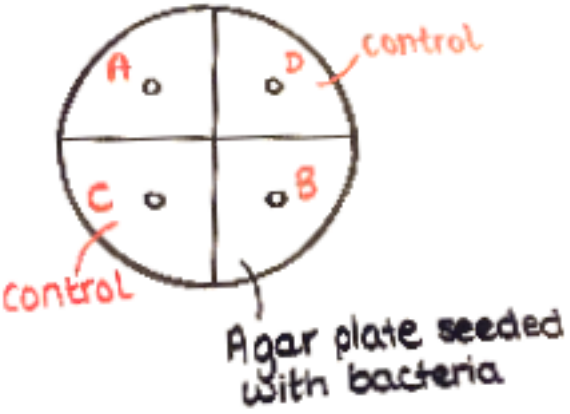
Investigate the antimicrobial properties of plants, including aseptic techniques for the safe handling of bacteria.

**Independent variable: The species plant used**
**Dependent variable: Area of inhibition zone**

## Equipment list

- Disinfectant spray
- Paper towels
- Syringe
- Agar plate already covered with bacteria
- Filter paper cut into 4 equally sized discs
- Incubator at 25 °C
- Sterile forceps
- Pestle and mortar
- Marker pen
- Tape
- Plant samples - mint and garlic
- Measuring beaker - 50 $cm^3$
- Ruler

## Method




1. Begin by using the disinfectant spray to clean the workbench and wash your hands thoroughly **minimise the risk of contamination** in the experiment.
2. Use the marker pen to split the seeded agar plate into 4 equal quarters and mark the letters A-D on the edge of the plate, one in each quarter.
3. Take the first plant sample - mint - and place it into the mortar. Use the pestle to grind the plant into a **fine paste** and then use a syringe to add 10 $cm^3$ of ethanol.
4. Use the sterile forceps to soak a filter paper disc in the solution created for 20 seconds and then place the disc on to the sterile agar plate to dry.
5. Once dry, use the forceps to transfer the disc to the second agar plate which has been seeded with bacterial culture and **quickly replace the lid** to the agar dish.
6. Wash the pestle and mortar and repeat steps 3-5 with the second plant sample - garlic.
7. Then fill the 50 $cm^3$ beaker with a small volume (around 5-10 $cm^3$) of alcohol. Use clean forceps to soak the last 2 filter paper discs in the alcohol, allow them to dry as before and finally transfer them to their relevant quarters on the seeded agar plate.
8. Tape the lid on at 4 points making it secure but also allowing oxygen to enter so the **bacteria may respire aerobically**. Then incubate the dish at 25 °C for 48 hours.
9. Disinfect the work surface again and wash your hands.
10. After the 48 hours have passed use a ruler to measure the **diameter of the inhibition zone (clear zone)** created around each disc.

## Risk assessment

| Risk | Hazard | Precaution |
|---|---|---|
| Microorganisms | 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.<br>Don't eat or drink during the course of the experiment.<br>Wear eye protection and wash hands before and after experiment.<br>Do not open any inoculated plates. |
| Disinfectant, agar jelly | Could cause skin irritation | Avoid skin contact, wear gloves if necessary |
| Plant samples | Potential allergic reaction | Wear gloves when handling |
| Ethanol | It is flammable and volatile | Do not use it near a naked flame<br>Replace stopper immediately after use |

## Results table

| Substance | Diameter of clear zone (mm) | Area of clear zone (Radius² x π) (mm²) |
|---|---|---|
| A - Mint | | |
| B - Garlic | | |
| C - Control 1 | | |
| D - Control 2 | | |

## Conclusion

The effectiveness of the antimicrobial properties of each plant 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 discs dipped in alcohol only acts as a **control** to show that the plants alone are causing the death of bacteria and not any other factor.