AQA Biology A-Level

Required Practical 6

Use of aseptic techniques to investigate the effect of antimicrobial substances on microbial growth.

Aseptic techniques are used to **avoid contamination** of the sample from outside substances such as microorganisms. This is important to get **reliable** and **repeatable** data.

Aseptic Techniques

- 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 it to transfer bacteria.
- **Flame the neck of any bottles** before using them 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 list

- Bacteria sample
- Disinfectant
- Bunsen burner
- Heatproof mat
- Ethanol
- Wire hoop
- Pipette
- Forceps
- Plastic spreader
- Prepared agar plate
- Multodisc antibiotic ring
- Ruler

Method

1. Carry out **aseptic techniques** detailed above.
2. Use a **sterile pipette** or **wire hoop** to transfer bacteria from **broth** (distilled water, bacterial culture, nutrients) to **agar plate** (petri dish containing agar jelly).
3. **Spread bacteria evenly** over plate using a **sterile plastic spreader**.
4. Use **sterile forceps** to place a **multi disc antibiotic ring** on the plate. Ring should only be moved by holding the centre, NOT the arms.
5. Lightly tape a lid on, **invert** and **incubate** at 25°C for 48 hours. DO NOT tape around the entire dish as this **prevents oxygen entering** and so promotes the growth of more harmful **anaerobic** bacteria.
6. **Sterilise equipment** used to handle bacteria and **disinfect work surfaces.**

After Incubation

7. Measure the **diameter** of the **inhibition zone** (clear circle) for each antibiotic. DO NOT remove the **lid** from the agar plate.
8. Work out the **area** of the inhibition zone using the formula:

$$A = \frac{\pi d^2}{4}$$

where d is the diameter.

NB: Bacteria sample is incubated at **25°C**. This is because 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 likeliness of bacteria sample used to cause infection)
Naked flame	Fire hazard; burns	Keep away from flammable materials; tie up long hair, wear goggles	Put out fire; seek assistance;run burns under cold water immediately	Low

Graph

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

Conclusion

- If there is a **larger inhibition zone** around the antibiotic, it has **killed more bacteria**. Therefore, the larger the inhibition zone, the better the antibiotic works.
- Some antibiotics will have no/very little inhibition zone. This shows that the bacteria are **resistant** to this antibiotic and are not killed by it.