# OCR (B) Biology A-level

## Module 1: Development of practical skills in Biology

## PAG 7: Microbial Techniques

Please note: You only need to do one from each PAG, and you don't need to do the PAGs listed here, as long as you show the same skills that these are testing (see 5f of the specification for more information). However, you need to at least be able to design your own method for most of these experiments in the exam.

**Effect of antibiotics on bacterial growth – method:**

Use sterile techniques:

- Wash your hands and disinfect your work area.
- Have Bunsen Burner on nearby to sterilise the air and prevent air-borne microorganisms settling.
- When opening the bottle of broth, pass the neck over the Bunsen Burner flame to prevent the microorganisms in the air entering the bottle.
- Only open a petri dish enough to allow you to introduce your desired organisms.
- All equipment should be sterilised by passing it over the flame before and after use..

*Method:*

1. Using a sterile pipette, add the same volume ($0.1cm^3$) of each antibiotic to a different Petri dish. Antibiotics used could include: penicillin, ampicillin, etc. Alternatively, you could use one antibiotic, and alter the concentration.
2. Dip an **inoculating loop** in the broth (the broth of your desired bacteria). Recap the broth bottle.
3. Spread a streak of broth over the agar surface, then close the Petri dish, and tape it shut. Repeat for the remaining Petri dishes. Two petri dishes with no antibiotic should also be inoculated and two uncultured petri dishes should be added to act as controls.
4. Label each petri dish.
5. Place the petri dishes in a warm incubator.
6. The plates should be incubated upside down to stop condensation forming on the lid from dropping onto the agar.
7. Leave all the plates for the same amount of time (e.g. a day) then observe the results.
8. If bacterial growth has occurred, colonies of bacteria on the surface of the agar should be seen.
9. Count the number of colonies that have formed on each plate and record results in a table.
10. Work out the **mean number of colonies formed for each antibiotic**.
11. If colonies overlap, make **serial dilutions** with the bacteria broth so there is less bacteria initially so less colonies are present and it is more manageable.