

Investigating Selection

Can Investigate the Effects of Antibiotics on Bacterial Growth

Need to know how to investigate the effect of antimicrobial substances (e.g. antibiotics, antiseptics or disinfectants) on microbial growth, using aseptic techniques.

Test the Effects of Antibiotics Using Agar Plates

The bacteria you will use are likely to have been grown in a liquid broth (a mixture of distilled water, bacterial culture and nutrients).

Use a sterile pipette to transfer the bacteria from the broth to an agar plate (a Petri dish containing agar jelly). Spread the bacteria over the plate using a sterile plastic spreader.

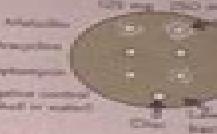
Use sterile forceps to place paper discs soaked with different antibiotics spaced apart on the plate. Make sure you add a negative control (one soaked only in sterile water).

Gently tap a bell jar, invert, and incubate the plate at about 25 °C for 48 hours. This allows the bacteria to grow (forming a 'lawns'). Anywhere the bacteria can't grow can be seen as a clear patch in the lawn of bacteria. This is called an inhibition zone.

The size of an inhibition zone tells you how well an antibiotic works. The larger the zone, the more the bacteria were inhibited from growing.

A similar technique can be used to test the effects of antiseptics or disinfectants on microbial growth.

An agar plate after incubation with discs of neomycin, tetracycline and streptomycin at different concentrations.



- The tetracycline discs have no inhibition zones, so the bacteria are resistant to tetracycline up to 250 µg.
- The streptomycin discs have small inhibition zones, with the zone at 250 µg slightly larger than the one at 125 µg. The streptomycin has some effect on the bacteria.
- The neomycin discs have the largest inhibition zones, so neomycin has the strongest effect on these bacteria.

Always Use Aseptic Techniques to Prevent Contamination of Microbial Cultures

Aseptic techniques are used to prevent contamination of cultures by unwanted microorganisms. This is important because contamination can affect the growth of the microorganism that you're working with. It's also important to avoid contamination with disease-causing microorganisms that could make you ill.

When carrying out the investigation above, you need to use the following aseptic techniques:

- Regularly disinfect work surfaces to minimise contamination. Don't put any chemicals on the work surface.
- Contaminated utensils should be placed in a beaker of disinfectant.
- Use sterile equipment and discard safely after use. E.g. glassware can be sterilised before and after use in an autoclave (which uses equipment at high pressure). Pre-sterilised plastic instruments are used once, then discarded.
- Work near a Bunsen flame. Hot air rises, so any microbes in the air should be blown away from your culture.
- Minimise the time spent with the lid off the agar plate, to reduce the chance of airborne microorganisms contaminating the culture.
- Briefly blast the neck of the glass container of teeth just after it's opened and just before it's closed — this causes air to move out of the container, preventing unwanted organisms from getting in.

You should also take steps to protect yourself, e.g. wash your hands thoroughly before and after handling cultures.

Arm-Up Questions

1. Describe how you could investigate the effects of antibiotics on bacterial growth.

Cam Question

2. A group of scientists monitored how the colour of oyster shells on a beach changed over time. The graph shows the colour of the oyster shells in the scientists' initial sample and in their final sample. The oysters were mainly found on the sand, which was a mid-brown colour.

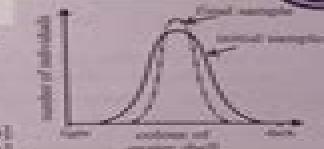
a) What type of selection is shown in the graph?

Explain your answer.

b) Suggest how the changes shown in the graph might have taken place.

[3 marks]

[4 marks]



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