Option B – Practical 3

Investigating the action of bactericides

Safety

- Bacterial cultures must be disposed of safely by autoclaving.
- Laboratory aprons should be worn.
- After the practical, clean benches with antibacterial wipes. Wash hands thoroughly, using soap.

Apparatus and materials

- 20 cm^3 culture solution of *Micrococcus luteus*
- dropping pipettes
- disposal beaker for used pipettes
- 250 cm^3 bottle of nutrient agar in a water bath at 60°C
- small discs of filter paper or blotting paper
- seven commonly available antibacterial products
- incubator at 25°C
- Bunsen burner
- eight sterile Petri dishes
- forceps
- distilled water
- sticky tape
- marker pen
- antiseptic wipes

Introduction

In this practical, you will investigate the effectiveness of some commonly available bactericidal or antibacterial products. You will assess each product's ability to inhibit the growth of a culture of common, harmless skin bacteria, *Micrococcus luteus*. The technique described to carry out the experiment is known as aseptic technique and is commonly used in microbiology.

Procedure

1 Take the bottle containing the *M. luteus* culture, remove the top and move the neck of the bottle across a Bunsen flame for 1 or 2 seconds to sterilise it. Take about 1 cm^3 of the culture in a plastic dropping pipette.

2 Carefully lift the lid of an empty, sterile Petri dish and pipette the culture into the dish. Place the pipette into the disposal beaker containing antiseptic solution.

3 Take the nutrient agar from the water bath, allow it to cool slightly (until hand hot) but not harden. Pour some of the agar into the Petri dish to a depth of about 5 mm. Close the lid and swirl the Petri dish gently to mix the microbe culture with the agar.

4 Repeat steps **1**–**3** for seven further Petri dishes.

5 Allow the agar to set (about 15 minutes) and select the antibacterial products to be tested.

6 When the agar has set, label each Petri dish on the underside with the product to be tested.

7 Flame the forceps in a Bunsen flame to sterilise them and use them to take a paper disc.

8 Dip the disc into the first antibacterial product, allow excess liquid to drain from it and place it into the centre of the agar in the first Petri dish. Replace the lid immediately.

9 Rinse the forceps and re-sterilise them, then repeat step **8** for each of the other products being tested. As a control, dip one paper disc into distilled water and place this on the final agar plate.

10 Use sticky tape to seal the lids of the Petri dishes (do not seal all the way round) and then incubate the plates at 25°C for approximately 48 hours.

11 Do not open the Petri dishes again as this may release potentially harmful microbes, which may have contaminated the plate, into the lab. Measure the diameter of the **zone of inhibition** (the clear zone around the paper disc) for each product. In this area, bacteria have been unable to grow in the agar. The size of the zone of inhibition indicates the strength of the antibacterial properties of each product. Measure the diameter of the zone in several places and obtain an average, since it is unlikely that the zone will be a perfect circle.

Questions and further work

1 Compare your results to identify the most effective antibacterial agent from those you have tested.

2 Design a further experiment to work out the concentration of each product that would most effectively kill bacteria.