Practical 6.3 Which antibiotic is most effective?

**Purpose**
- To investigate the effect of different antibiotics on bacteria.

**Safety**
- Wear eye protection.
- The microorganisms are a potential biological hazard.
- Use aseptic techniques when transferring the bacteria to the Petri dishes. Clean the bench with antibacterial disinfectant. Do NOT open the Petri dishes once they have been incubated.

**Introduction**
When a bacterial infection is diagnosed it is useful to be able to tell to which antibiotics it is most susceptible. In some cases this information is known, but in other cases tests need to be carried out to find out which antibiotic will be most effective. In this activity you will be testing the effectiveness of several types of antibiotics on bacteria.

The standard method of doing this is to put discs of chromatography blotting paper soaked in the various antibiotics onto an agar plate that has been inoculated with the bacteria. Alternatively a Mast ring (a ring of paper with several ‘arms’, each treated with a different antibiotic) can be used.

**Procedure**

You will need:
- Agar plate seeded with a known bacterium
- Bunsen burner
- Bench spray of disinfectant, 1% Virkon or equivalent
- Soap or handwash
- Paper towels
- Marker pen
- Autoclaved forceps
- Mast ring or antibiotic-impregnated paper discs
- Adhesive tape
- Eye protection

1. Wash your hands with the soap or handwash. Spray the working area thoroughly with the disinfectant spray. Leave for at least 10 minutes, then wipe with a paper towel.

2. Work very close to a lit Bunsen burner. Prepare an agar plate seeded with bacteria. This may have already been done for you. If not, follow the instructions in the section ‘Pouring agar plates’ in Practical 4.3 Edexcel AS Biology. Label the Petri dish on the base at the edge with your name, the date and the type of bacterium it is inoculated with.

3. Flame the forceps and then use them to pick up an antibiotic disc or Mast ring. Raise the lid of the Petri dish and place the Mast ring firmly in the centre of the agar; if individual discs are used they will need to be spaced evenly around the dish.

4. Tape the dish securely with two pieces of adhesive tape (but do not seal it completely), then keep it upside down at room temperature for 48 hours.
Practical 6.3 (cont.) Which antibiotic is most effective?

5 Wash your hands with soap or handwash and clean the bench again using the Virkon spray.

6 After incubation, look carefully at the plate but do not open it. Where bacteria have grown the plate will look opaque, but where the antibiotics have inhibited growth, clear zones called inhibition zones will be seen. Measure the diameter of the inhibition zones in millimetres and use this information to decide which antibiotic is most effective at inhibiting the growth of the bacterium.

7 Collect data from other members of the class who used the other bacterial cultures.

8 Write a brief report of the results, comparing the different antibiotics and the effects on the different bacterial cultures.

Questions

1 Are the inhibition zones circular? If not, what is a sensible measuring strategy?

2 What factors determine the diameter of the inhibition zones?

3 If class data are shared:
   a what is the overall spread of the data
   b do all individual results show the same trends – if not, why not, and how could this variability be represented on your graphs?

4 If you were working in a hospital laboratory, and you had just carried out this test on bacteria isolated from sick patients, would you always choose the antibiotic that gave the biggest inhibition zone? Are there any other factors you would need to consider?