Objectives

B5.16 Explain that antibiotics can only be used to treat bacterial infections because they inhibit cell processes in the bacterium but not the host organism.

B5.18B *Core Practical: Investigate the effects of antiseptics, antibiotics or plant extracts on microbial cultures.*

B5.20 Describe that the process of developing new medicines, including antibiotics, has many stages including discovery, development, preclinical and clinical testing.

Maths requirements

1a Recognise and use expressions in decimal form.

5c Calculate areas of triangles and rectangles, surface areas and volumes of cubes.

Learning outcomes

5th **SB5.16** Define the term antibiotic (as medicines that inhibit cell processes in bacteria).

6th **SB5.16** Explain why antibiotics are useful for treating bacterial infections (because they do not damage human cell processes).

6th **SB5.16** Explain why antibiotics cannot be used to treat infections by pathogens other than bacteria.

6th **SB5.20** Describe the stages of development of new medicines.

7th **SB5.20** Explain why each stage of the development of a new medicine is needed.

Exploring

1. Core practical – Investigating the effect of antibiotics

This practical forms part of the core practical requirement of the specification. It is supported by the information on *Students' sheet CP5 (Investigating the effect of antibiotics).*

Full instructions are given on Students' sheet CP3. Discs of different concentration of the same antibiotic could be used. Alternatively, different antibiotics could be compared. The method can be adjusted to look at the effect of different antiseptics or plant extracts if preferred. (Note that the effect of plant extracts is covered in detail by the practical work in Exploring 1 of *SB5g Plant defences*.)

The method allows students to pour their own agar plates then inoculate them with bacteria, to develop their skills in aseptic technique. However, nutrient agar plates could be prepared for students just before the lesson. Seal plates in plastic bags if they are not used straight away, to stop the agar drying out. Show students the preparation of agar in the autoclave to sterilise it. If students will not be preparing their own agar plates, demonstrate the process and discuss the various parts of the process that contribute to aseptic technique.

If students pour their own plates, after the sterile agar has been melted in bottles in boiling water, allow it to cool for 5–10 minutes before placing the bottles in a water bath at 50 °C to keep the agar molten until students need it. Warn students that the bottles are warm.

Some parts of the method are very fiddly and may benefit from a little practice beforehand. Alternatively, students could work in pairs to pour and inoculate plates. Make sure, though, that they understand the need for aseptic techniques and work as effectively as possible.

Students should work on a surface that has been disinfected for at least 10 minutes with 1% VirKon and the surface should be re-disinfected after the activity. The disinfected surface can be most easily achieved by placing an impervious surface (e.g. ceramic tile, piece of plastic, or laminated sheet of A4 paper) into 1% VirKon in a bowl so that its surface is completely covered. The surface should be left in place for at least 10 minutes before use and then blotted dry using a paper towel. The impervious surface should be returned to the disinfectant after the practical.

After the first lesson, the plates should be incubated upside down at 20–25 °C for two to three days. If the second lesson is later than this, further growth can be slowed by refrigerating the plates. Take plates out of the fridge at least 30 minutes before the lesson, to allow them to come to room temperature and for any condensation to evaporate.

Before students record their results, discuss which method to use. Measurement of the diameter of the clear area can be done using a ruler or by measuring against millimetre graph paper. Each diameter should be converted to a radius using $d = r/2$ before using the formula πr^2 to calculate the area where there is no growth on and around each disc.

⚠ Safety

1% VirKon solution should be made up shortly before use in the practical. Wear eye protection and nitrile protective gloves when removing impervious surfaces from the tray/bowl of 1% VirKon. Concentrated VirKon solutions and the solid are irritant to skin and eyes. 1% VirKon solution is presently not considered hazardous.

Ethanol (IDA) is hazardous, harmful and highly flammable.

Check before the practical that no student is taking immunosuppressive medication that may increase their risk of infection with bacteria. Warn students not to rub their eyes and to wash any splashes on skin immediately with water and soap. Make sure students wash their hands thoroughly before beginning the practical and again before leaving the laboratory.

Plates must be taped closed as shown in the worksheet to allow air in and discourage the growth of pathogenic bacteria. Never incubate plates above 25 °C, as this encourages growth of pathogenic bacteria. Secure the lids with additional tape before giving them to students, leaving a few gaps so that some oxygen can still enter and condensation can escape.

Sterilise all equipment before and after use with microorganisms.

Support: Work with students to discuss the answers to the questions. Students may need support with the calculations in step 2 of the results on the worksheet. Alternatively they could use a qualitative method of recording, such as photography.

Stretch: Remove the numbered questions from the worksheet and ask students to complete their own write-up.

Expected results

There should be a relationship between increasing concentration of antibiotic and increasing diameter of clear area around the disc. Or if comparing types of antibiotics, a comparison of the relative effectiveness of each (compared with the control) should be possible.

Course resources

Bio Students' sheet CP5

Equipment

per group: pre-poured agar plate with lid, bacterial culture in screw-top bottle (e.g. *Bacillus subtilis*, *Micrococcus luteus*), sterile pipette in wrapper, sterile spreader in wrapper, suitable container (e.g. wide-necked screw-top bottle) of 1% VirKon, two small filter paper discs of different antibiotic concentration or type (available pre-prepared from biological suppliers), sterile (e.g. autoclaved in foil) disc of filter paper (same diameter as antibiotic discs), sticky tape, marker pen, forceps, ethanol (IDA), Bunsen burner and heat-resistant mat, ruler

Optional (if students pour their own plates): Petri dish with lid, screw-top bottle of sterile liquid nutrient agar kept in water bath at 50 °C (for different antiseptics), small filter paper discs dipped in different antiseptics (for plant extracts), see Exploring 1 *SB5g Plant defences*