Student's Guide

Health and hygiene

Effects of antiseptics on microbes

Antiseptics are used to reduce the numbers of microbes in living tissue, e.g. from a cut or graze. They are milder than disinfectants that are used to clean objects and surfaces. You are going to investigate the effect of different dilutions of an antiseptic on microbes.

Learning objectives

To show:

- ▷ the effects of antiseptics on microbial growth
- ▷ the effects of dilution of antiseptics on microbial growth

Techniques required

See *Basic Practical Microbiology*

- ▷ using a pipette (p. 10)
- ▷ flaming the neck of a bottle (pp. 10–11)
- ▷ sterilizing forceps (p. 21)

Procedure

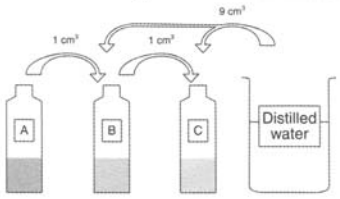
1. Light your Bunsen burner. Remove the cap from the bottle containing the culture of *Bacillus subtilis*. Pass the neck through the Bunsen burner flame 2–3 times. Remove a few drops of culture with the sterile dropping pipette. Flame the bottle neck again and replace the cap.

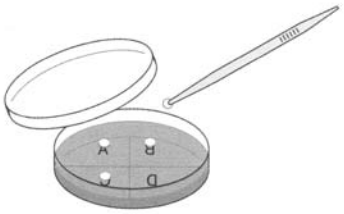
2. Lift the lid of the Petri dish. Place 2–5 drops of culture in the centre of the dish. Replace the lid as quickly as possible. Discard the dropping pipette into the beaker of disinfectant.

3. Take the bottle of melted agar. Remove the cap and pass the neck through the Bunsen burner flame 2–3 times. Pour the contents into the Petri dish carefully. Replace the lid as quickly as possible. Flame the neck again and replace the cap on the bottle.

4. Keeping the Petri dish flat on the bench, mix the agar thoroughly by gently swirling the plate as directed by your teacher. Avoid splashing the agar on to the lid or over the edge of the dish. Allow the agar to set. This is known as a pour plate.

5. Use a pipette and filler (or syringe) to place 9 cm^3 distilled water into empty bottles B and C. Using the same pipette, transfer 1 cm^3 antiseptic from bottle A to bottle B. Gently shake B to mix the contents, then transfer 1 cm^3 from B into C.





Safety! *Do not open the plates.*

6. When the agar has set, turn the dish upside down. Divide the base into four sections by drawing a cross on it with a marker pen. Label the sections A, B, C and D. Turn the dish over again.

7. Take sterile forceps, or sterilise them by dipping in alcohol (keeping the points facing downwards) and passing quickly through the Bunsen burner flame.

8. Using the sterile forceps, dip a paper disc into the remaining distilled water. Drain excess liquid from it, then place it on section D of the agar. Replace the lid as soon as possible.

9. Repeat for the other three sections using the samples in the order C, B, A. Tape and label the dish with your name and the date. It will be incubated until the next lesson.

Next lesson...

10. Examine your agar plate. Place it on a sheet of graph paper and record the diameter of any clear areas around the discs. Answer the questions.

Teacher's Guide

Health and hygiene

Effects of antiseptics on microbes

Antiseptics are used in the disinfection of living tissue. They may be used prophylactically (i.e. to prevent infection) or therapeutically (i.e. to treat infection). Any given antiseptic is usually more effective against some microbes than others and its activity may be greatly affected by factors such as dilution, temperature, pH or the presence of organic matter or detergent.

Learning objectives

To show:

- ▷ the effects of antiseptics on microbial growth
- ▷ the effects of dilution of antiseptics on microbial growth

Age range

Year 9 and above

Duration

Session 1	50 minutes
Session 2	20 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. The culture of *Bacillus subtilis* should be inoculated in nutrient broth and incubated at 25 °C at least 48 hours before the lesson.
2. Any proprietory antiseptic can be used.
3. The plates should be incubated preferably at 20–25 °C for 2–3 days, but can be kept at room temperature.
4. Filter paper discs (Whatman Antibiotic Assay discs) are available from school science suppliers or they can be cut from Whatman No. 1 filter paper with a cork borer (ca 6 mm diameter) or a hole punch.
5. When students are mixing the agar and the culture in the Petri dish, they should be instructed to swirl the contents **gently** a few times clockwise, anti-clockwise, forwards and sideways.

Notes

1. Other suitable bacteria include e.g. *Micrococcus luteus*, *Escherichia coli* and *Bacillus subtilis*.
2. Inhibition of growth is shown by a clear area around the disc. Growth appears either as many small colonies visible to the naked eye or, if a large volume of cells was added, a confluent area of turbidity.
3. The same approach can be used to investigate the effects of other antimicrobial compounds – see p. 43.

Materials (each group)

- ▷ *Bacillus subtilis* culture in nutrient broth
- ▷ Universal bottle containing ca 20 cm^3 molten nutrient agar held at ca 45–50 °C
- ▷ 70 % (v/v) industrial denatured alcohol in a small beaker or glass Petri dish covered in foil (*CAUTION: flammable*)
- ▷ Petri dish
- ▷ sterile dropping pipette
- ▷ ca 10 cm^3 antiseptic, e.g. TCP, Dettol, in Universal bottle labelled A
- ▷ 2 Universal bottles labelled B and C
- ▷ distilled water in a beaker
- ▷ beaker of disinfectant
- ▷ 10 cm^3 syringe or pipette and filler
- ▷ metal forceps (can be pre-sterilised by autoclaving)
- ▷ 4 sterile paper discs
- ▷ Bunsen burner
- ▷ adhesive tape
- ▷ marker pen
- ▷ graph paper

Questions

Session 1

1. What are antiseptics?
2. What is the difference between antiseptics and antibiotics?
3. What might affect the growth of microbes between now and the next lesson?
4. What is the concentration of antiseptic in bottles B and C?
5. Why are the discs added in the order suggested?
6. What do you expect the appearance of your agar plates to be next lesson?

Session 2

7. Do your results support the predictions you made last lesson?
8. Describe the range of results obtained by the class for each dilution. Suggest possible reasons for any differences between the groups.
9. Do the class results all indicate the same overall pattern? If so, what conclusions can you draw from them?
10. What further experiments could you do to confirm these conclusions?

Open-ended investigation

Investigating the effects of antimicrobials

Research brief

Using this information and other facts you have researched, plan an investigation into some aspect of antimicrobial substances. The basic procedure for testing the effects of antimicrobials is described on p. 39. You may wish to modify this according to the type of investigation you are planning. For example, antibiotic production by soil bacteria can be investigated by inoculating a nutrient agar plate with some soil, incubating it and looking for zones of inhibition.

Background

- Antimicrobial substances include disinfectants, antiseptics and antibiotics. Disinfection is a procedure that destroys or inactivates microbes. It usually involves the treatment of non-living objects such as surfaces or liquids with chemicals (disinfectants) e.g. chlorine, phenols and hypochlorites. Antisepsis is the disinfection of living tissues with chemicals (antiseptics) e.g. hydrogen peroxide, iodine and diluted alcohol. Antibiotics are chemicals that, even at very low concentrations, inhibit or kill certain microbes. Penicillins are a well-known group of antibiotics.
- Disinfectants and antiseptics that kill bacteria are said to be bactericidal. Others merely halt the growth of bacteria and if inactivated, e.g. by dilution, bacterial growth may be resumed. These are said to be bacteriostatic. A bactericidal disinfectant or antiseptic may become bacteriostatic when diluted.
- Antibiotics are produced by microbes as a natural defence against other microbes. Some are still produced commercially using micro-organisms, although a large number are manufactured chemically. Some antibiotics are active against a narrow range of species whilst others affect a broad spectrum of organisms. The ability to make antimicrobial substances is not limited to microbes; most animals have antibacterial substances in their bodily secretions, such as lysosyme in sweat and tears. Plant materials such as garlic (*Allium sativum*), tea tree oil (*Melaleuca alternifolia*) and oil of cloves (*Syzyium aromaticum*) also have antimicrobial properties.

Designing your investigation

In designing your investigation you should consider the experimental and investigative skills to be assessed. These are:

- planning
- implementing
- analysing evidence and drawing conclusions
- evaluating evidence and procedures

(*See your coursework guide for further details*).

Points to consider when planning your investigation:

- Any hazards involved in doing the experiment and how the risks of the procedure can be minimised. (see *Basic Practical Microbiology: A Manual*)
- Whether you can make a prediction that you can test (e.g. higher concentrations of antimicrobial substance will be more effective).
- What variables need to be taken into account (e.g. incubation temperature, pH).
- How many tests you need for reliability (e.g. how many replicates of each treatment).

Examples

Here are some ideas that could be explored:

- The antimicrobial effects of different plant extracts
- The effect of concentration on the inhibitory effects of different antimicrobial agents
- Antimicrobial effects of different toothpastes, mouthwashes or deodorants
- Effects of antimicrobials on different microbes

Writing Up

Your report should include the following sections, in this order: Introduction/plan; Hypothesis; Prediction; Materials and Methods (including a risk assessment); Results; Discussion; Evaluation/Conclusion.

Safety!

Your plan MUST be checked by your teacher before starting your investigation.