Chemical Methods of Control: Disinfectants and Antiseptics

One nineteenth-century method of avoiding cholera: Wear a pouch of foul-smelling herbs around your neck. If the odor is bad enough, disease carriers will spare you the trouble of avoiding them.

ANONYMOUS

Objectives

After completing this exercise, you should be able to:

1. Define the following terms: disinfectant and antiseptic.
2. Describe the use-dilution test.
3. Evaluate the relative effectiveness of various chemical substances as antimicrobial agents.

Background

A wide variety of chemicals called **antimicrobial agents** are available for controlling the growth of microbes. Chemotherapeutic agents are used internally and will be evaluated in another exercise. **Disinfectants** are chemical agents used on inanimate objects to lower the level of microbes on their surfaces; **antiseptics** are chemicals used on living tissue to decrease the number of microbes. Disinfectants and antiseptics affect bacteria in many ways. Those that result in bacterial death are called **bactericidal agents.** Those causing temporary inhibition of growth are **bacteriostatic agents.**

No single chemical is the best to use in all situations. Antimicrobial agents must be matched to specific organisms and environmental conditions. Additional variables to consider in selecting an antimicrobial agent include pH, solubility, toxicity, organic material present, and cost. In evaluating the effectiveness of antimicrobial agents, the concentration, length of contact, and whether it is lethal (*-cidal*) or inhibiting (*-static*) are the important criteria. The standard method for measuring the effectiveness of a chemical agent is the **American Official Analytical Chemist's use-dilution test.** For most purposes, three strains of bacteria are used in this test: *Salmonella enterica* Choleraesuis, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. To perform a use-dilution test, metal rings are dipped into standardized cultures of the test bacteria grown in liquid media, removed, and dried. The rings are then placed into a solution of the disinfectant at the concentration recommended by the manufacturer for 10 minutes at 20°C. The rings are then transferred to a nutrient medium to permit the growth of any surviving bacteria. The effectiveness of the disinfectant can then be determined by the amount of resulting growth. The use-dilution test is limited to bactericidal compounds and cannot be used to evaluate bacteriostatic compounds.

In this exercise, we will perform a modified use-dilution test.

Materials

Petri plates containing nutrient agar (2)

Sterile water

Sterile tubes (3)

Sterile 5-ml pipettes (2)

Sterile 1-ml pipettes (2)

Test substance: chemical agents such as bathroom cleaner, floor cleaner, mouthwash, lens cleaner, and acne cream. Bring your own.

Culture

Staphylococcus aureus

Techniques Required

Inoculating loop technique, Exercise 10

Aseptic technique, Exercise 10

Pipetting, Appendix A

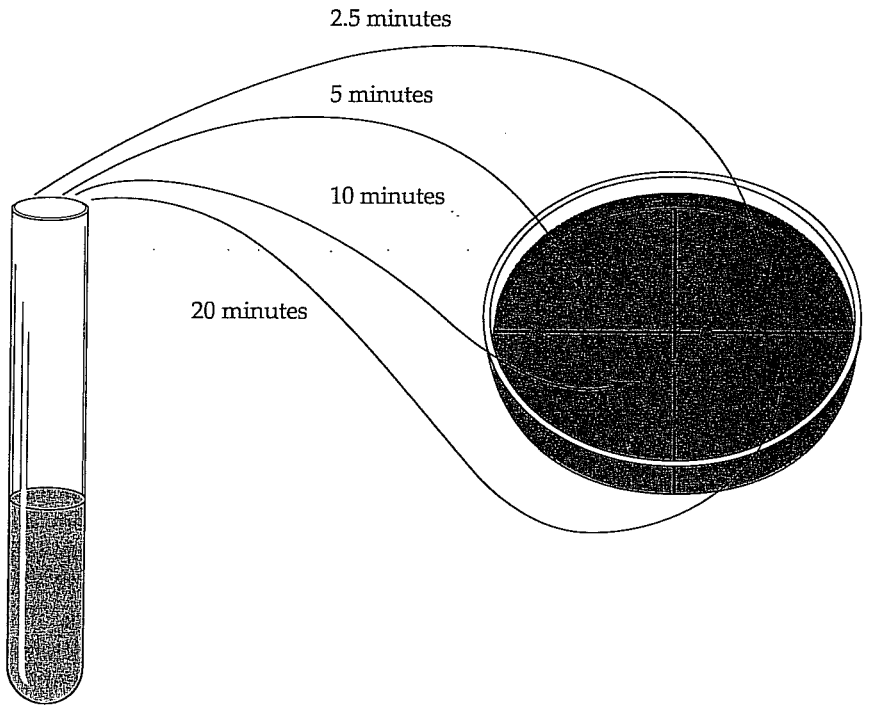

Figure 24.1

Transfer a loopful from the tube containing the chemical and *Staphylococcus* to the appropriate sector at the time intervals shown. Repeat the procedure with a loopful from the tube containing the test substance and *Staphylococcus* onto the appropriate sector of the second nutrient agar plate.

Procedure

1. Using sterile water, prepare a dilution of the test substance in a sterile tube, diluted to the strength at which it is normally used. If it is a paste, it must be suspended in sterile water.
2. Transfer 5 ml of the diluted test substance to a sterile tube. If the test substance is normally used at full strength, then don't dilute it for this experiment. Label the tube. Add 5 ml of your laboratory disinfectant to another sterile tube. What is the disinfectant you use to disinfect your lab bench?

   _____

   Label the tube.
3. Divide one plate of nutrient agar into five sections. Label the sections "0," "D-2.5," "D-5," "D-10," and "D-20." The D stands for laboratory disinfectant.
4. Label the other nutrient agar plate for the other chemical and divide it into four sections. Label the sections "2.5," "5," "10," and "20."
5. Inoculate the 0 sector with a loopful of *S. aureus*.
6. Aseptically add 0.5 ml of the *S. aureus* culture to each tube prepared in step 2.
7. Transfer one loopful from each tube to a corresponding sector at 2.5 minutes, 5 minutes, 10 minutes, and 20 minutes (Figure 24.1).
8. Incubate the plates, inverted, at 35°C until the next lab period. (Discard the chemical/bacteria mixtures in the To Be Autoclaved area.)
9. Observe the plates for growth. Record the growth as (−) = no growth, (+) = minimum growth, (2+) = moderate growth, (3+) = heavy growth, and (4+) = maximum growth.

Exercise 24

LABORATORY REPORT

Chemical Methods of Control: Disinfectants and Antiseptics

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

Time of Exposure (min)	Amount of Growth		
	Control	Lab Disinfectant	Chemical: _____
0			
2.5			
5			
10			
20			

Conclusions

_____

_____

_____

Questions

1. Was this a fair test? Is it representative of the effectiveness of the test substance? _____

_____

_____

2. Read the label of the preparation you tested. What is (are) the active ingredient(s)? _____

_____

Using your textbook or another reference, find the method of action of the active ingredient(s) in the test substance.

_____

_____

_____

3. What is the use-dilution method? _____

_____

_____

Critical Thinking

1. How could the procedures used in this experiment be altered to measure bacteriostatic effects?

2. In the use-dilution test, a chemical is evaluated by its ability to kill 10^6 to 10^8 dried *Clostridium sporogenes* or *Bacillus subtilis* endospores. Why is this considered a stringent test?

3. The effectiveness of disinfectants can be measured in DRT values. DRT, or decimal reduction time, is the length of time it takes to kill 90% of a test population of bacteria. The DRT values for contact lens disinfectants against *Serratia marcescens* are as follows:

Disinfectant	DRT Value (min)	Disinfectant	DRT Value (min)
Chlorhexidine, 0.005%	2.8	Thimerosal, 0.002%	138.9
Hydrogen peroxide, 3%	3.1	Polyquaternium-1, 0.001%	383.3

Which disinfectant is most effective? _____ What is the minimum time that lenses with 10^2 bacteria should be soaked in chlorhexidine? _____

In polyquaternium-1? _____ What if the lenses are contaminated with *Staphylococcus* or *Acanthamoeba*? _____

_____

Why isn't a higher concentration of disinfectant used? _____