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 an antimicrobial agent, the concentration, length of contact, and whether it is lethal (-cidal) or inhibiting (-static) are the important criteria. Before a disinfectant is selected, the decimal reduction time (DRT) for the most common and persistent microbes identified at a health care facility should be determined. The DRT is the time it takes to kill 90% of the test microbial population. 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: Salmontella 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 next 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

First Period

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.

Cultures (as assigned)

Escherichia coli
Staphylococcus epidermidis
Pseudomonas aeruginosa **BSL-2**
Staphylococcus aureus **BSL-2**

Techniques Required

Inoculating loop technique (Exercise 4)
Aseptic technique (Exercise 4)
Pipetting (Appendix A)
PROCEDURE First Period

Work with another student group so that you test the effects of the same disinfectant or antiseptic against the two bacteria available.

Wear safety goggles when pipetting.

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. If the test substance is normally used at full strength, then don’t dilute it for this experiment.

2. Transfer 5 ml of the test substance prepared in step 1 to a sterile tube. 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.

FIGURE 24.1 Procedure for testing the effectiveness of a disinfectant or antiseptic.

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 your assigned bacteria.

6. Aseptically add 0.5 ml of the assigned 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.)

PROCEDURE Second Period

Observe the plates for growth. Record the growth as (−) = no growth, (+) = minimum growth, (2+) = moderate growth, (3+) = heavy growth, and (4+) = maximum growth. Observe the results of students using the other organism.
LABORATORY REPORT
Chemical Methods of Control: Disinfectants and Antiseptics

PURPOSE

1. How could the procedure used in this experiment be modified to increase bacterial killing time?

HYPOTHESIS

The disinfectants tested will kill all bacteria in ______ minutes.

RESULTS

1. What organism did you use?

Be sure to record the chemical or product you tested.

<table>
<thead>
<tr>
<th>Times of Exposure (min)</th>
<th>Amount of Growth (Record growth on a scale of — to 4+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
2. Classmates' results for: __________________________ bacteria

<table>
<thead>
<tr>
<th>Time of Exposure (min)</th>
<th>Amount of Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td></td>
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<tr>
<td>2.5</td>
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<td>10</td>
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<tr>
<td>20</td>
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</table>

CONCLUSIONS

1. Do your results confirm your hypothesis?

2. Was your test chemical effective? Briefly explain.

3. Was it more effective than the lab disinfectant? Briefly explain.

4. Was your test chemical bactericidal or bacteriostatic?

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)?
EXERCISE 24: CHEMICAL METHODS OF CONTROL: DISINFECTANTS AND ANTISEPTICS

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

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 $10^8$ to $10^9$ dried *Clostridium sporogenes* or *Bacillus subtilis* endospores. Why is this considered a stringent test?

BACKGROUND

The observations that were made during the experiment may have influenced the outcome of this study. It is important to note that the results obtained in this experiment may not be applicable to all situations. The success of the experiment depends on the quality and accuracy of the procedures used.

CLINICAL APPLICATION

The effectiveness of disinfectants can be measured in DRT values. The DRT values for contact lens disinfectants against *Serratia marcescens* are as follows:

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>DRT Value (min)</th>
<th>Disinfectant</th>
<th>DRT Value (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine, 0.005%</td>
<td>2.8</td>
<td>Thimerosal, 0.002%</td>
<td>138.9</td>
</tr>
<tr>
<td>Hydrogen peroxide, 3%</td>
<td>3.1</td>
<td>Polyquaternium-1, 0.001%</td>
<td>383.3</td>
</tr>
</tbody>
</table>

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?

MATERIALS

First Period

- Petri plate containing Mueller-Hinton medium
- Drug streak plate
- Alcohol
- Antimicrobial discs
- Prepared slides

Second Period

- Ruler
- Preclot plate containing horse blood
- Test tubes

Intelligent, spot-administer the appropriate dose
Intelligent, spot-administer the appropriate dose
Intelligent, spot-administer the appropriate dose