Bacteria Sensitivity
Lab Activity
Student Study Guide

BACKGROUND

Bacteria, which inhabit nearly every portion of the earth where water and nutrients are available, are in part responsible for food spoiling and materials deteriorating, as well as numerous diseases. The control of bacteria is therefore extremely important in medicine and many industries. By using both chemical and biological substances, scientists are able to slow or stop the spread of many types of harmful bacteria without destroying or poisoning the substances in and on which these organisms grow.

Disinfectants are chemical agents that affect the growth of bacteria by either microcidal (microorganism killing) or microstatic (growth inhibiting) activity. These activities are relative; a substance that is microcidal at a certain combination of temperature, pH, concentration, and exposure may be only mildly inhibiting under different conditions.

Disinfectants can create unfavorable conditions for bacteria in two ways: interfering with cell processes, such as enzyme activity and the passage of material in and out of the cell membrane and wall; and interfering with cell structures, such as wall proteins and nucleic acids. Because of the way disinfectants work, and because concentration affects performance, they can be harmful to all living cells under certain conditions. As a result, although disinfectants continue to be very important tools in sanitation, they have been largely replaced in medical use by antibiotics, which are more specific.

Many bacteria and fungi produce antibiotics as a means of preventing the growth of other microorganisms helping to preserve the supply of available water and nutrients. Antibiotics generally affect some aspect of an organism’s metabolism. Tetracycline and streptomycin, for example, act at the ribosomes, the location of protein synthesis. By blocking RNA molecules carrying essential amino acids, or by preventing their binding to ribosomes, these antibiotics interrupt the formation of proteins critical to the bacteria’s survival.

Antibiotics can also inhibit bacteria by altering the cell wall to prevent reproduction. Penicillin is one of the most well known examples of this type of antibiotic. Other antibiotics function by interrupting important processes within the nucleus, such as the replication of DNA.
Antibiotics do not pose as great a danger to the patient as more generally acting chemicals and are preferred medicines, due to the highly specific way in which they function—affecting only the disease-causing organism. This specific method of action also means that an antibiotic’s effectiveness against different bacteria species will vary.

Antibiotics are often divided into two groups: those affecting bacteria that give a negative reaction to the Gram stain and those which affect positively reacting organisms. This kit contains two common bacterial species. *Bacillus subtilis*, a soil organism, is rod shaped, nonpathogenic, Gram positive, and forms spores. *Escherichia coli*, a normal inhabitant of the human intestinal tract, is rod shaped, Gram negative, and does not form spores. Other species of *Escherichia* are frequently the cause of appendicitis, peritonitis, and sporadic infections of the internal organs.

The effectiveness of antibiotics and disinfectants can be demonstrated by placing discs impregnated with antibiotics or disinfectants on a bacteria culture. If the substance is effective against the bacteria, a clear zone will appear around the disc where the substance inhibits growth. The relative effectiveness of each antibiotic or disinfectant can then be obtained by comparing the area of the clear zones (zones of inhibition).
OBJECTIVES

- Examine the microcidual abilities of several antibiotics applied to two different bacterial species
- Test the microcidual abilities of several disinfectants applied to two different bacterial species
- Compare and contrast the effectiveness of each agent on a Gram-positive bacterium versus a Gram-negative bacterium

MATERIALS

MATERIALS NEEDED PER GROUP

2  Agar plates inoculated with *E. coli*
2  Agar plates inoculated with *B. subtilis*
14  Blank discs
1  Pair of forceps
1  Ruler

SHARED MATERIALS

- Iodine
- Mouthwash
- Peroxide
- Hand soap
- Dish soap
- Antibiotic test discs

DID YOU KNOW?
The term "disinfect" has Latin roots. It is a combination of *dis*, meaning "apart" and *infectere*, meaning "to corrupt".

DID YOU KNOW?
Antibiotics only affect bacteria. Viral illnesses, such as the common cold or flu, are not affected by antibiotics. Taking an antibiotic for these illnesses can actually contribute to bacterial antibiotic resistance, as can not taking the entire dose of antibiotic as prescribed by your doctor.
PROCEDURE

This procedure should be performed in a sterile environment such as a laminar-flow hood designed for bacterial culture. If a laminar-flow hood is not available, perform the activity in a clean, draft-free part of the room. Be sure to wear protective gloves and observe proper sterile technique through the entire procedure. Also be sure to wipe your entire work area down with 70% isopropyl alcohol before performing the procedure.

1. Place one of your *E. coli* plates upside down (with the lid on) on the template that follows and trace the pattern onto the bottom of your plate with a permanent marker or wax pencil. Repeat for one of the *B. subtilis* plates as well. Label these two plates ‘Antibiotics’.

2. Repeat the previous step using both of your remaining plates. Label both of these plates ‘Disinfectants’.

3. Label all four plates with your group name.

DID YOU KNOW?
Disinfection to prevent the spread of pathogens is a practice that can be traced back to the ancient Egyptians who used to smoke and salt foods, burn wastes, and expose bedding to sunlight to combat bacterial infection.
4. Using sterile forceps, remove a chloroamphenicol antibiotic disc from the magazine. Place the disc over the circle marked ‘1’ on the surface of the agar of the *E. coli* plate labeled ‘Antibiotics’.

   **NOTE**

   To avoid contaminating the plates, flame the forceps after each application, and uncover the plates as little as possible when placing the discs on the plates.

5. Repeat for the remaining antibiotic discs, using the chart below as a guide. Be sure to flame the forceps and allow them to cool before applying the next disc.

<table>
<thead>
<tr>
<th>Circle</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>#2</td>
<td>Penicillin</td>
</tr>
<tr>
<td>#3</td>
<td>Streptomycin</td>
</tr>
<tr>
<td>#4</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>#5</td>
<td>Tetracycline</td>
</tr>
</tbody>
</table>

6. Place a blank sterile disc in the center of the plate, over the circle marked ‘C’.

7. Repeat the above steps for the *B. subtilis* plate labeled ‘Antibiotics’.

8. Using sterile forceps moisten a blank sterile disc in the disinfectant iodine. Do not ‘soak’ the disc in the iodine. Remove any excess iodine by touching the disc to the inside of the mouth of the bottle.

9. Place the disc over the circle marked ‘1’ on the surface of the agar of the *E. coli* plate labeled ‘Disinfectants’.

   **NOTE**

   To avoid contaminating the plates, flame the forceps after each application, and uncover the plates as little as possible when placing the discs on the plates.

DID YOU KNOW?

Bacteria rapidly evolve resistance to antibiotics when the drugs are used improperly. Therefore, it is very important to take antibiotics only when absolutely necessary. Bacterial resistance to antibiotics is a widespread problem with approximately 70% of bacteria that cause infections in hospitals being resistant to at least one of the antibiotics commonly used to treat infections. More than 13,000 people die each year due to infections by antibiotic resistant bacteria.
10. Using four more blank sterile discs, repeat for the remaining disinfectants. Use the chart below as a guide. Be sure to flame the forceps and allow them to cool before applying the next disc.

<table>
<thead>
<tr>
<th>Circle</th>
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</tr>
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<tbody>
<tr>
<td>#1</td>
<td>Iodine</td>
</tr>
<tr>
<td>#2</td>
<td>Mouthwash</td>
</tr>
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<td>#3</td>
<td>Peroxide</td>
</tr>
<tr>
<td>#4</td>
<td>Hand soap</td>
</tr>
<tr>
<td>#5</td>
<td>Dish soap</td>
</tr>
</tbody>
</table>

11. Place a blank sterile disc in the center of the plate, over the circle marked ‘C’.

12. Repeat the above steps for the B. subtilis plate labeled ‘Disinfectants’.

13. Incubate all four of your plates, agar side up, at 30°C for 24 hours.

14. Measure the diameter of the clear area (zone of inhibition) around each disc on the plate. Record the data in the Analysis section.

**NOTE**

If students will not be able to analyze their plates the next day, place them in a refrigerator until the next lab period.
### ANALYSIS

**Inhibition Zone (mm)**

<table>
<thead>
<tr>
<th>Antibiotic Name</th>
<th><em>E. coli</em></th>
<th><em>B. subtilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chloroamphenicol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Penicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Streptomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Erythromycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Tetracycline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
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</table>
WARD’S
Bacteria Sensitivity
Lab Activity

Name:
Group:
Date:

ASSESSMENT

1. Which antibiotic was most effective against the Gram-negative *E. coli*? How do you know? How about against the Gram-positive *B. subtilis*?

2. Which disinfectant was least effective against the Gram-negative *E. coli*? How do you know?

3. Which disinfectant was least effective against the Gram-positive *B. subtilis*? How do you know?

4. Did your results with a specific disinfectant or antibiotic differ from those obtained by other lab groups? Why might this occur?

5. Why are antibiotics, rather than disinfectants, used in treating diseases?

6. Why are disinfectants, rather than antibiotics, used to decontaminate inanimate objects?
7. Which substances would you use to treat an object contaminated with an unknown bacterium? Why?

8. Disinfectants are not the only method used in the sterilization of contaminated surfaces. Using the internet or your school library, research another method used to sterilize objects and explain it below.

9. Often times, physicians will see patients and suspect the patient is not feeling well due to an infectious agent they are carrying. Before receiving the lab results from the tests they perform, the physicians will issue a preventative prescription for antibiotics. Microbiologists often criticize this approach. Explain why you believe microbiologists are opposed to this practice.

10. Many products on the market claim to be antibacterial. Visit a local supermarket or drugstore and find at least three such products. Write down the product name and examine the list of ingredients and record the antibacterial agent each product contains (and the concentration if possible). Bring your information in and share it with the class.