Carolina BioKits®

Disinfectant and Antiseptic Sensitivity

TEACHER’S MANUAL
AND STUDENT GUIDE

CAROLINA®
World-Class Support for Science & Math
## Carolina BioKits®: Disinfectant and Antiseptic Sensitivity

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*Photocopy the Student Guide as needed for use in your classroom.

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Carolina BioKits®:
Disinfectant and Antiseptic Sensitivity

Overview
Disinfectants and antiseptics are chemical compounds that inhibit or kill microorganisms. During this experiment, students observe the relative effectiveness of five common antimicrobial agents on a gram-negative bacterium, *Escherichia coli*, and a gram-positive bacterium, *Bacillus cereus*. The materials supplied in the Classroom Kit are sufficient for 30 students working in 10 groups of three. The materials in the Demonstration Kit are sufficient for one teacher demonstration or a single student or group; where appropriate, modify the preparation and procedure instructions accordingly.

Objectives
Students will

- test the effectiveness of five disinfectants and antiseptics on two common bacteria.
- on the basis of observed results, discuss the advantages of one disinfectant or antiseptic over the others.

Correlation to Science Standards
To view the national and local standards met by this kit, visit www.carolina.com/correlations.

Science and Engineering Practices
- Asking questions and defining problems
- Planning and carrying out investigations
- Analyzing and interpreting data
- Constructing explanations and designing solutions
- Engaging in argument from evidence
- Obtaining, evaluating, and communicating information

Crosscutting Concepts
- Cause and effect
- Structure and function
- Stability and change
**Time Requirements**

Preparation................................................................. 75 minutes  
Day 1: Adding Disks to Plates........................................ 40 minutes  
Day 2: Measurements and Observations (after 24 hours).... 15 minutes  
Day 3: Measurements and Observations (after 48 hours).... 15 minutes  

This experiment requires portions of three class periods on three consecutive days. On Day 1, students receive *E. coli* and *B. cereus* cultures and place the disinfectant and antiseptic disks in them. On Day 2 (after 24 hours) and Day 3 (after 48 hours), students observe bacterial growth, note areas of inhibition, and analyze the results of the disinfectant and antiseptic sensitivity tests.

**Materials**

*Included in the kit:*

<table>
<thead>
<tr>
<th>Item</th>
<th>Classroom Kit</th>
<th>Demonstration Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order Form for the following perishable items (if not shipped with the order):</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> broth culture</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> broth culture</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>4 125-mL bottles</td>
<td>2 20-mL tubes</td>
</tr>
<tr>
<td>Petri dishes</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>30-mL bottle of alcohol (2-propanol)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>20-mL bottle of bleach solution</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>20-mL bottle of surface cleaning solution</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>30-mL bottle of vinegar</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Small bottle of mouthwash</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Forceps</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Alcohol prep wipes</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>Autoclavable disposal bag</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10-mL tube of sterile water</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Filter paper</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td><em>Techniques for Studying Bacteria and Fungi</em> manual</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hole punch</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Metric rulers</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Twist tie</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1-mL sterile pipet</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Teacher's Manual and reproducible Student Guide</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Needed, but not supplied:

<table>
<thead>
<tr>
<th>Item</th>
<th>Classroom Kit</th>
<th>Demonstration Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>water bath with thermometer (or pan of boiling water)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>plastic cups or beakers</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>heat resistant gloves</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>examination gloves</td>
<td>varies</td>
<td>1 pair</td>
</tr>
<tr>
<td>labeling markers</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>70% ethanol or isopropanol (for cleaning prep area)</td>
<td>varies</td>
<td>varies</td>
</tr>
<tr>
<td>clear tape</td>
<td>varies</td>
<td>varies</td>
</tr>
<tr>
<td>incubator (optional)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>autoclave (optional)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>plastic bucket (optional); for alternative disposal method</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Order Form for Perishable Items

If the kit with perishables is ordered, there will be no Order Form—the perishables will be shipped at the same time as the other kit materials. Otherwise, follow the instructions on the Order Form for prepaid delivery of the perishable items.

Perishable Materials Handling

Upon receipt of the perishable items, store *B. cereus* and *E. coli* cultures at room temperature.

Safety

Use this kit only in accordance with established laboratory safety practices, including appropriate personal protective equipment (PPE). Ensure that students understand and adhere to these practices.

When working with bacteria it is crucial to use sterile technique and proper laboratory practices to reduce contamination. Sanitize lab benches before and after the lab. Ensure that students wash their hands before and after conducting the lab exercises, and that they wear personal protective equipment such as safety glasses or goggles, gloves, and aprons when appropriate.

Know and follow all federal, state, and local regulations as well as school district guidelines for the disposal of laboratory wastes.

If you prefer to microwave the agar in this kit, first loosen the cap on the bottle (or tube, if you are using the demo kit). Then place the bottle in the microwave for 1-minute intervals, swirling the agar between each interval to ensure even melting, until the agar is completely melted. The bottles of melted agar will be hot. Use appropriate personal protective equipment. Watch the agar carefully to ensure that it does not boil out of the bottle. **Caution: Do not microwave the bottle of agar without first loosening the cap, as this could make the glassware explode.**
Background

Like other microorganisms, many types of bacteria can cause disease and food spoilage. To control the growth and spread of potentially harmful microorganisms, we use antimicrobial agents—chemical compounds that inhibit or kill microbes. Disinfectants are a class of antimicrobials that damage or kill bacteria and other microbes, but also might kill or damage other living cells; therefore, many disinfectants should only be used to remove microbial contaminants from inanimate objects. Another class of antimicrobials, known as antiseptics, safely can be applied to living tissue. The bleach and cleaning solutions supplied in this kit are disinfectants, and the mouthwash, vinegar, and alcohol are antiseptics.

Different disinfectants and antiseptics act on microorganisms in different ways. Some inhibit growth and reproduction, while others kill the microorganism outright. Scientists group disinfectants and antiseptics into categories according to their mode of action:

1. Interference with the cell wall and/or cell membrane;
2. Interference with enzyme function;
3. Denaturation of proteins.

Knowledge of how antimicrobial agents work on the cellular level can be extremely useful in predicting the most appropriate chemical to use against a particular microbe in a given situation. A disinfectant that is highly effective against one strain of bacteria may not be nearly as effective against a different strain. Manufacturers often combine several chemicals to produce disinfectants that are effective against a variety of microbes and, therefore, have a variety of uses. The same is true of antiseptics.

In this activity, students will test a total of five disinfectants and antiseptics on two common strains of bacteria, *Bacillus cereus* and *Escherichia coli*. *B. cereus* is commonly found in soils. It is a gram-positive spore forming bacillus, or rod-shaped bacterium. It causes food spoilage of cooked meats, raw and cooked vegetables, and desserts. It is also known to cause gastroenteritis. *E. coli* is a coliform, a normal inhabitant of the intestines of vertebrates. Coliforms are gram-negative, nonspore-forming bacilli that ferment lactose rapidly, producing a gaseous by-product. Although *E. coli* is usually nonpathogenic, some strains are opportunistic and may pose a risk to compromised patients such as the elderly, newborns, or those with a weakened immune system.

The radial diffusion technique used in this exercise is one of the methods employed to determine the antimicrobial activity of a chemical compound. The chemical diffuses from the disk into the surrounding agar. Zones of inhibition around the disinfectant or antiseptic disk indicate the activity of the disinfectant or antiseptic. The diameter of the zone of inhibition is directly proportional to the antimicrobial effectiveness of the chemical. This method can be useful, but is imprecise. Chemicals diffuse at different rates through agar, depending on their molecular weights. They also might be inactivated by the organic culture medium. Nevertheless, the results are useful for comparing disinfectants and antiseptics at the introductory level.
Preparation: Demonstration Kit

1. If your kit included an Order Form for delivery of perishable items, follow its instructions to ensure that the items arrive when you need them.

2. Review the content of the Teacher’s Manual and the Student Guide. Familiarize yourself with the activity instructions, required materials, and assessments.

3. Photocopy the Student Guide for each student.

4. Gather the materials that are needed but not supplied.

5. Use the hole punch to create small disks from the filter paper. You will need a minimum of 12 disks. Place the disks into a plastic cup or beaker.

6. Pour a small sample of each solution to be tested into a small plastic cup or beaker labeled with the solution’s identity.

7. Decide where and how you will incubate the petri dishes. Optimal incubation temperatures are 30°C for B. cereus and 37°C for E. coli. However, room temperature should provide adequate conditions for growth.

Preparation of Agar Plates for the Demo Kit (no more than 2 hours before the lab)

1. Disinfect work surfaces with 70% alcohol before preparing the plates.

2. Slightly loosen the caps on the 2 tubes of nutrient agar, and then place them in the boiling water bath to melt the agar. Make sure the water level is even with the agar level. Melting requires 20–30 minutes. Swirl the agar inside each tube to be sure that it has melted completely. Use a heat-resistant glove to remove the tubes.

   **Note:** Water must be kept at a constant boil to melt the agar in 20–30 minutes. Otherwise, it will take significantly longer.

3. Cool the agar in the tubes to 45°C by allowing the water bath to cool to that temperature, or by removing the tubes and letting them cool for several minutes. The tubes should feel comfortably hot to the touch.

4. Place the broth culture tubes in a 30°C water bath for 10 minutes.

   **Important:** Do not heat the tubes to above 30°C, which might heat shock the B. cereus. Failure to complete this step may cause clumping of agar.

5. Label the underside of a petri dish with the name of the bacteria.

6. Invert the B. cereus broth culture to distribute its contents. Remove the cap and use a sterile pipet to transfer 1 mL of broth culture into one agar tube. Cap the tube. Then, label the tube with the name of the bacteria.

7. Gently swirl it to distribute the bacteria throughout the medium. Pour the seeded agar from the tube to a petri dish. Do not put the cover of the petri dish on the lab table. Instead, hold it in your hand and use it as a shield between you and the bottom of the dish. To prevent contamination, cover