LAB 4
Using Antibiotics and Antiseptics to Control the Growth of Bacteria

Objectives:
1. Learn some techniques for working with live bacteria in a lab environment.
2. Know the difference between antibiotics and antiseptics.
3. Be able to measure the response of bacteria to growth regulating agents.

Warning Note: The bacteria used in this lab are alive and growing vigorously. To protect yourself and your group members from infection or contamination, wash your hands before and after the lab this week. Wear protective gloves when working with the bacteria. Absolutely no eating or drinking at any time during this lab! Dispose of all contaminated waste in the appropriate containers.

Introduction:
An antibiotic is a chemical produced by a fungus or a bacteria that has the potential to limit the growth of another bacteria. Antiseptics are chemicals produced by humans that are used to prevent the growth of bacteria or fungi on living tissues (disinfectants prevent growth on inanimate objects). Many antibiotics are selective in terms of which species they will control, and the mechanism of how they control the growth. Likewise, antiseptics rarely kill all bacteria present on living organism, rather they just reduce the overall number of bacteria present.

In this lab, we will apply an assortment of antibiotics and antiseptics to a species of bacteria that is actively growing on a plate of agar. The response of the bacteria to these agents will help us conclude which agents are best able to control their growth. The bacteria will be spread out evenly on the agar plate, this is known as a lawn. In this way, we can assume that equal numbers of bacteria are present in all portions of the agar plate, and that the antibiotics or antiseptics are the only things that will affect their growth.

Today we will work with two common bacteria: Bacillus cereus is widely distributed in nature and in foods. It is commonly found in soil, milk, cereals, starches, herbs, spices, and other dried food stuffs. Foods most often implicated in outbreaks include meat pies, fried rice and puddings. Individuals may become ill from B. cereus when foods are prepared and held without adequate refrigeration for several hours before serving.

Escherichia coli is a common intestinal bacteria that occurs in the intestines of humans and. Although, most strains of this bacteria are harmless, several are known to produce toxins that can cause diarrhea. The most common routes of infection by E. coli are: eating undercooked ground beef (the inside is pink), drinking contaminated (impure) water, drinking unpasteurized (raw) milk, or person to person due to poor personal hygiene after using the bathroom.
**Application of Bacterial Lawn:**
1. Sterilize your work area by spraying and wiping a solution of dilute bleach or 70% ethanol (we will use ethanol for today’s lab). Do not contaminate this area with non-sterile equipment.

2. Obtain a sterile agar plate from supplies bench. The agar medium contains nutrients and space in which the bacteria can grow. You will need a sterile swab to transfer the bacteria onto this plate.

3. Insert the sterile swab into the bacterial culture. Allow any excess to drip off before removing it from the flask.

4. Carefully lift the lid of your plate about 45° and swab the entire surface of the agar. You do not have to press down for the bacteria to be placed on the plate. Also, the bacteria are microscopic, so the plate will not look different once they have been applied.

5. Rotate the plate by 90° and swab again at right angles to the original streaks. **NOTE:** It is important that you cover as much of the surface area as possible in order to obtain good results. You won’t have to dip your swab more than once (there are thousands upon thousands of bacteria on it)

6. Dispose of the swab in the BIOHAZARD bag in the front of the room. Wait two minutes to let the bacterial solution soak into the agar.

**Application of Growth Regulators:**

1. You are going to apply six pre-sterilized disks to your agar plate, right on top of the bacterial lawn. The disks have been saturated with several types of antiseptics and antibiotics. The plate itself must be labeled to indicate which disks have been placed where.

2. On the bottom of the plate (the half with the agar), draw 6 small circles and number them sequentially. They should not come close to each other! Place them around the edge of the plate. See fig 1 for an example.

![Figure 1](attachment:image1.png)

*Figure 1:* A Petri dish with circles marked to indicate position of antibiotic and antiseptic disks. Circles should be numbered sequentially.
3. Using sterile forceps, take a disk from each of the solutions in the front of the room. Shake off any excess liquid. Place the disk above one of the circles. List below which treatment you have assigned to each numbered disk.

   Disk 1:  Sterile Water
   Disk 2:     
   Disk 3:     
   Disk 4:     
   Disk 5:     
   Disk 6:     

4. Replace the lid. Seal the plate shut with lab tape or parafilm. Make sure to label the lid with your group name and lab section. These plates will be placed in an incubator for 24-48 hours to provide the bacteria with a warm environment in which to grow. They should double their population size every 20-40 minutes!

5. Next week we will measure the zone of inhibition around each disk. The more the chemical inhibits the bacterial growth, the wider the “non-growth” will be around that disk. From this data, you can determine the most effective reagents for controlling the growth of this bacteria.

Data Analysis:

1. Examine your plate for bacterial lawn growth. This should appear as a milky-white smear in all the places in which you spread the bacteria.

2. From the underside of the plate, examine the area of the disks to see if any growth has been inhibited. This should appear as a “clear” area. If the disk itself is preventing you from clearly seeing the bacteria, remove it with sterile forceps and drop the disk in the bleach solution for disposal.

3. Measure the diameter of all of the inhibition regions. Record this in Table 1. Use the arbitrary criteria below to determine the bacterial sensitivity to the chemical.

   NS = not sensitive, no inhibition zone
   S = sensitive, zone between 0 and 1 cm
   VS = very sensitive, zone greater than 1 cm.
Table 1  Results of Sensitivity Tests for Antibiotics and Antiseptics.

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<tr>
<th>Chemical Reagent</th>
<th>Inhibition Zone Diameter</th>
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