

## LAB 5 EVALUATION OF ANTIMICROBIAL CHEMICALS

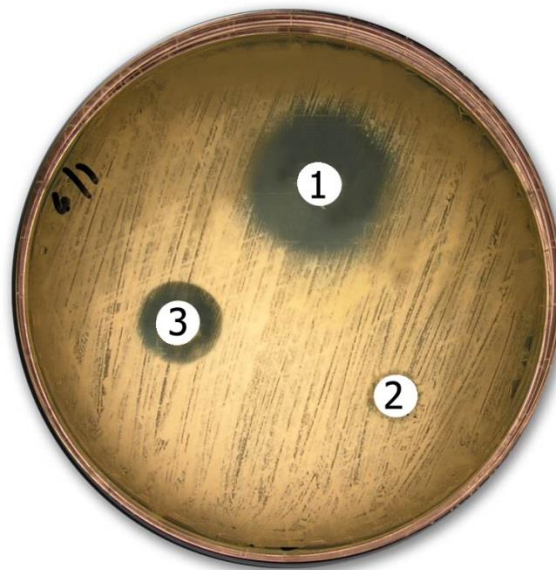
### Objectives

- In this lab you will learn how to:
  - discern between antibiotics, disinfectants, and antibiotics
  - test the sensitivity of bacteria to antibiotics, disinfectants, and antibiotics
  - perform the Kirby-Bauer (Disk Diffusion) test
  - sterilize forceps using ethanol and flame

### Introduction

In both clinical and household settings, **antimicrobial chemicals** are used on an everyday basis. **Antiseptics** are microbe-killing chemicals applied to living tissue, and include hydrogen peroxide, mouthwash, rubbing alcohol, antibacterial soap, and iodine. **Antibiotics** are microbe-killing chemicals taken internally (in pill or liquid form), such as Penicillin, Ampicillin, Streptomycin, and Chloramphenicol. **Disinfectants** are microbe-killing chemicals applied to inert surfaces, and include chlorine bleach (sodium hypochlorite) and Lysol, among many others.

The effectiveness of these substances in killing microbes can be tested using the **Disk-Diffusion Test**, or **Kirby-Bauer Method**. This is a valuable and standard microbiological technique in which a small filter paper disk is impregnated with the chemical/product being tested. The disk is placed on a culture medium in a Petri plate, which has been inoculated with a heavy dose of a single species of microbe. The plates are then incubated to allow growth of the bacteria. The product diffuses away from the disk into the culture media, forming a concentration gradient (the highest concentration of the chemical is closest to the disk). If the microbe is susceptible to the chemical, a clear zone will appear around the disk where the growth was inhibited (illustrated below).



**Disk Diffusion Test, illustrating the effect of various chemicals on bacterial growth. Disks #1 and #3 on the Petri plate contain chemicals that inhibit bacterial growth -- note the clear zone of inhibition around each of those disks. In contrast, disk #2 contains a chemical that does not inhibit bacterial growth; there is no clear zone of diffusion around that disk.**

The size of this **zone of inhibition** depends on the sensitivity of the microbe to the chemical. In other words, the larger the zone of inhibition, the more sensitive the microbe is to the chemical.

A problem with the Disk Diffusion test is the presence of a lot of organic matter (in the nutrient agar), which can reduce the effectiveness of the chemical. For example, organic matter in the agar reacts with bleach (sodium hypochlorite) to form a less harmful set of chemical compounds, including salt (sodium chloride).

### Procedures

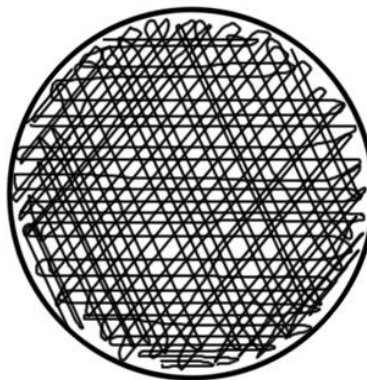
Obtain:

- Petri dishes
- nutrient agar deeps
- broth cultures of *E. coli* or *B. megaterium*
- sterile swabs
- sterile paper disks
- disinfectants, an antiseptic, and an antibiotic disk
- forceps
- 95% ethanol
- small beaker

Pour a Petri plate of nutrient agar. Proceed only after it is cooled and solidified, Label the bottom of the Petri plate with a location for disks #1-3 (see blackboard for labeling instructions). Gently swirl a broth culture of *E. coli* or *B. megaterium* to suspend the bacteria. Dip a sterile swab into the broth culture and replace the cap.

\*\* Bacterial species used: \_\_\_\_\_

Inoculate the plate by streaking the entire surface of the agar with the swab in a zig-zag pattern. Rotate the plate slightly, and continue streaking it by overlapping/crosshatching the streaks from before. Keep streaking until the whole surface is streaked in an even crosshatch pattern, as shown:



Next, flame-sterilize the forceps by dipping the forceps ends in the beaker of ethanol, then igniting the ethanol over the flame. After ignition, quickly move the flaming forceps away from the burner flame.

**IMPORTANT:**

- DO NOT KEEP THE FORCEPS IN THE FLAME! •
- POSITION THE BEAKER OF ETHANOL AWAY FROM THE BURNER! •

Briefly open the container of sterile disks and grab one disk with the forceps. Close container. Add one drop of antiseptic to disk #1. The 3 disks will be:

Antiseptic (disk #1) = \_\_\_\_\_ (name of antiseptic used)

Disinfectant (disk #2) = \_\_\_\_\_ (name of disinfectant used)

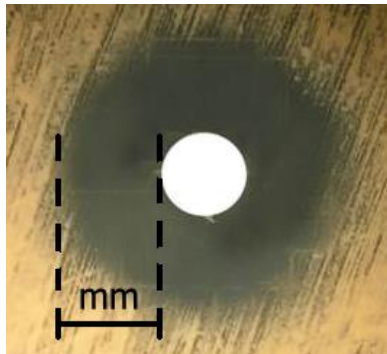
Antibiotic (disk #3) = \_\_\_\_\_ (name of antibiotic used)

Gently press the disk onto the agar surface. Make sure it contacts the agar evenly.

Put the forceps back into beaker of ethanol. Add the other 2 disks to the plate, repeating the steps described above.

When finished, allow the Petri plate to sit on the table for several minutes to allow the fluids to absorb into the agar.

Incubate the plate for 24 hours at 37°C. After incubation, measure the zone of inhibition around each disk, if any exists. Measure from one side of the zone to the other side (the diameter of the circle), as shown below.



Record your results, in mm. Compare to the other students' results.

Disk #1 = \_\_\_\_\_ mm (zone of inhibition)

Disk #2 = \_\_\_\_\_ mm (zone of inhibition)

Disk #3 = \_\_\_\_\_ mm (zone of inhibition)