Zone of Inhibition

Objective: The goal of this project is to measure the effectiveness of different antimicrobial agents by measuring zones of inhibition on bacterial culture plates.

Introduction: Antimicrobial agents are chemicals that are used against bacteria. There are many such agents available. Because there are many different situations where bacterial control is important, no antimicrobial agent is effective in all situations. For example, you wouldn't use the same compound to fight an ear infection as you would use to sterilize surfaces in an operating room. The situations are completely different. In one case, you are trying to assist the body to fight off an internal infection, and in the other case, you are trying to eliminate bacteria from inanimate surfaces.

There are many additional factors that you would have to consider in order to choose an appropriate antimicrobial agent for a given situation. For example, are the chemical properties of the agent (e.g., pH and solubility) appropriate for the situation? You would want to know whether the compound is toxic—to humans, other animals, plants, or beneficial bacteria. Finally, you would definitely want to know that the compound is effective against the organism(s) you are trying to eliminate.

This project shows you one method of measuring the effectiveness of an antimicrobial agent against bacteria grown in culture. This is called the Kirby-Bauer disk-diffusion method, and here is how it works. The bacteria of interest is swabbed uniformly across a culture plate. Then a filter-paper disk, impregnated with the compound to be tested, is placed on the surface of the agar. The compound diffuses out from the filter paper into the agar. The concentration of the compound will be higher next to the disk, and will decrease gradually as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow wherever the concentration in the agar is greater than or equal to that effective concentration. This region is called the "zone of inhibition." Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound. Figure 1, below, illustrates the idea.

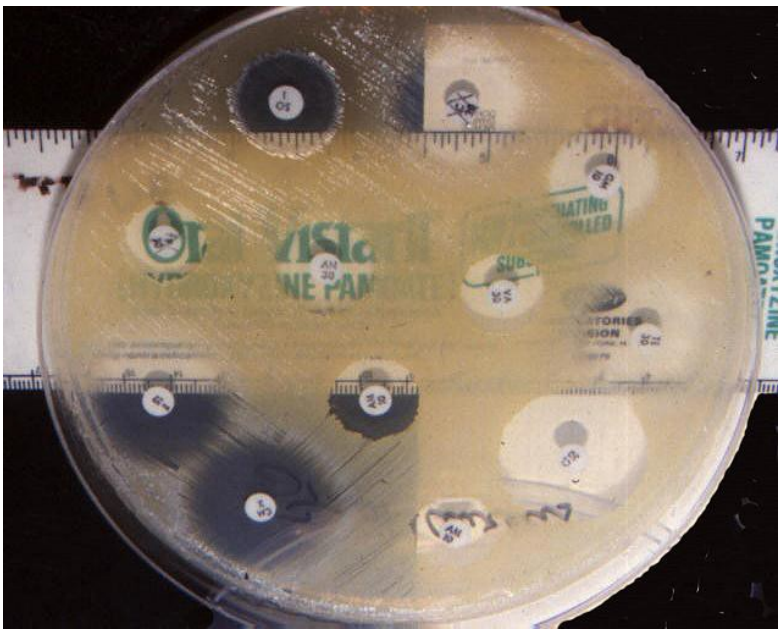

Figure 1. The illustration shows zones of inhibition around filter paper disks saturated with anti-microbial compounds. The diameter of the zone of inhibition is a measure of the effectiveness of an anti-microbial compound (Rollins and Joseph, 2000).

You can use this method to compare the effectiveness of different disinfectants or different antibiotics against a strain of bacteria. Since this method depends on diffusion of the compound, it is important to keep several factors constant when you make your comparisons, including:

- the size of the filter disks,
- the temperature of incubation,
- the composition and thickness of the agar, and
- the uniformity of bacterial plating.

With careful attention to making your conditions consistent, this method will produce reliable results for comparing antimicrobial effectiveness.

Materials and Equipment (Per team)

2 agar plates:
1 plate will serve as your control, with no disinfectants,
1 plate will serve as your test plate, with disinfectant
live bacteria
sterile swabs
filter paper disks.
hole punch
forceps

Disinfectants (use 4) some ideas:

- solution of garlic powder,
- liquid cleaners
- mouthwash
- contact lens cleaner
- anti-acne product
- household bleach (sodium hypochlorite).

Procedure

1. Prepare sterile filter disks by using a hole punch to make small circular disks from filter paper.
2. Use pencil to label each disk with a code for the disinfectant to be used for that disk (up to six). Keep track of the codes in your lab notebook.
3. Use a permanent marker or crayon to mark the bottoms of the three test plates with as many sections as you have disinfectants (four). The sections should all be equal in size. Number the sections sequentially.
4. Label the control plate. The purpose is to show that the bacteria consistently grow uniformly over the plate in the absence of disinfectant disks confirming that your inoculation technique is consistent, and that the plates support uniform bacterial growth.
5. To inoculate a plate, dip a sterile swab in water and rub it on the surface where you suspect bacteria are found.
6. Gently wipe the swab over the surface of the plate, swabbing in three directions (120° apart) to insure complete coverage of the plate. Cover the plate and wait at least five minutes for the plate to dry.
7. Hold a single sterile disk by the edge with sterile forceps and dip it into the disinfectant solution to be tested (make sure it matches with the label on the disk). Touch the disk against the side of the container to drain off excess liquid.
8. Use sterile forceps to place a single disinfectant disk in the center of each of the marked sections on your test plates. Use the forceps to gently press each disk against the agar surface to insure good contact. Remember to use the exact same technique for each disk consistency is very important for this experiment. Take notes in your lab notebook to keep track of which disinfectant is tested in each numbered section.
9. Incubate all of the plates, inverted, (agar on top) overnight. Use a longer incubation time if necessary (for example, for incubation at lower temperature).

Measuring Zones of Inhibition

1. After overnight incubation, examine your plates (keep them covered at all times).
2. The control plates should show uniform colonies over the entire surface of the plate. If the distribution is highly uneven, you will need to improve your inoculation technique and repeat the experiment.
3. If your disinfectants are effective at the concentrations you tested, you should see zones of inhibition around the disinfectant disks. The clear zones around each disk should have a uniform diameter, since diffusion of the compounds through the agar should be uniform in every direction. If this is not the case, suspect either your impregnation technique, or poor contact of the filter paper with the agar.
4. Measure the diameter of the zone of inhibition around each disk. Keeping the lid of the plate in place, use a ruler to measure the diameter of the clear area in millimeters. You will get three separate measurements for each disinfectant, one from each of the three test plates. Are the diameters consistent across all three plates?
5. Use the values from Table 1 (below) to evaluate the bacterial response to each compound (Johnson and Case, 1995).

Diameter of zone of inhibition (mm)	
Resistant	10 or less
Intermediate	11–15
Susceptible	16 or more