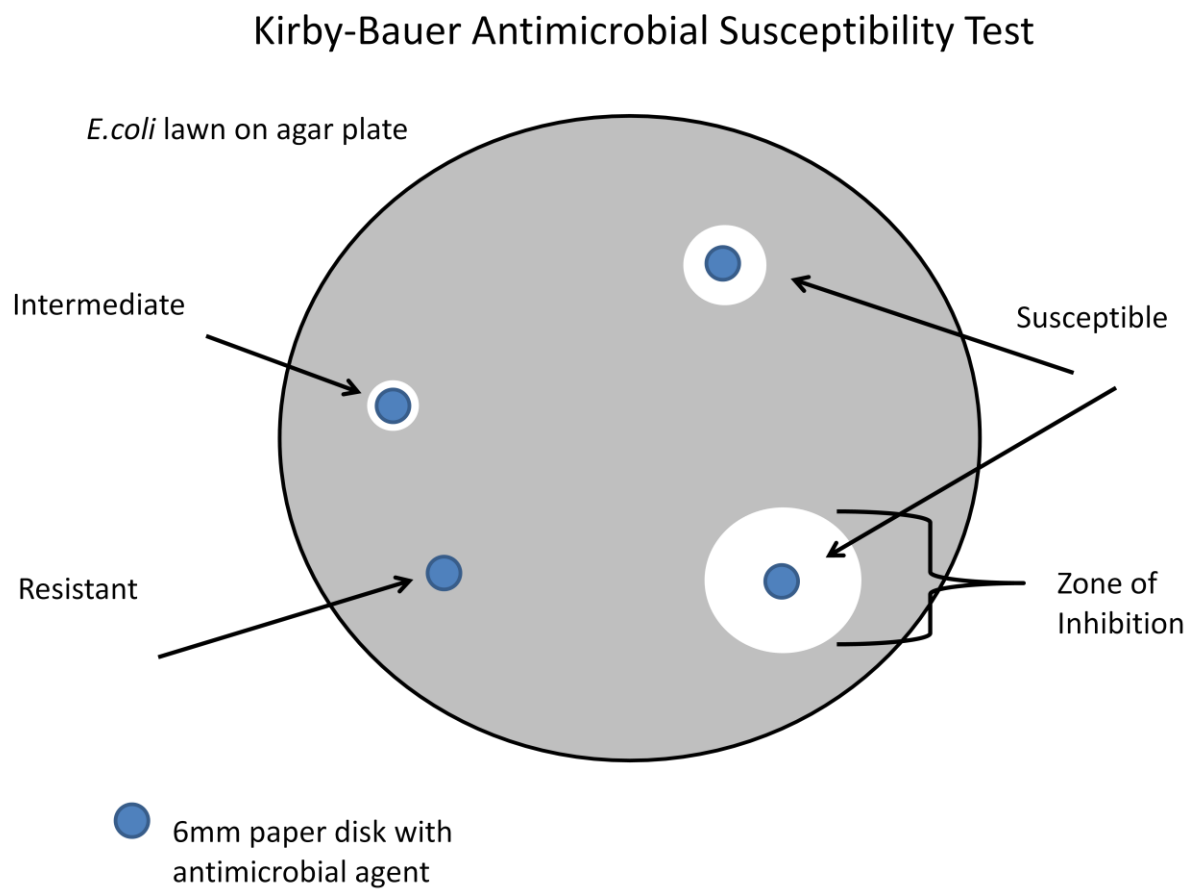
Microbiology Lab: Antibacterial Agents *Teacher notes italicized*

Goal: Compare bactericidal activity of different common household agents using the Kirby-Bauer disk method.

In this lab you will learn the Kriby-Bauer disk method of determining the strength of antibacterial agents. You will be streaking bacteria on an agar plate to create a bacterial lawn of growth. You will soak paper disks in antibacterial agents and determine how effective each agent is at inhibiting growth of the bacterial lawn in the area around each disk. This is a common method used by microbiologists to determine the effectiveness of antibiotic compounds and to see how sensitive different kinds of bacteria are to antibiotic agents. You will be testing different concentrations of bleach, hand sanitizer, water, and antibiotic ointment.



Hypothesis: Write down your hypothesis of which substances will be most effective at inhibiting bacterial growth.

Lab Materials *Teachers will need to have LB agar plates and E.coli overnight culture prepared ahead of time*

Marker

Cotton swabs (*optional sterile*)

Liquid bacteria culture

Luria Broth Nutrient Agar plates

Overnight E.coli culture

6 millimeter paper disks (*optional sterile: you can get some from your three-hole punch!*)

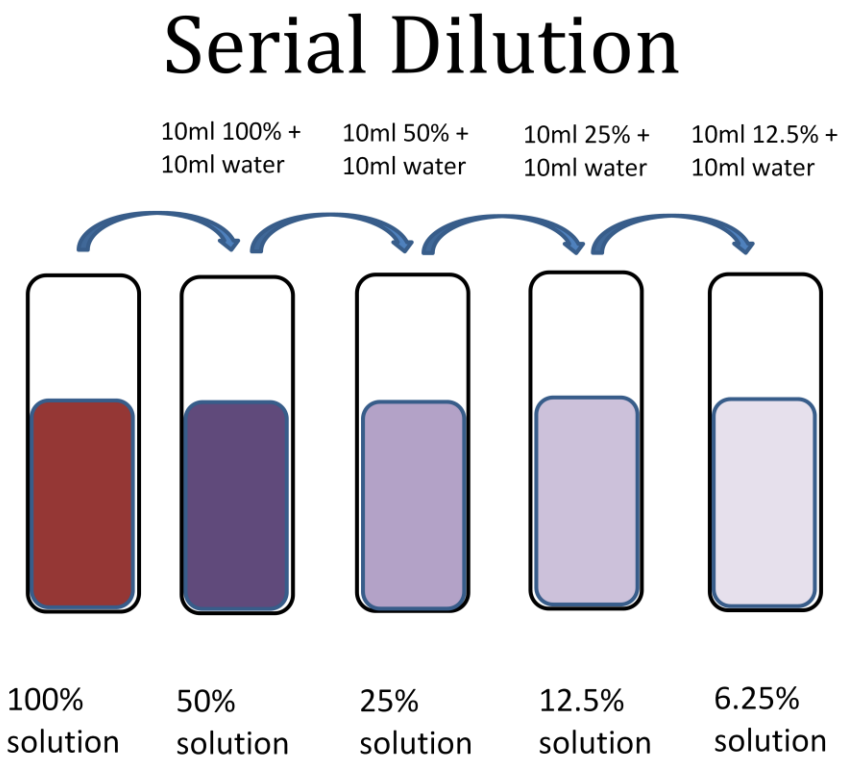
Tweezers

Paper Towel

ruler

Bleach stock solutions (100%, 50%, 25%, 12%, 6% 0%) *To make these stock solutions, you may use beakers a do a serial dilution. For example, label 6 beakers as above, and add 10 ml of water to each of the 50%, 25%, 12%, 6%, and 0% beakers. Add 20 ml of bleach to the 100% beaker, and transfer 10ml to the 50% beaker. Now you have 20ml of 50% solution in the 50% beaker. Pour 10 ml of the 50% solution*

into the 25% solution beaker to make 20 ml of the 25% solution. Continue with the 12% and 6%



Water

Hand sanitizer (65% ethanol)

Triple antibiotic cream mixed with water (bacitracin, neomycin, polymixin B)

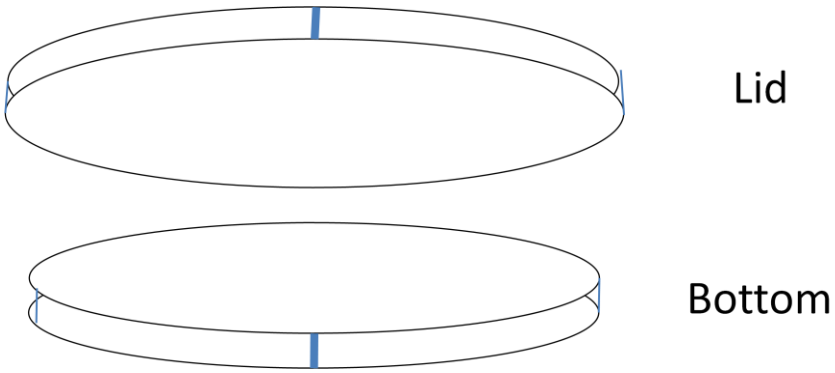
Other products to test could include: hydrogen peroxide, rubbing alcohol (isopropyl versus ethanol?)or food componenets such as onion, garlic, cilantro, jalapeno, or other spices

Day One

Take a cotton swab and dip it into the liquid bacteria culture, getting rid of excess liquid by gently squeezing against the inside of the culture container.

Starting at one edge of the plate, streak the swab tightly from side to side over the plate, trying to cover the entire surface. Repeat two more times, turning the plate 45 degrees each time to ensure the plate is equally covered. Run the swab around the edges of the plate, too. Cover the plate and let it dry for 5 minutes.

With the cover on the plate, draw a line on side of the lid and the bottom of the plate.



This is to help you keep track of what solution is on each disk, which you will write on the cover above each disk as you place them. When the marks are aligned, your labels will match up with the correct disk.

Using tweezers, select a paper disk and wet it in the water. This will be your negative control, the solution that should not inhibit bacterial growth.

Dab the disk and your tweezers on the paper towel, making sure all excess liquid is gone. The paper should be damp, not dripping. If the disk it too wet, it will wash the bacteria away and confuse your results.

Then, place the disk gently in the center of the plate, and label the top of the dish "Water" over the disk.

Repeat with the 6%, 12%, 25%, 50%, and 100% bleach solutions, spacing the disks evenly around the plate, making sure each disk is about an inch apart from the others.

Don't forget to label the concentrations on the top of your plate! You can also dip a paper disk into hand sanitizer or triple antibiotic cream mixed with water, dabbing away the excess and placing the disk on the plate. When your plate is complete, put it in a 37 degree Celsius incubator, which helps the bacteria grow.

Day Two

Collect your plate and observe the bacterial growth near the disks compared to the rest of the plate. You should see clear rings around the paper disks that were soaked in the antibacterial substances. This ring is called a zone of inhibition, because it shows how far away from the disk the agent was able to inhibit the growth of the bacterial lawn.

1) What substances were able to inhibit bacterial growth? What substances did not inhibit growth? What substance had the largest zone of inhibition?

2) Measure the diameter of the zone of inhibition of each of the substances that inhibited bacterial growth.

Bleach Concentration	Zone of Inhibition Diameter
100%	
50%	
25%	
12.50%	
6%	

3) Use the concentration of bleach as the x coordinates and the diameter of the zone of inhibition as the y coordinates to graph the relationship between bleach concentration and bacterial growth inhibition. Describe the relationship.

Depending on the level of the students, they can also estimate the slope of the line relating percent bleach to Zone of Inhibition diameter, or even calculate the molarity of bleach in each solution and graph molarity versus diameter of zone of inhibition.

4) How did the other inhibitory substances compare to the bleach series? Discuss how the results compare to your hypothesis.