

**COMPARING ANTIBACTERIAL POTENCY
DEMONSTRATION OF BACTERIAL GROWTH INHIBITION**

page 48

David B. Fankhauser, PhD

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http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Bacterial_Inhibition/Growth_Inhibition.htm

See protocol on *Agar Overlay Technique* for the preparation of the plates for this experiment.

EQUIPMENT AND SUPPLIES:

Fresh overnight cultures of *E coli* B and *Staphylococcus aureus*
2 pre-warmed nutrient agar plates (or tryptone soy agar)
melted top agar (Nutrient Broth + 0.6% agar)
sterile 13 x 100 mm capped test tubes
45°C hot block
10 uL micropipetters, box of sterile white tips
sterile cotton swabs
assorted putative anti-bacterials
sterile (thick) filter paper plugs, 5 mm in diameter

Prepared discs:

1 Chloramphenicol
2 Erythromycin
3 Kanamycin
4 Vancomycin
5 Neomycin
6 Novobiocin
7 Penicillin
8 Streptomycin
9 Tetracycline
etc?

1. **Compile a table of the seven putative anti-bacterial agents you are testing.** Describe in detail how each is prepared and quantities applied to the plate. Leave an empty column to record the zone of inhibition observed. Seven spots should be equally spaced on a plate. Apply carefully and do not allow them to spread across the plate. Pick four prepared antibiotic test discs (see list above). Then choose three other putative antiseptics such as: copper (penny), silver, ethyl alcohol, isopropyl alcohol, salt, Lysol, mouthwash, soap, iodine solution, Merthiolate, Neosporin, garlic, onion juice, saliva etc. The list of possible agents which you could test is very long. Use your imagination.
2. **Mark the bottom of the plates** (as instructed) with seven well-spaced circles 1/4 inch in diameter (one in center, six arranged hexagonally around it). Label each circle with the name of agent to be tested. Write your initials and the date in small letters at the upper edge of the plate. **Draw two copies of the plate plan** on facing pages in your book, (one for *Staph* and one for *E. coli*). Make them life-sized and identically configured to original.
3. **Prepare the plate with an agar overlay:** Add 0.1 mL of a fresh overnight culture of *E. coli* B or *S. aureus* to 2 mL of 45°C melted top agar. Mix and pour over the surface of a pre-warmed nutrient agar plate, immediately rock slightly to spread evenly. Let sit undisturbed for several minutes to gel. (See protocol: *Agar Overlay Technique*.)
4. Apply appropriate putative antiseptics: prepared antibiotic disc, blank filter paper discs for liquid agents, or apply a pin-head quantity of solids directly to the surface.
5. **Apply the putative anti-bacterials:** Add 10 µL of each liquid to the filter paper plugs with a 10 uL pipet (diluted if the directions call for it). Deliver slowly so as not to overshoot the pad. If they are viscous, either apply a thin layer to the disc or dilute them 10x so they can be pipetted. If solid, for known poisonous agents, place a crystal about the size of a head of a pin at the spot, 10x that much for probable non-toxics. Carefully note the preparation in your notebook or the quantities applied. Allow the liquids to absorb.
6. **Invert the plate, incubate at 37°C for 24 to 48 hours.**
7. **Measure the zones of inhibitions around each agent** in millimeters (from edge of disc to edge of inhibition zone). Draw the zones of inhibition onto your previously drawn notebook illustrations of the plates (from step 2). Use circles to show the zone of inhibition. Note on the illustration the size in mm of the zone of inhibition, and record of this number in your table.
8. Enter into **class data table**.