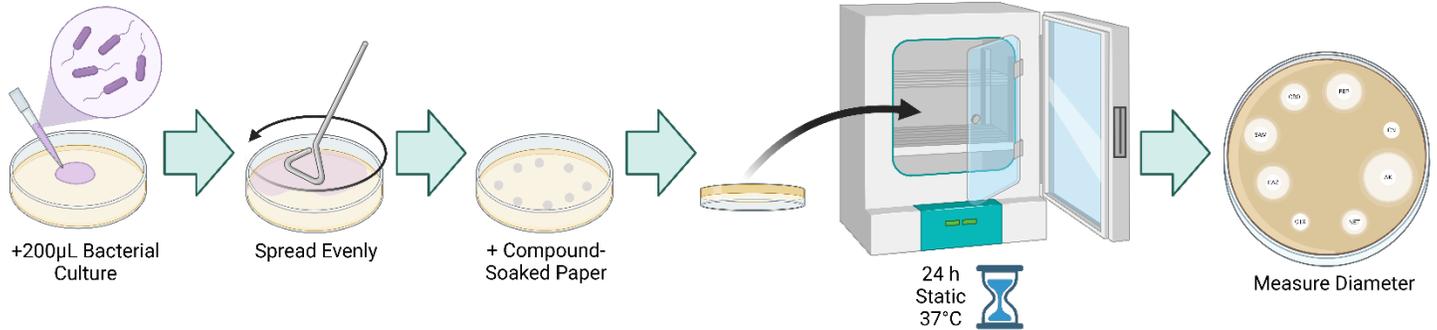


Disk Diffusion (Kirby-Bauer) Assay

Graphical Schematic of Procedure:



Materials:

- Sterile LB
- 14 ml bacterial culture tubes with lids
- Pipette tips
- LB agar plates
- Compounds for testing at desired concentration
- Bacterial strain *E.coli*-AR3110 (or other) (-80°C stock)
- Eppendorf Bio Photometer
- Plastic cuvettes 1 ml
- Sterile circle filter paper, prepared ahead by punching holes into Whatman paper and the sterilizing the hole cut-outs in the autoclave.

Work under sterile conditions

Methods:

Day 1 → Streak out (single-colony method) bacterial strain (*E. coli*) on LB Agar plate as shown below and allow to incubate overnight (ON) at 37°C.

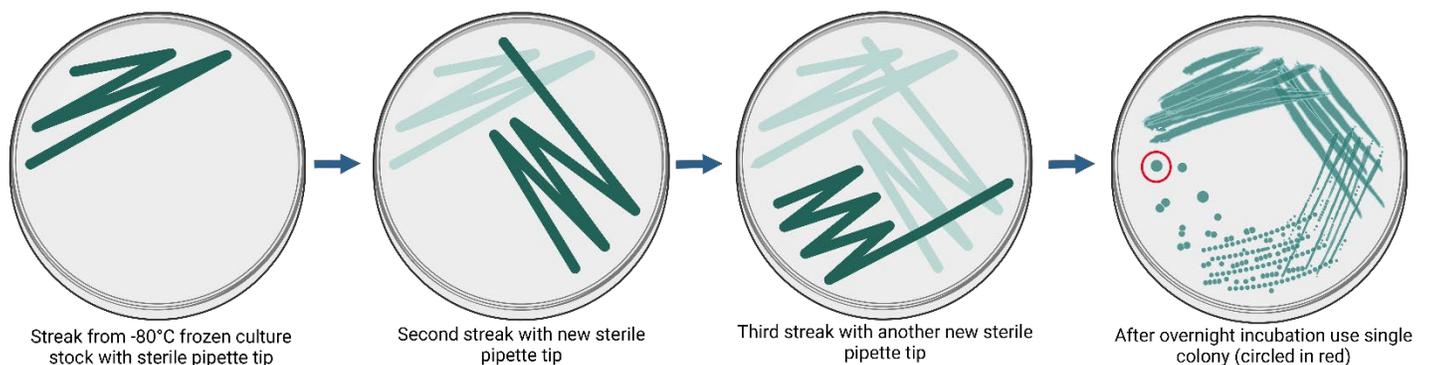


Figure 1: Single colony streak out method
Tatyana L. Povolotsky
Disc Diffusion (Kirby-Bauer) Assay

Day 2 → Prepare bacterial culture by inoculating 5mL LB with single colony from plate. Prepare negative control of only LB (3mL). Grow shaking 180-250 rpm at 37°C and allow to grow 16 hrs.

Day 3 → Confirm that the negative control LB is still clear and sterile. Measure OD_{600} and dilute to an OD_{600} of 1.2 using the $C_1V_1=C_2V_2$ formula. In the clean bench, working under sterile conditions, dry LB plates. Add 200 μ L of the 1.2 OD_{600} culture and spread equally over plate using the plate spreader and mini turn-table. Apply either solid compound to be tested directly to the culture-coated LB plate or liquid compound to a sterile circle filter (allow compound to be fully soaked up by the filter paper) and the place onto the culture-coated LB plate as pictured below. Close lid and mark on lid where compounds were placed and the orientation of the lid top to the plate bottom. Place in the 37°C incubator for 24 hours.

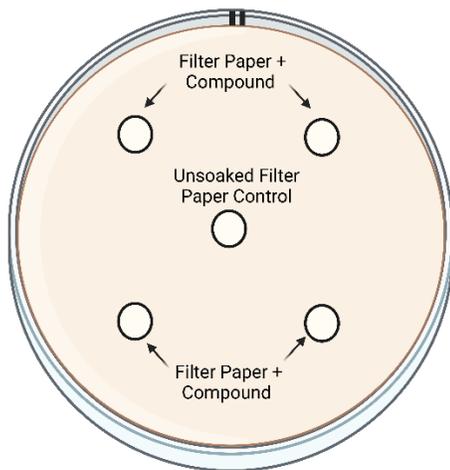


Figure 2: Recommended layout for compound testing

Day 4 → Take pictures and measure the diameter of the growth inhibition zone and convert diameters into bar graphs.

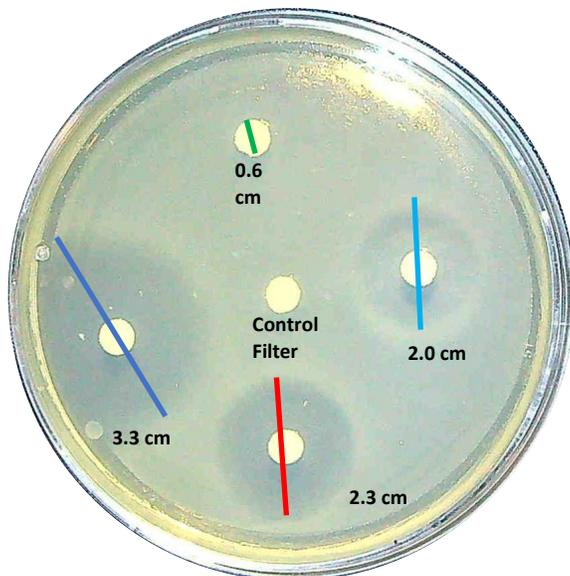


Figure 3: Example of results after 24 hours.