AGAR OVERLAY TECHNIQUE

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

This technique allows you to produce a homogeneous lawn of bacteria within a thin layer of agar across the surface of a plate. Bacteria are added to a soft top agar (0.60% agar, as opposed to the usual 1.5% for agar plates) which has been melted at 100°C and cooled to 45°C. This is warm enough so the agar remains liquid, but cool enough so that the bacteria are not killed (for a short time of exposure). Bacteria are added to the melted 45°C agar, the bacterial suspension is mixed and poured evenly across the top of a warmed agar plate and allowed to solidify.

The bacteria distributed through the top agar layer will grow to produce a homogeneously **turbid lawn**. If the freshly seeded lawn is exposed to various antibacterial agents and then incubated at 37°C, any inhibition of bacterial growth will cause a reduction in the turbidity of the lawn near the agent: the greater the antibacterial action, the wider the zone of inhibition. Thus, the antibacterial strength of the agent may be judged by the width of the zone of inhibition around it.

EQUIPMENT:

- sterile capped 13x100 mm tubes
- hand repipettor, two syringes available:
  - sterile 5 and 50 mL syringes
- hot block, 45°C, warmed up
- vortex
- Bunsen Burner

SUPPLIES:

- melted top agar, (0.6%) about 60°C
- fresh culture of indicator bacteria (such as *E. coli* B and/or *S. aureus*)
- Each student pre-labels two plates:
  - pre-warmed nutrient agar plates (37°C) (or tryptone soy agar plates, etc)

PROTOCOL:

THE PREVIOUS NIGHT:

1. For 50 plates, inoculate about 6 mL of nutrient broth or tryptone soy broth with the desired indicator bacteria (*E. coli* B, and *S. aureus* in this case). Grow as a stationary cultures ON at 37°C in hot block.

THE DAY OF USE:

2. Pipet about 2 mL of hot **melted top agar** into sterile capped 13x100 mm tubes in a 45°C hot block. A sterile repipettor with a 50 mL syringe simplifies this step. Allow to cool to 45°C (several minutes).
4. Pipet about **0.1 mL of an ON culture** of indicator bacteria into the melted agar (down the inside of the tube is OK). Again, a sterile repipettor (5 mL syringe) simplifies this step.

Overlay technique:

5. **Label plate:** up to seven spots on the plate bottom, draw the plate map in your notebook.
6. a. **Vortex to mix** the bacteria into the melted top agar
   b. immediately **pour out onto *pre-warmed* labeled agar plate**
   c. immediately **tilt back and forth, shake gently to evenly distribute**. Avoid bubbles, and stop agitating *before* agar begins to gel. Let set on a cool desk undisturbed to gel fully, about five minutes.
7. When fully gelled, perform desired operations such as: application of antibacterial agents, antibiotics, exposure to UV, etc. Apply 5 uL of liquids to 5 mm sterile filter discs placed on top of the top agar. Do not let run.
8. **Invert, incubate ON at 37°C.**

THE NEXT DAY:

9. Next AM, **read the plates**. Where growth is thickest, there was the least antibacterial action. Where the thinnest, the greatest. Illustrate the plate, measure the zones of inhibition (width of the zone from edge of agent to edge of zone), record data.