

Laboratory 1: Bacterial Abundance

Method 1: SERIAL DILUTION- AGAR PLATING TO QUANTIFY VIABLE CELLS

You will be using water from your Winogradsky column for two assays: 1 ml for a dilution series and 9.0 ml to preserve for a direct count of bacterial numbers to be done in Thursday's lab. Use sterile technique throughout.

- Label a set of four 14 mL snap cap tubes with your Winogradsky column number and "10⁻¹" to "10⁻⁴". Fill the tubes ("10⁻¹" to "10⁻⁴") with 9 mL phosphate buffered saline (PBS) using serological pipette and sterile technique.
- Use a 15 mL syringe to withdraw a little more than 10 mL of water from the top port of your Winogradsky column and transfer 10 mL to an empty 14 mL snap cap tube; label it "10⁰".
- Serially dilute (10:1) the water samples. Take 1 mL from the "10⁰" tube and add it to the "10⁻¹" tube, then swirl test tube. Repeat the serial dilution with a new sterile pipette tip transferring 1 mL from the "10⁻¹" tube to the "10⁻²" tube. Repeat the procedure to get to the 10⁻⁴ dilution
- Plate 0.1 mL from each tube (5 in all) on separate Reasoner-Geldreich medium plates using a 100 µL pipettor (use a clean sterile tip for each sample) and spread with a flamed glass rod. Cool glass rod on agar plat before spreading drop.
- Incubate the five plates at room temperature. Check and count them on Thursday and next Tuesday.
- Add 1.0 mL of 37% formaldehyde (wear gloves when handling formaldehyde and work under the hood) to the remaining 9.0 ml in the 10⁰ tube. Save this sample in the refrigerator for a direct count during the next lab. Make sure it is properly labeled. Note, saturated formaldehyde (37%) is called formalin. So our 3.7% formaldehyde fix is sometimes called 10% formalin.
- Top off the Winogradsky column with water from the extra bottles in the refrigerator if necessary.

Method 2: COLIFORM COUNTS

- Place a 47 mm, 0.45 µm gridded filter in the sterile Nalgene filter unit (use flame sterilized forceps).
- Filter 100 mL of the water sample that was assigned to you (or you choose) through the Nalgene filter. You can use the volume marks on the Nalgene filter assembly.
- Soak absorbent pad with one ampoule of mMF endo broth in a sterile petri dish. (Media from Fisher, [MHA00FCR2](#))
- Place filter on top of the medium pad using sterile forceps, label petri dish with your name and water sample and volume, and incubate at 44.5 °C.
- Count blue (fecal) and total (white/red + blue) colonies after ~24 hr