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 Winograsky column and transfer 10 mL to an empty 14 mL snap cap tube, and label it "10^{0}".

- Serially dilute (10:1) the water samples. Take 1 mL from the "10^{0}" 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 an ethanol-flamed glass rod. Cool glass rod on agar plat before spreading.

- Incubate the five plates at room temperature. Check and count them on Thursday and next Tuesday.

- Add 1.0 mL of 25% glutaraldehyde (wear gloves when handling formaldehyde and work under the hood) to the remaining 9.0 ml in the 10^{0} tube. Save this sample in the refrigerator for a direct count during the next lab. Make sure it is properly labeled.

- 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 MF endo broth in a sterile petri dish.

- 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