Module 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 today 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 (or test) 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 (or test) tube; 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 a flamed glass rod. Cool glass rod on agar plat before spreading drop.

- Incubate the five plates at room temperature. Check them on Thursday and count the number of colonies on each plate (where possible) next Tuesday.

- Add 1.0 mL of 37% formaldehydeto the remaining 9.0 ml in the 10^{0} tube (wear gloves and safety glasses when handling formaldehyde and work under the hood). 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 making sure to use either seawater of freshwater as appropriate.

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