

## Laboratory 1: Bacterial Abundance

### **Method 1: SERIAL DILUTION- AGAR PLATING TO QUANTITATE VIABLE CELLS**

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You will be using water from your Winogradsky column for two assays: 1 ml for a dilution series and 9.5 ml to preserve for a direct count of bacterial numbers to be done in Thursday's lab. Use sterile technique throughout.

1. Label a set of five 14 ml snap cap tubes with your Winogradsky column number and  $10^0$ ,  $10^{-1}$  to  $10^{-4}$ . Fill the  $10^{-1}$  to  $10^{-4}$  tubes with 9 ml phosphate buffered saline (PBS) using a serological pipette.
2. Use a 15 ml syringe to withdraw a little more than 10.5 ml of water from the top port of your Winogradsky column and transfer it to the  $10^0$  tube.
3. Serially dilute (10:1) the water samples by taking 1 ml from the  $10^0$  tube and adding it to the  $10^{-1}$  tube using a 1 ml pipettor, then Vortex and continue serial dilutions.
4. Plate 0.1 ml from each tube (5 in all) on separate Reasoner-Geldreich medium plates using a pipettor (use a clean tip for each dilution) and spread with an ethanol-flamed and cooled glass rod.
5. Incubate the five plates at room temperature. Check and count them on Thursday and next Tuesday.
6. Add 0.5 ml 50% glutaraldehyde (wear gloves when handling glutaraldehyde and work under the hood) to the remaining 9.5 ml in the  $10^0$  tube. Save this sample in the refrigerator for a direct count during the next lab.
7. Top off the Winogradsky column with water from the extra bottles in the refrigerator if necessary.

### **Method 2: COLIFORM COUNTS**

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1. Place 47 mm, 0.45  $\mu\text{m}$  gridded filter in the sterile Nalgene filter unit (use sterile forceps, throw away the blue paper)
2. Filter 100 mL of the water sample that was assigned to you through the Nalgene filter unit (don't let pressure on hand pump exceed 12 cm Hg).
3. Soak absorbent pad with one ampoule of MF endo broth in a sterile petri dish (wipe the outside of the ampule with ethanol prior to breaking it open).
4. Place filter on top of pad using sterile forceps, label petri dish, incubate at 44.5 °C.
5. Count blue (fecal) and total (white + blue) colonies after ~20 hr, and again on Thursday at the beginning of class.