

## **Bacterial Examination of Water: Multiple Tube Test and Standard Plate Count**

Many intestinal pathogens can be waterborne and transmitted by drinking contaminated water. It's important to be able to test drinking water sources for contamination by pathogens, but it's not very practical or expedient to look for the many types of pathogens that could be found (and in small numbers and often hard to grow in culture). Methods have been developed to detect organisms which are normally found in the human gut but are not normally found in soil or water. The presence of these organisms in water indicates that there may be fecal contamination of the water and, therefore, that intestinal pathogens might also be present. These organisms are called "indicator" organisms and the group of choice is the coliform group – Gram negative, facultative anaerobic, non-endospore forming rods that ferment lactose to produce acid and gas in 48 hours at 35°C. Typical coliforms that we have observed in lab are *Enterobacter aerogenes* and *Escherichia coli*. The following tests are conducted to detect the presence of coliforms, particularly *E. coli*, in water samples.

### **Multiple Tube Test**

#### Materials:

3 Durham tubes of double strength lactose broth (DSLБ)  
6 Durham tubes of single strength lactose broth (SSLB)  
1.0mL and 10mL pipettes  
1 EMB plate

#### Presumptive Test:

1. Set up three DSLB and six SSLB tubes as shown by your instructor. Label each tube with the amount of water that is to be dispensed into it: 10mL, 1.0mL, and 0.1mL.  
  
(10mL in each DSLBs, 1.0mL in 3 SSLBs, 0.1mL in remaining 3 SSLBs as directed below)
2. Shake water sample 25 times if possible.
3. With a 10mL pipette, transfer 10mL of water to each of the DSLB tubes.
4. With a 1.0mL pipette, transfer 1.0mL of water to each of the middle set of SSLB tubes, and 0.1mL to each of the last three SSLB tubes.
5. Incubate the tubes at 35°C for 24 hours.
6. Examine the tubes and record the number of tubes in each set that have gas present.
7. Determine the MPN by referring to MPN Determination Table.

### Confirmed test:

If a water sample is positive for gas then it is presumed that the sample contains coliforms and the confirmed test is done by inoculating EMB from a gas positive tube. In this lab you will conduct the confirmed test prior to getting results from the presumptive test.

1. Inoculate an EMB plate with your original sample of water.
2. Incubate at 35°C for 24 hours.
3. Observe plate for coliforms; purple colonies with dark centers. *E. coli* may exhibit a green sheen. You can compare this to your Membrane Filtration test.

### Completed test:

Coliform colonies from EMB would be inoculated again into Lactose Broth with a Durham tube and checked for gas, and inoculated on NA or TSA and checked via Gram stain for GNRs. If these tests are positive it shows that coliforms (not another gas producer) are present and indicates that the water sample is contaminated. Due to time constraints you will not be performing the Completed Test.

## **Standard Plate Count**

A standard plate count can be done to determine total numbers of bacteria in a sample, but is not specific for coliforms. Depending on the water source a dilution series might be appropriate. However, since we don't suspect your water samples to have high numbers of bacteria you will plate directly from your water sample.

### Materials:

- 1 TGEA pour
- 1 empty petri dish
- 1 -1.0mL pipette

Transfer 1.0mL of your sample to the empty petri dish and add the TGEA pour. Incubate at 35°C for 24 hours. Count total colonies.