EXPERIMENT ON MOST PROBABLE NUMBER

1. AIM OF EXPERIMENT

To detect coliform bacteria in water by the most probable number (MPN) method.

2. GENERAL INFORMATION

Microorganisms pathogenic to humans that are transmitted by water include bacteria (including blue-green algal toxins), viruses, and protozoa. Most of the microorganisms transmitted by water usually grow in the intestinal tract of man and leave the body in the feces. Fecal pollution of water used for swimming and drinking can then occur resulting in transmission of infectious microorganisms. The significance of this was recognized at the turn of the century when filtration and disinfection of drinking water was begun in the USA. This resulted in the almost complete elimination of waterborne cholera and typhoid in the country. Routine examination of water for the presence of intestinal pathogens would be a tedious and difficult, if not impossible, task. It is much easier to demonstrate the presence of some of the nonpathogenic intestinal bacteria such as Escherichia coli. The organisms are always found in the intestines and normally are not present in soil or water; hence, when they are detected in water, it can be assumed that the water has been contaminated with fecal material.

Coliform bacteria (of which Escherichia coli is a member) are often associated with enteric pathogenic organisms and have been shown to be useful indicators of the presence of fecal contamination. Coliform bacteria occur normally in the intestines of humans and other warm-blooded animals and are discharged in great numbers in human and animal waste. In polluted water, coliform bacteria are found in densities roughly proportional to the degree of fecal pollution. When members of the coliform group are present, other kinds of microorganisms capable of causing disease also may be present.

The coliform group includes all aerobic and facultatively anaerobic, gram negative, non-spore-forming, rod-shaped bacteria which ferment lactose with gas production in prescribed culture media within 48 hours at 35°C. Coliform bacteria include Escherichia coli,Citrobacter, Enterobacter, and Klebsiella species. MPN test and the membrane filter test have been the methods most commonly used for the detection of coliforms in water. The membrane filter cannot be used easily with turbid waters.

To envision the theoretical basis for the Most Probable Number (MPN) method, think of a ten-fold dilution series being made of a water sample with one mL of each dilution being inoculated into a separate tube of an all-purpose broth medium.

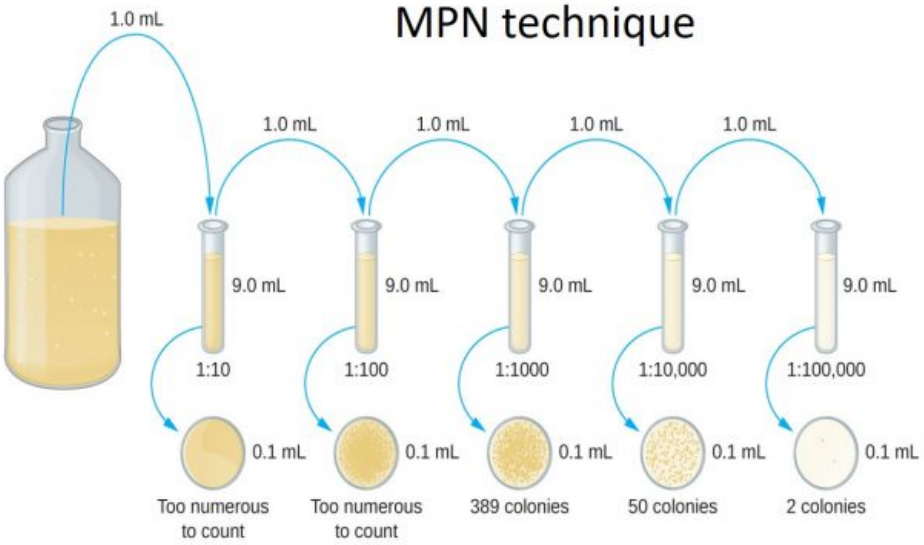

Figure 1. MPN experiment set-up

3. EXPERIMENT PROCEDURE

3.1. MATERIALS REQUIRED

3.1.1. Apparatus Required

- 3 test tubes containing Durham tubes for Double strenght lactose broth (DSLB)
- 6 test tubes containing Durham tubes for Single strenght lactose broth (SSLB)
- 10-ml pipette
- 1-ml pipette

3.1.2. Chemical Required

- Lactose broth
- Peptone water
- Double strenght lactose broth (DSLB)
- Single strenght lactose broth (SSLB)

3.2. PROCEDURE

- Set up three DSLB and six SSLB tubes. Label each tube according to the amount of water that is to be dispensed to it: 10 ml, 1.0 ml, and 0.1 ml, respectively.
- Mix the bottle of water to be tested by shaking 25 times.
- With a 10 ml pipette, transfer 10 ml of water to the DSLB tubes.
- With a 1.0 ml pipette, transfer 1 ml of water to each of the middle set of SSLB tubes and 0.1 ml to each of the last three SSLB tubes.
- Incubate the tubes at 35°C for 48 h.
- Observe gase output inside durham tubes, color and turbidity.

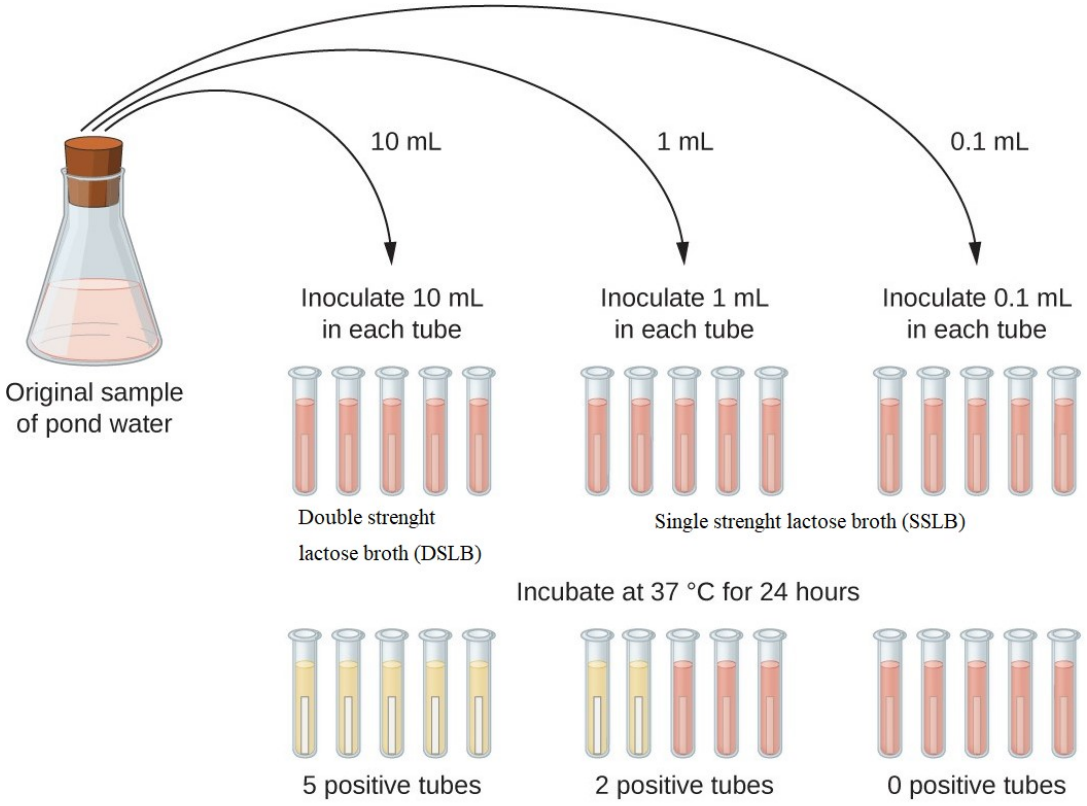

Figure 2. MPN procedure

4. CALCULATION

After incubation, the broth tubes are observed for the presence or absence of growth according color, turbidity and gase output inside durham tubes. Theoretically, if at least one organism had been present in any of the inocula, visible growth should be seen for that

particular tube. If the broth inoculated from the 10^{-3} dilution shows growth, but the broth from the 10^{-4} does not, it is then possible to say that there were greater than 1×10^3 organisms per mL of the original sample but less than 1×10^4.