**Introductory Medical Microbiology**

**Laboratory Notes**

**BIOL 2161L**

# SERIAL DILUTIONS & FECAL COLIFORM TESTING

Fecal coliform bacteria are non‑disease causing organisms, which are found in the intestinal tract of all warm‑blooded animals. Each discharge of body wastes contains large amounts of these organisms. The presence of fecal coliform bacteria in a stream or lake indicates the presence of human or animal wastes. The number of fecal coliform bacteria present is a good indicator of the amount of pollution present in the water.

Most waterborne disease-causing organisms originate in human or animal bodies and are discharged as part of body wastes. Due to the relatively small numbers of disease‑causing organisms, it is very difficult to isolate and identify specific disease‑causing bacteria. Since fecal coliform bacteria originate in the same location, they are used as an indicator of possible disease hazards in a body of water. The presence of very few fecal coliform bacteria would indicate that a water source probably contains no disease‑producing organisms, while the presence of large numbers of fecal coliform bacteria would indicate a very high probability that the water source could contain disease‑producing organisms. For this reason, regulatory agencies with responsibility for protection of public health have established water quality standards, which include maximum levels of fecal coliform bacteria.

The accepted standard for drinking water is that there should be no coliforms present after the water is filtered or treated. Natural waters will nearly always contain some form of bacteria. That is why you should never drink untreated water from a river or lake. Currently, the most common measurement for surface waters is fecal coliform.

When interpreting data from fecal coliform tests, it is important to remember that there can be a high degree of randomness of distribution within a sample. A large number of data points are necessary to obtain statistically significant data. Fecal coliform is measured in colony forming units per 100 mL, CFU/100 mL, of water tested.

|  |  |
| --- | --- |
| **US EPA Permissible level (CFU/100mL)** | |
| Drinking Water | 0 |
| Swimming | < 1,000 |
| Boating or Fishing | < 4,000 |

**Materials:**

* + Sample of lake water
  + Eight 9-ml water blanks per team
  + Eight agar plates per team
  + Ten sterile disposable 1ml pipettes per team
  + Spreading rods
  + Colony counter available for next (evaluation) lab

**Procedure:**

**Serial Dilution of Water Samples**

Each group will get a sample of lake water collected from a nearby source and use the techniques learned to perform a set of serial dilutions and spread plates for the sample.

1.       Using you sample, transfer 1ml into the first sterile water blank. Label this tube 1:10.

2.       Mix the tube contents using the vortex mixer on the end of each table.

3.       Using another transfer pipette, transfer 1ml of the 1:10 tube to the next sterile blank tube. Label this tube 1:100.

4.       Continue making transfers until 1:100 and 1:10000 dilutions have been made.

5.       After all dilution have been completed, Transfer ½ ml from the original sample and place it on a plate labeled 1:1

6. Then, transfer ½ ml from the 1:10 dilution and place it on a plate labeled 1:20.

7.       Spread the liquid across the surface of the plate with a clean spreading rod.

8.       Continue making plates in this fashion from each of the dilution tubes until you have created four plates: 1:1, 1:20, 1:200, 1:2000.

9.       Place the inverted plates in a rack to be incubated.

**Spread Plate Counts**

Students should count the organisms growing on their plates to determine the number of colonies. Then a calculation of the viable cells in the original sample can be performed. A colony is the result of rapid division of one or a few cells giving rise to a visible mass. This allows you to estimate the number of cells or colony forming units (CFUs) in the original sample by counting the number of colonies and multiplying by the dilution factor of the plate.

**Example:  If the 1:200 plate shows 148 living colonies:  
               (148 x 200) x 100=29,600,000 CFUs in 100ml of the original sample**

*Note\* Any plate containing less than 200 colonies but more than 30 should be counted.  Plates containing under 30 colonies are considered statistically insignificant and subject to sampling error.  Plates containing over 300 colonies are overcrowded and colonies are likely to merge in growth, preventing accuracy in counting.*

**Results:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Number of colonies counted in your sample | Number of colonies in Group 2’s sample | Number of colonies in Group 3’s sample | Number of colonies in Group 4’s sample | Average number of colonies counted | CFUs |
|  |  |  |  |  |  |

Is the water safe for drinking?

Is the water safe for swimming?

Is the water safe for boating/fishing?