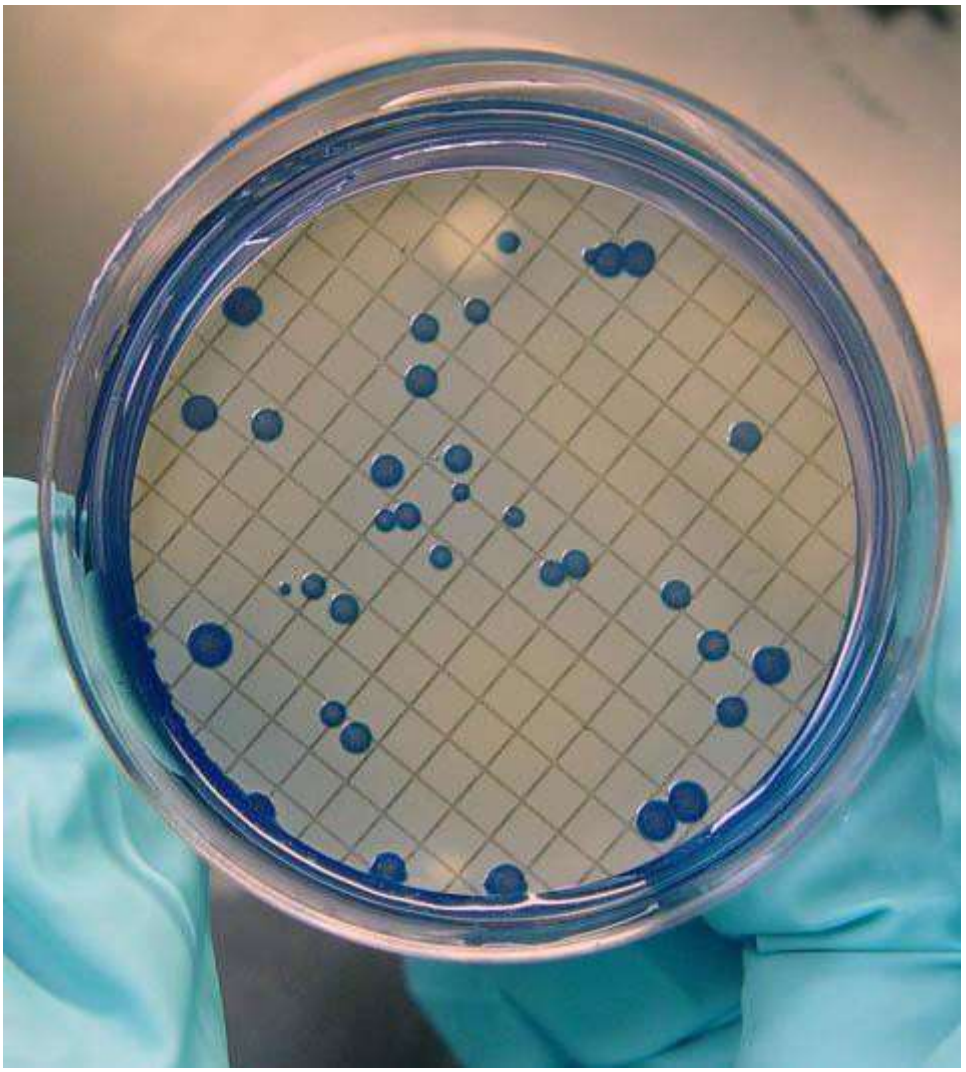
Experiment 15
Determination of Total and Fecal Coliform in water



Introduction:

A variety of different microorganisms are found in untreated water. Most of these organisms do not pose a health hazard to humans. Certain organisms, referred to as pathogens, cause disease to humans which include species of bacteria, viruses and protozoa. These organisms are not native to aquatic systems and usually require an animal host for growth and reproduction. Pathogens are likely to gain entrance sporadically, and they do not survive for very long period of time; consequently they could be missed in a sample submitted to the laboratory. Although it is possible to detect the presence of various pathogens in water, the isolation and identification of many of these is often extremely complicated, time-consuming and expensive proposition. Hence in most cases (except when presence of any particular microorganism is suspected) the microbiological quality of water is checked using some indicator organisms.

An *indicator organism* is one whose presence presumes that contamination has occurred and suggests the nature and extent of the contaminants. An indicator organism should be a microorganism whose presence is evidence of fecal contamination of warm blooded animals. Indicators may be accompanied by pathogens, but typically do not cause disease themselves. The ideal indicator organism should have the following characteristics:

- Always be present when pathogens are present
- Always be absent where pathogens arc absent
- Numbers should correlate the degree of pollution
- Be present in greater number than pathogens
- There should be no after-growth or re-growth in water
- There should be greater or equal survival time than pathogens
- Be easily and quickly detected by simple laboratory tests
- Should have constant biochemical and identifying characteristics
- Harmless to humans

No organisms or group of organisms meet all of these criteria; but the *coliform* bacteria fulfill most of them, and this group is most common indicator used in microbial examination of water. Total coliforms are grouped into two categories (*1) Fecal coliform* (thermo-tolerant coliform^{-}) and (*2) Non- Fecal coliform*

Total coliforms are defined as gram negative bacteria which ferment lactose at 35° or 37° C with the production of acid, gas and aldehyde within 24 or 48 hours. *Fecal coliform* are a subgroup of total coliforms, which live in the warm blooded animals and have the same properties as the *total coliform* but tolerate and grow at higher selective temperature range of 44° to 44.5°C.In addition, they form indole from tryptophan. And these combined properties, when positive, are regarded as presumptive *Escherichia coli* (presumptive E. coli).Some coliform species are frequently associated with plant debris or may be common inhabitants in soil or surface waters which arc called non-fecal coliforms.

Total coliform (TC) = Fecal coliform (FC) + Non-fecal coliform

Thus, the total coliform group should not be regarded as an indicator of organisms exclusively of fecal origin. The use of total coliforms as an indicator may therefore be of little value in assessing the fecal contamination of surface water, unprotected shallow wells etc. where contamination by coliforms of non-fecal origin can occur. The measurement of total coliforms is of particular relevance for treated and / or chlorinated water supplies; in this case the absence of total coliforms would normally indicate that the water has been sufficiently

treated / disinfected to destroy various pathogens. Measurement of focal coliforms is a better indicator of general contamination by material of fecal origin. The predominant species of fecal coliform group is *Escherichia coil*(E. coil), which is exclusively of fecal origin, but strains of *Klebsella pneumonia* and *Enterobacter* species may also be present in contaminated water.

Using coliform as indicators of the presence and absence of pathogens sometimes may cause the following drawbacks:

- False positive result can be obtained from the bacterial genus aeromonas, which can biochemically mimic the coliform group
- False negative result can be obtained when conforms are present along with high population of other bacteria. The latter bacteria can act to suppress coliform activity.
- A number of pathogens have been shown to survive longer in natural waters and / or through various treatment processes than coliform.

But the use of coliforms was established first and there does not appear to be any distinct advantages to warrant shifting to other indicator organisms. Since bacteria are used as indicator organisms, the microbiological examination of water is commonly called bacteriological examination.

Apparatus:

1. Petri Dish
2. Incubator
3. Measuring Cylinder, beaker, dropper etc.

Reagents:

1. Appropriate culture medium (broth)
2. Distilled water

Methods of Bacteriological Examination of Water:

Basically there two methods of bacteriological analysis of water: (a) Multiple Tube or Most Probable Number (MPN) method, and (b) Membrane Filter (MF) method.

(a) Multiple Tube/ Most Probable Number (MPN) method:

MPN is a procedure to estimate the population density of viable microorganisms in a test sample. It's based upon the application of the theory of probability to the numbers of observed positive growth responses to a standard dilution series of sample inoculums placed into a set number of culture media tubes. Positive growth response after incubation may be indicated by such observations as gas production in fermentation tubes or visible turbidity in broth tubes, depending upon the type of media employed.

(b) Membrane Filter Method:

In contrast to the multiple-tube (MT) method, the membrane filter (IVIF) method gives a direct count of total coliforms and fecal coliforms present in a given sample of water. The method is based on the filtration of a known volume of water through a membrane filter consisting of a cellulose compound with a uniform pore diameter of 0.45 μm; the bacteria are retained on the surface of the membrane filter. When the membrane containing the bacteria is incubated in a sterile container at an appropriate temperature with a selective differential culture medium, characteristic colonies of coliforms and fecal coliforms develop, which can be counted directly. This technique is popular with environmental engineers. This method is not suitable for turbid waters, but otherwise it has several advantages. Its particular advantages and limitations are as follows:

Advantages:

- Results are obtained more quickly as the number of coliforms can be assessed in less than 24 hours, whereas the multiple tube technique requires 48 hours both for a negative or a presumptive positive test;
- Saving in work, certain supplies and glassware;
- Method gives direct results;
- Easy to use in laboratories, or even in the field if portable equipment is used.

Disadvantages:

- High turbidity caused by clay, algae, etc. prevents the filtration of a sufficient volume of water for analysis and it may also produce a deposit on the membrane which could interfere with bacterial growth;
- Presence of a relatively high non-coliform count may interfere with the determination of coliforms:
- Waters containing particular toxic substances which may be absorbed by the membranes, can affect the growth of the coliforms.

Test Procedure (For MF method):

This section describes the general procedures, it should be noted that different types of filtration units and equipment are available in the market for performing the tests.

Determination of Total Coliforms (TC):

1. Connect the Erlenmeyer (side-arm) flask to the vacuum source (turned off) and place the porous support in position. if an electric pump is used, it is advisable to put a second flask between the Erlenmeyer and the vacuum source; this second flask acts as a water trap and thus protects the electric pump.
2. Open a Petri-dish and place a pad in it.
3. 'With a sterile pipette add 2 mL of selective broth (culture) medium to saturate the pad.
4. Assemble the filtration unit by placing sterile membrane filter on the porous support, using forceps sterilized earlier by flaming.
5. Place the upper container in position and secure it with the special clamps. The type of clamping to be used will depend on the type of equipment.
6. Pour tide volume of sample chosen as optimal, in accordance with the type of water, into the upper container. If the test sample is less than 10 mL, at least 20 ml of sterile dilution water should be added to the top container before filtration applying the vacuum.
7. After the sample has passed through the filter, disconnect the vacuum and rinse the container with 20-30 mL of sterile dilution water. Repeat the rinsing after all the water from the first rinse has passed through the filter.
8. Take the filtration unit apart and using the forceps, place the membrane filter in the Petri-dish on the pad with the grid side up. Make sure that no air bubbles are trapped between the pad and the filter.
9. Invert the Petri-dish for incubation.
10. Incubate at 35°C or 37°C for 18-24 hours with 100% humidity (to ensure this, place a piece of wet cotton wool in the incubator). If ointment containers or plastic dishes with tight-fitting lids are used, humidification is not necessary.

Bacterial Colony observation:

Colonies of coliform bacteria are a medium red or dark red color, with a greenish gold or metallic surface sheen. This sheen may cover the entire colony or appear only in the centre of

the colony. Colonies of other types should not be counted. The colonies can be counted with the aid of a lens. The number of total coliforms per 100 mL is then given by:

Determination of Fecal Coliforms (FC):

The procedure for fecal coliforms is similar to that used for determining total coliforms. Filter the sample as described, and place the membrane filler on the pad saturated with appropriate culture medium.

1. Place the dishes in an incubator at 44±0.5 °C for 24 hours at 100% humidity. Alternatively, tight-fitting or sealed Petri-dishes may be placed in water-proof plastic bags for incubation.
2. Submerge the bags in a water-bath maintained at 44±0.5°C for 24 hours. The plastic bags must be below the surface of the water throughout the incubation period. They can be held down by means of a suitable weight, e.g., a metal rack.

Bacterial Colony observation:

Colonies of fecal coliform bacteria are blue in color. This color may cover the entire colony, or appear only in the center of the colony. Colonies of other types should not be counted. The colonies can be counted with the aid of a lens. The number of fecal coliforms per 100 ml is then given by:

Calculation:

$$\text{Total coliform (CFU/ 100 mL)} = \frac{\text{No.of coliform colonies counted x 100}}{\text{mL of sample filtered}}$$

$$\text{Fecal coliform (CFU/ 100 mL)} = \frac{\text{No.of coliform colonies counted x 100}}{\text{mL of sample filtered}}$$

Assignment:

1. What do you understand by "indicator organisms"? Why water samples are usually tested for indicator organisms instead of specific pathogenic organisms?
2. Define and differentiate between total coliform (TC) and fecal coliform (FC)?
3. What are the major advantages of "membrane filtration method" over "multiple tube method"?