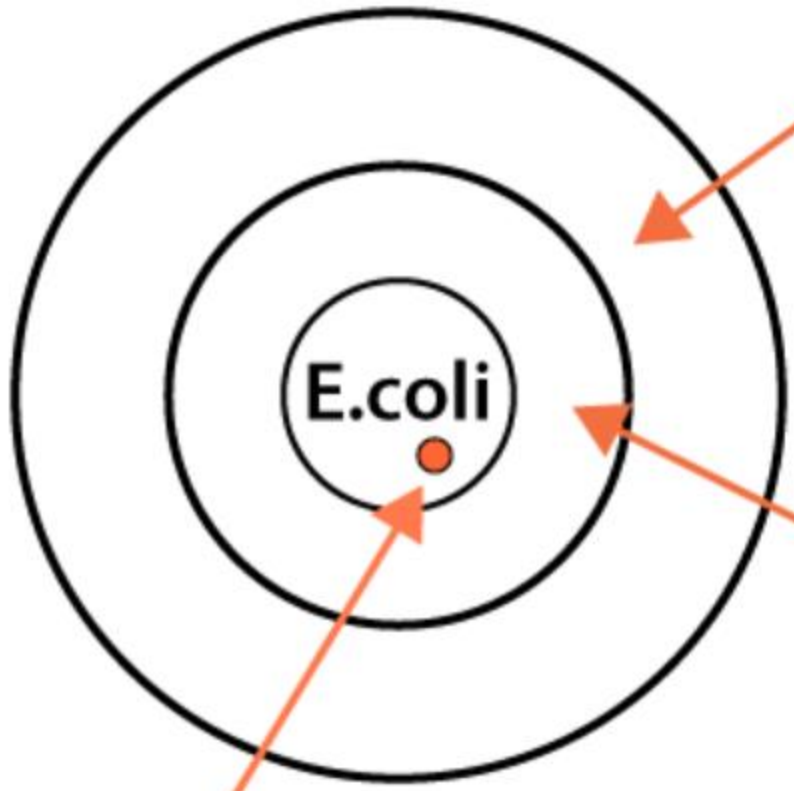
DETERMINATION OF TOTAL AND FECAL COLIFORM IN WATER



Microorganisms: TC and FC



Total Coliform, Fecal Coliform and E.coli



Total Coliform

Total Coliform =
Environmental Contamination

Fecal Coliform

Fecal Coliform + E.coli =
Fecal Contamination

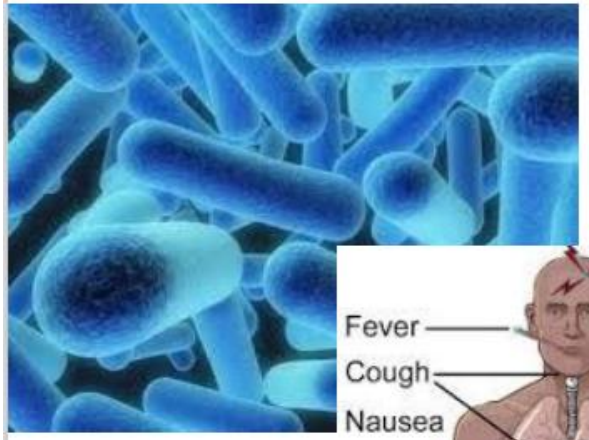
E.coli 0157:H7

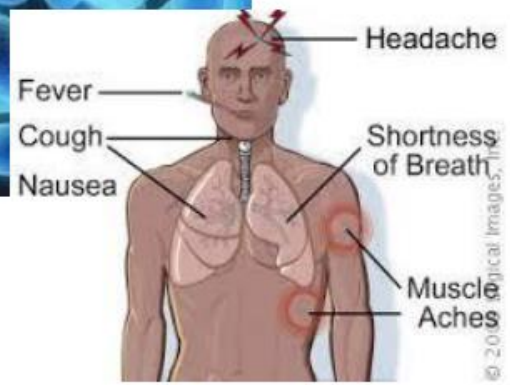
Coliforms in Water and Public Health





Cholera





Legionellosis

Giardia lamblia





INTRODUCTION

- 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 do not survive for very long period of time; consequently they could be missed in a sample submitted to the laboratory.
- Identification of such pathogens are quite time consuming, complicated and expensive approach.
- Hence in most cases, the microbiological quality of water is checked using some indicator organisms.

INDICATOR ORGANISM

- 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***.
- The ideal indicator organism should have the following characteristics:
  - Always be present when pathogens are present
  - Always be absent where pathogens are 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

INDICATOR ORGANISM

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

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

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

METHODS FOR DETERMINATION OF INDICATOR ORGANISM

- ***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.

- ***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.

Membrane Filter Method

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 (MF METHOD)

Determination of Total Coliform

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.

TEST PROCEDURE (MF METHOD) (cont…)

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 Petridish 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.

TEST PROCEDURE (MF METHOD)

Determination of Fecal Coliform

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

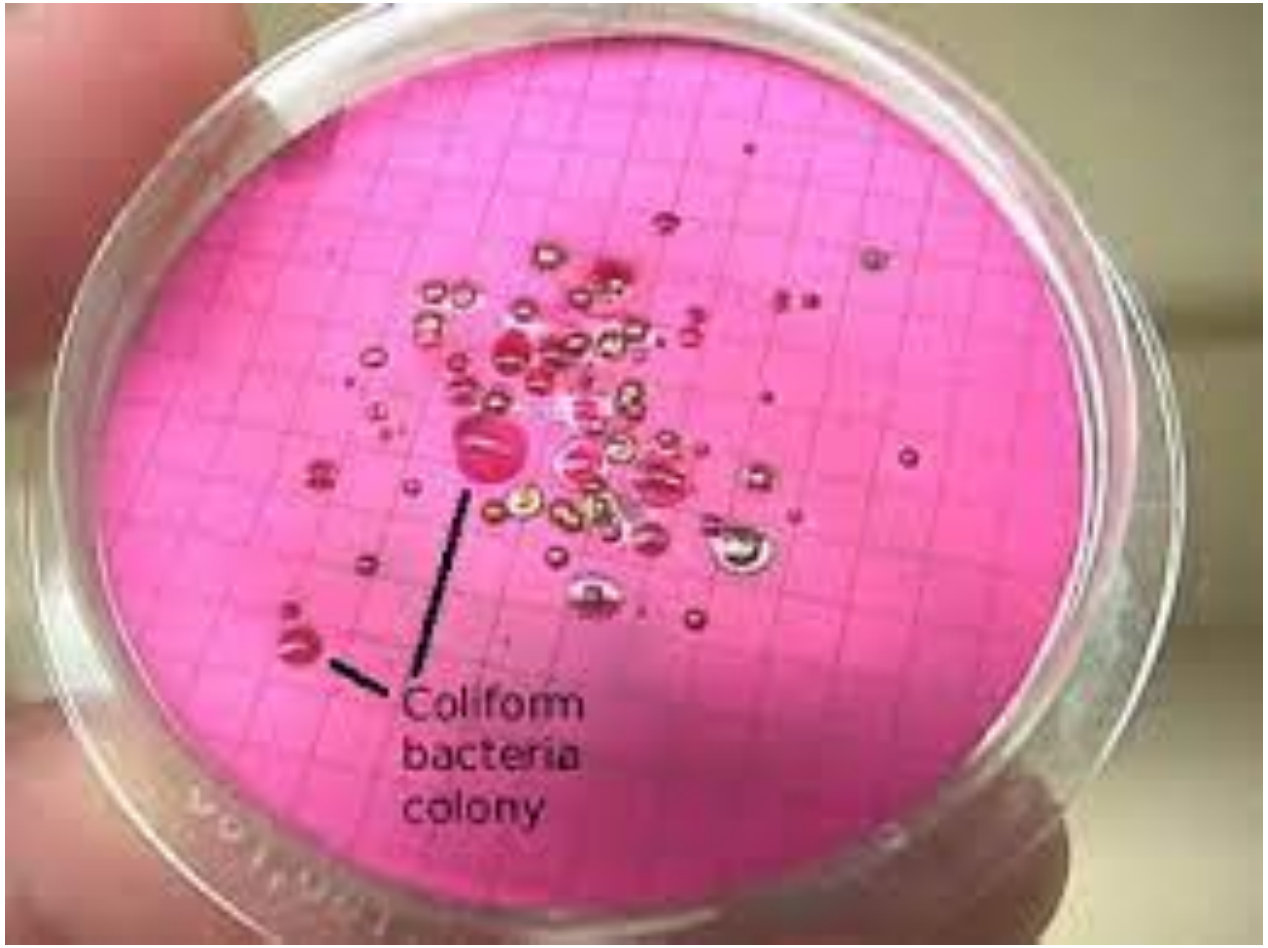
For Total Coliform:

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 center of the colony. Colonies of other types should not be counted. The colonies can be counted with the aid of a lens.

For Fecal Coliform:

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.

Identification of TC



CALCULATION

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

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

THANK YOU !