Bacteriology of Drinking water

The Quality of Water is polluted with many Factors

Bacteriology of water to identify the bacterial contamination

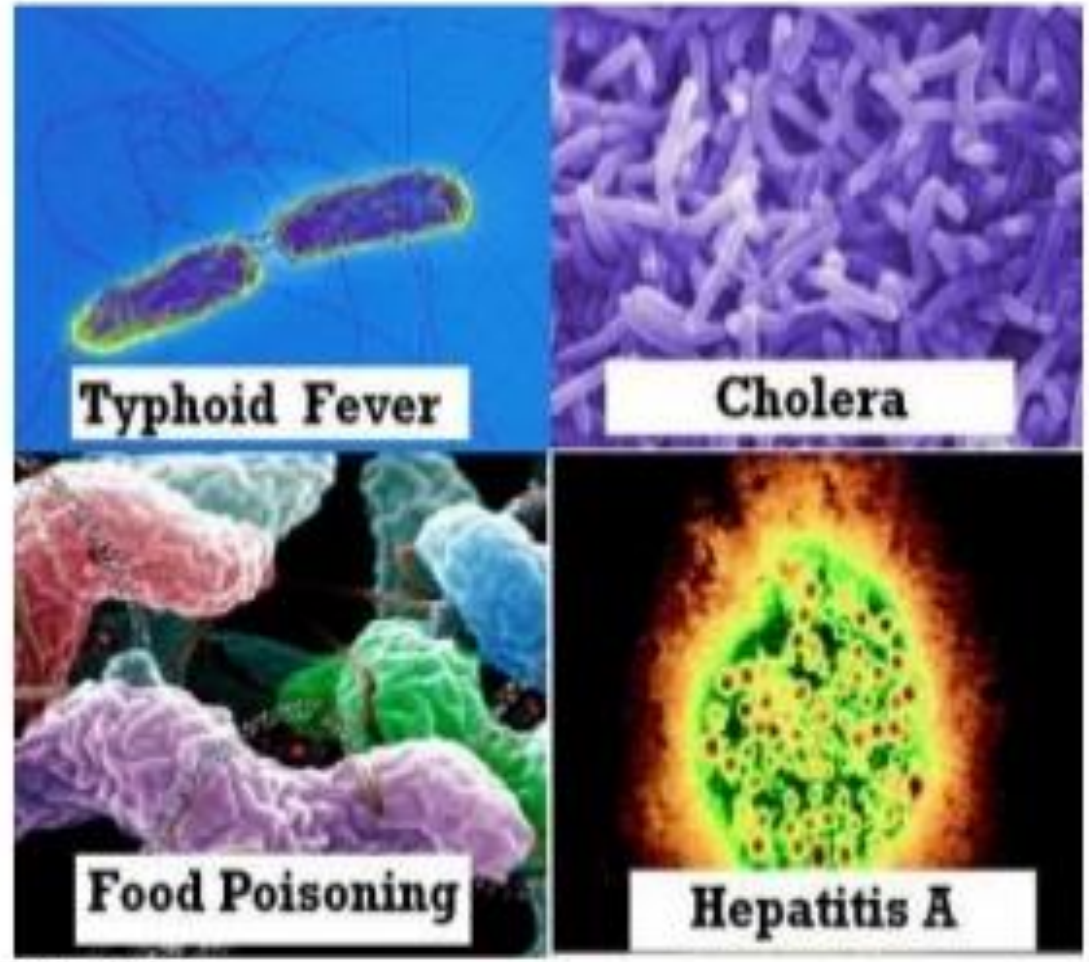
- The bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms.

How the water gets contaminated

- Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery

Common Diseases Spread by Water

- Typhoid fever
- Cholera
- Diarrhoeal diseases
- Polio myelitis
- Viral hepatitis A and E



Natural Water contaminated with

- Pseudomonas spp
- Flavobacterium
- Chromobacterium
- Acinetobacter spp

Water we drink should be Free from

- Should be free from pathogenic bacteria
- The primary criteria is the water should be free from Coliforms if they are present it indicates faecal contamination
- Thermotolerant E.coli bacteria indicates the presence of contamination
- Faecal Streptococci thermos tolerant indicates contamination

Bacteriological Analysis of Water

- Bacteriological water analysis is a method of analysing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality.

How the bacteriological analysis of water Helps

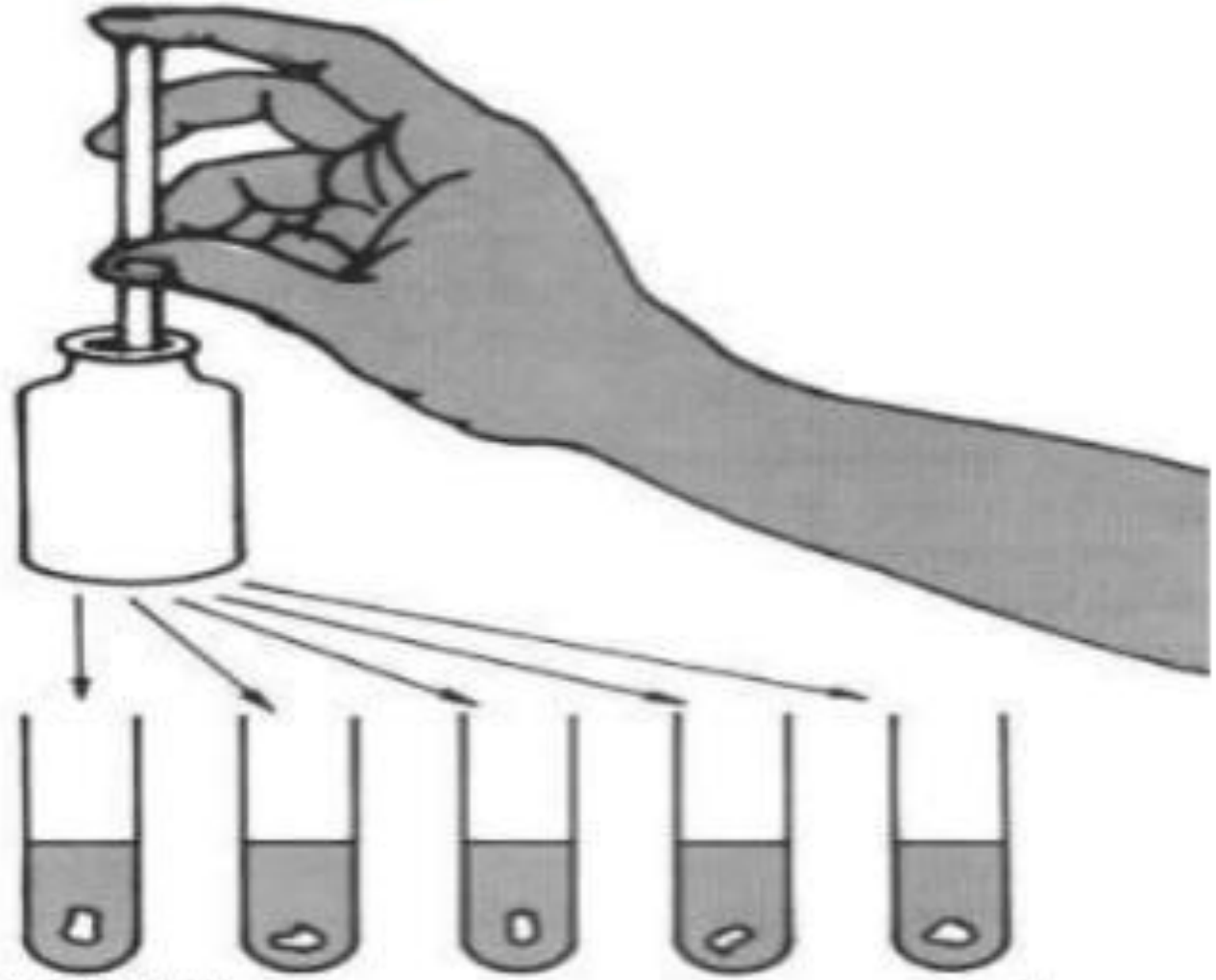
- It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations.

What are the Indicator organisms contaminate water

- The common feature of all these routine screening procedures is that the primary analysis is for indicator organisms rather than the pathogens that might cause concern. Indicator organisms are bacteria such as non-specific coliforms, Escherichia coli and Pseudomonas aeruginosa that are very commonly found in the human or animal gut and which, if detected, may suggest the presence of sewage

Methods used in Culturing of Water

- Analysis is usually performed using culture, biochemical and sometimes optical methods. When indicator organisms levels exceed pre-set triggers, specific analysis for pathogens may then be undertaken and these can be quickly detected (where suspected) using specific culture methods or molecular biology.



Multiple tube method

- One of the oldest methods is called the multiple tube method. In this method a measured sub-sample (perhaps 10 ml) is diluted with 100 ml of sterile growth medium and an aliquot of 10 ml is then decanted into each of ten tubes. The remaining 10 ml is then diluted again and the process repeated. At the end of 5 dilutions this produces 50 tubes covering the dilution range of 1:10 through to 1:10000.

Methodology of Bacterial analysis of Water

- The tubes are then incubated at a pre-set temperature for a specified time and at the end of the process the number of tubes with growth in is counted for each dilution. Statistical tables are then used to derive the concentration of organisms in the original sample. This method can be enhanced by using indicator medium which changes colour when acid forming species are present and by including a tiny inverted tube called a Durham tube in each sample tube. The Durham inverted tube catches any gas produced. The production of gas at 37 degrees Celsius is a strong indication of the presence of Escherichia coli.

Laboratory pictures show how the Water is Analysed

Pour plate method

- When the analysis is looking for bacterial species that grow poorly in air, the initial analysis is done by mixing serial dilutions of the sample in liquid nutrient agar which is then poured into bottles which are then sealed and laid on their sides to produce a sloping agar surface. Colonies that develop in the body of the medium can be counted by eye after incubation.

Pour plate method

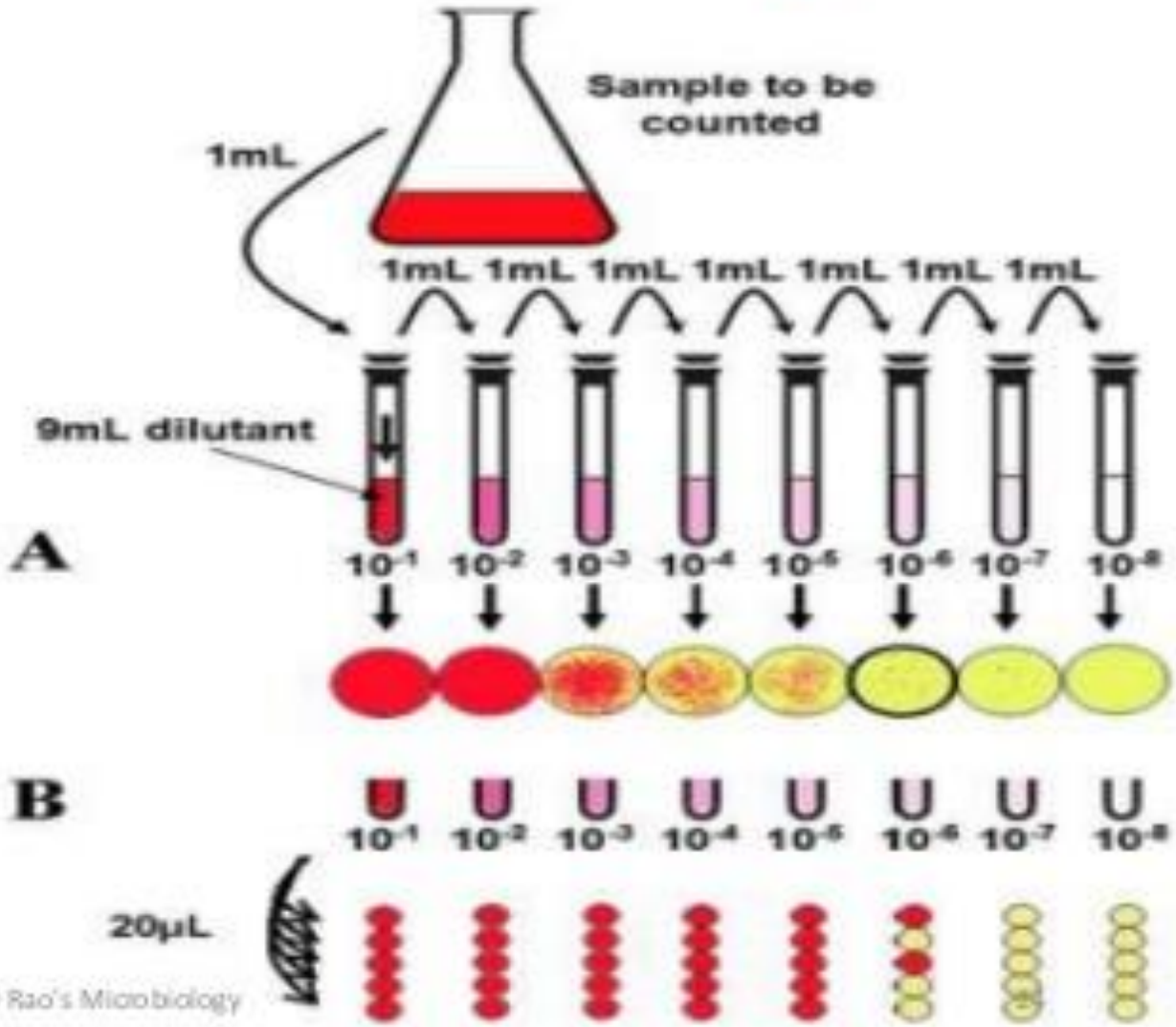
- The total number of colonies is referred to as the Total Viable Count (TVC). The unit of measurement is cfu/ml (or colony forming units per millilitre) and relates to the original sample. Calculation of this is a multiple of the counted number of colonies multiplied by the dilution used.

Reporting the Results of Water Analysis

- When a water sample arrives at the laboratory, two tests, the plate count and the coliform test by the multiple tube method, are made and reported to the operator.

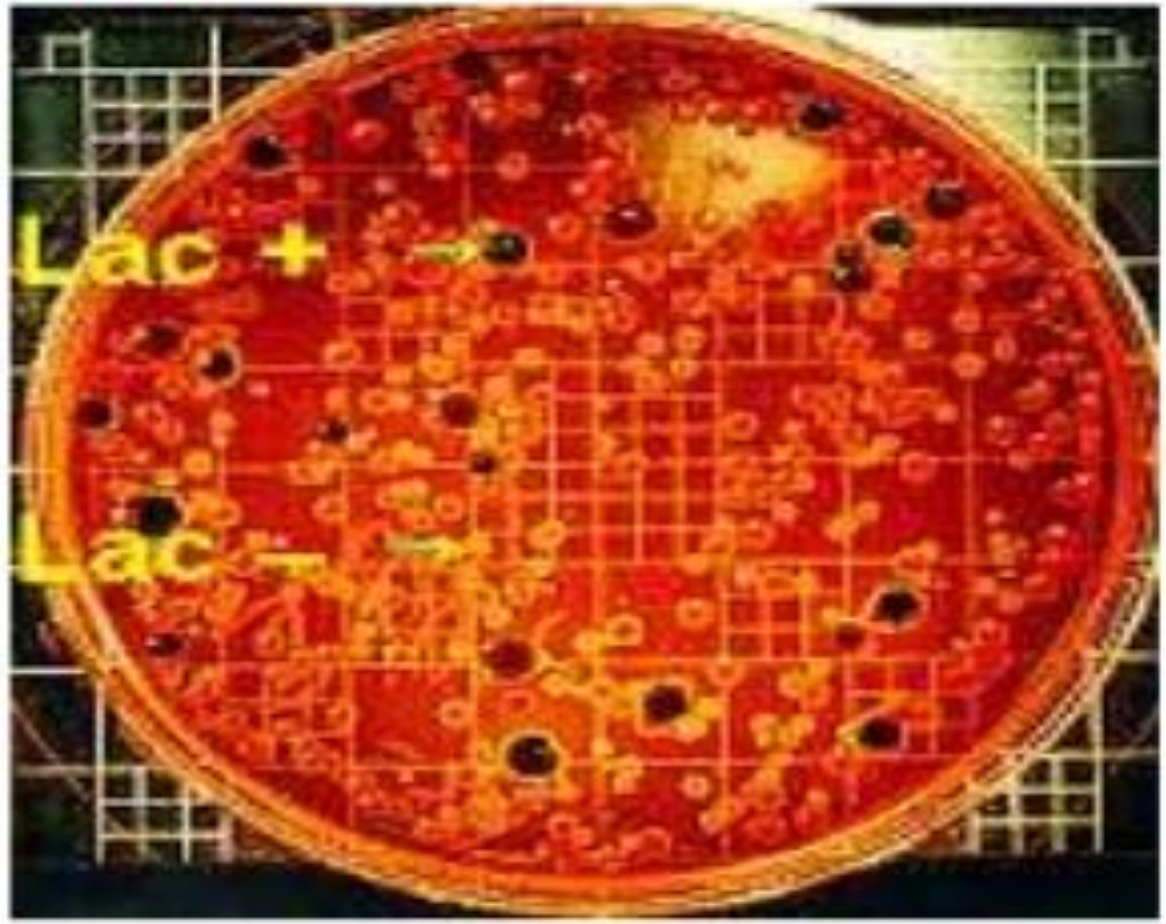
Reporting the Results of Water Analysis

- The coliform test actually consists of two steps known as the presumptive test and the confirmed test. Under certain conditions, it is necessary to go one step further and make a completed test; however, this step is not always necessary. To make the tests, small portions of the water sample are used in accordance with the following procedures.



Making the Microbes to grow on the Petri dish

- A portion of the water sample is placed in the petri dish along with the agar. It is then placed in an incubator with the temperature at 37°C or 98.6°F, which is body temperature. After 24 hours, the plate is removed, examined, and the colonies in and on the agar are counted and recorded on the report form as "Bacteria per ml at 37°C".



Types of Nutrient media used in analysis

- **MacConkey agar is culture medium designed to grow Gram-negative bacteria and stain them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria), neutral red dye (which stains microbes fermenting lactose), lactose and peptone.**

Presence of Bacteria Means

- The presence of the Coli-aerogenes group of bacteria in the above tests does not definitely mean that harmful bacteria are present. Coliform bacteria are normally present in great numbers in the human intestine and, except in unusual circumstances, are not harmful to humans.

What the Indication of presence of Bacteria

- When present in a water sample, they do, however, indicate the presence of faecal contamination and the possibility that harmful (pathogenic) organisms, such as typhoid fever germs, may be present. Therefore, the tests are not measures of actual disease-producing organisms, but rather are indicators of the possibility that they are present.

Public Health Concept

- The coliform group has been used extensively as an indicator of water quality and has historically led to the public health protection concept.

Methods in use for Bacteriological analysis

- The simple and inexpensive membrane filter technique is the most widely used method for routine enumeration of coliforms in drinking water.The detection of coliforms based on specific enzymatic activity has improved the sensitivity of these methods. The enzymes beta-D galactosidase and beta-D glucuronidase are widely used for the detection and enumeration of total coliforms and Escherichia coli, respectively. Many chromogenic and fluorogenic substrates exist for the specific detection of these enzymatic activities, and various commercial tests based on these substrates are available

Bacteriological Identification with molecular methods

- The immunological, polymerase chain reaction (PCR) and in-situ hybridization (ISH) techniques. In the immunological approach, various antibodies against coliform bacteria have been produced, but the application of this technique often showed low antibody specificity. PCR can be used to detect coliform bacteria by means of signal amplification: DNA sequence coding for the lacZ gene (beta-galactosidase gene) and the uidA gene (beta-D glucuronidase gene) has been used to detect total coliforms and E. coli, respectively.

Is the PCR is precise in analysis of Water

- However, quantification with PCR is still lacking in precision and necessitates extensive laboratory work. The FISH technique involves the use of oligonucleotide probes to detect complementary sequences inside specific cells. Oligonucleotide probes designed specifically for regions of the 16S RNA molecules of Enterobacteriaceae can be used for microbiological quality control of drinking water samples