

Bacteriological Analysis of Water

Introduction:

Many of the microorganisms that cause serious disease, such as typhoid fever, cholera, and dysentery, can be traced directly to polluted drinking water. These disease-causing organisms called pathogens are discharged along with fecal wastes and are difficult to detect in water supplies. Fortunately, less harmful, easily isolated bacteria called indicator organisms can be used indirectly to detect pathogens. Among these indicators are coliform bacteria. They live in the intestine of man and other animals and are almost always present, even in healthy persons. The presence of coliforms in water is a warning signal that more dangerous bacteria may be present.

By definition coliform bacteria are those, that are aerobic and facultative, gram-negative, non-spore forming, rod shaped and ferment lactose with the gas formation within 48 hours at 35°C. Those that have the same properties at a temperature of 44°C or 44.5°C are described as fecal coliform organisms. It is convenient to express the result in replicate tubes and dilutions in terms of the Most Probable Number (MPN). This term is actually an estimate based on certain probability formula.

Intestinal bacterial pathogens that are present in contaminated drinking water include Salmonella, Shigella, E.Coli, Vibrio Cholera, etc. These organisms may cause disease that vary in severity from mild gastroenteritis to severe and sometimes fatal dysentery, cholera, or typhoid.

Potable water used for drinking and bathing, if it contains excessive numbers of organisms such as Pseudomonas, Klebsiella, may produce a variety of infections involving the skin and mucous membranes of the eye, ear, nose, and throat.

For Sampling use a sterile bottle or sterile bag to collect sample. Sample containers should not be filled completely. Maintain an air space of at least $\frac{1}{4}$ the total volume.

Objectives:

The objective of this experiment is to learn enumeration technique of coliform bacteria.

Materials:

Fermentation tubes with aluminium caps, Durham tubes, lactose broth, brilliant green bile broth, platinum loop, bunsen burner, disposable pipettes, 10 ml, and 1ml. An incubator adjusted at 35 °C is also required.

Procedure:

MPN test is performed in three stages: Presumptive test, confirmative test, and completed test. Presently we need only the first two.

I. Presumptive Test:

- Prepare lactose broth and distribute 15 ml sterilized media in each fermentation tube containing an inverted Durham tube.
- Distribute 5 fermentation tubes in three lines and mark 10 ml, 1ml, and 0.1 ml in each line.
- Shake well effluent and distribute specific volume 10 ml, 1 ml, and 0.1 ml in each tube under sterile conditions by sterilized pipettes.
- Place the tubes in the incubator for 48 hours.
- After incubation record positive tubes indicated by the presence of trapped gas bubble inside Durham tubes.

II. Confirmative Test:

- Transfer a loopful of sample from positive tubes to a BGB tube under aseptic condition and mark the tubes as in the earlier test.
- Incubate the tubes for 48 hours
- Record positive tubes after incubation
- Convert the reading to MPN index / 100 ml using MPN index table presented in the Standard Methods.