Laboratory (3) Water Microbiology Dr Duaa Suhail

Isolation and Enumeration of Water Bacteria

The presence of large number of bacteria in water has no effect on water potability, thus a water sample with large microbial content could be safe to drink. The crucial consideration from a microbiological perspective is the microbial kinds present in water. As long as human pathogens are not existed in water, it is potable even if it contains variety of autotrophs and saprophytic heterotrophs. Water contamination with human fecal material is considered as an enormous sanitary problem. Therefore, regular examining of water for the presence of fecal microorganisms is important for water purity.

B- Most Probable Number Technique (MPN)

Method Definition:

Most Probable Number (MPN) is a qualitative technique most commonly applied for the quality test of water to detect coliform and thereby to determine simply the potability or safety of water.

Method Principle:

A group of bacteria commonly referred to as "fecal coliforms" act as an indicator of fecal contamination of water because the source of these bacteria is human gut. Both *E. coli* (Gram-negative coliform) and *Enterococcus faecalis* (Gram positive enterococcus) are classified as good "sewage indicators". The principle of method is illustrated as following:

1. Water to be tested is serially diluted.
2. Water is inoculated in lactose broth.

3. If coliforms are present in water, they utilize the lactose present in the medium to produce acid and gas.
4. The presence of acid is indicated by the color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted Durham tube present in the medium.
5. The number of total coliforms is determined by counting the number of tubes giving positive reaction (i.e both color change and gas production) and comparing the pattern of positive results (the number of tubes showing growth or turbidity at each dilution) with standard statistical tables.

Note: Both *E. coli* and *Enterococcus faecalis* have characteristics which make them good indicators of fecal contamination:

1. They are usually not present in water or soil.
2. Their identification is easy.
3. Their survival in water is a little longer than enteric pathogens.

Note: As both bacterial species are non-spore former, their persistence in water is not broad. If they are resistant, surviving a long time in water, they would make the water purity test too sensitive.

What are coliforms? Coliforms are defined as Gram-negative, facultative anaerobes, non-spore forming rods that ferment lactose to produce gas. The coliform count is the test used for the detection of water contamination in which the number of the colonies of coliform-bacteria *Escherichia coli* (*E. coli*) per 100 milliliter of water is counted. The result is expressed as “Coliform Microbial Density” and indicates the extent of fecal matter present in it.

Inadequate processing of the water may result in gastroenteritis (i.e., diarrhea, bacillary dysentery, typhoid, cholera etc.) in the consumers caused by the enteric pathogens including *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and others.

Detection of Coliforms in Water

The standard analysis of the water for the detection of coliform consists of three sequential tests: presumptive test, confirmed test and completed test. The detectable gas production in the presumptive test is recognized as coliform positive from which the presence of *E. coli* can be confirmed by observing green metallic sheen on eosin methylene blue (EMB) agar plate followed by Gram staining procedure as the completed test.

However, MPN method can only determine the presence of coliform bacteria, but cannot estimate the actual load of coliform and other pathogenic bacteria in drinking water. The enumeration and identification of the pathogens are usually carried out by means of cultural and biochemical techniques, often followed by molecular studies or specific antigen detection.

1) Presumptive test

Presumptive test is the first part which is primarily performed for detection of the Gram-negative coliform bacteria in the water samples.

Requirements:

-Water sample

-Five Durham tubes of DSLB (2X)

-Ten Durham tubes of SSLB (1X)

-One pipette (10 ml)

-One pipette (1 ml)

Note: DSLB is the double strength lactose broth which contains twice the as much as lactose as SSLB (single strength lactose broth).

Working Steps:

1. Different water samples such as river, sea, sewage are collected, 100 ml for each sample.
2. Three sets of test tubes containing 10 mL of sterile lactose fermentation broth are needed for each sample. Each set of five tubes resulting in a total of 15 tubes for each sample of water. Each tube is incorporated with a Durham tube indicating gas formation after lactose fermentation by coliform bacteria.
3. Label each tube according to the amount of water to be dispensed to it: 10 ml, 1.0 ml and 0.1 ml, respectively.
4. Mix the water sample by shaking several times to ensure homogeneity
5. Ten ml, 1 ml and 0.1 ml samples are added sequentially in 5 test tubes containing 10 ml lactose fermentation broth 2X, 5 tubes containing 10 ml lactose fermentation broth 1X and 5 test tubes containing 10 ml 1X lactose fermentation broth.
6. Incubate the tubes at 37º C for 24hrs.
7. Check the tubes for positive result (positive presumptive test) and compare the number of tubes giving a positive reaction to a standard chart and record the number of bacteria present in it.

Results Reading:

If gas has not been formed by the bacteria, leave the tubes incubated for another 24hrs. Production of gas after 24hrs, it is presumed that coliform is present in the water sample. However, no gas production after 48hrs indicates negative presumptive test. This test is also conducted to determine the most probable number (MPN) of coliforms present per 100

ml of water. A table for determination this value from the positive tubes of lactose broth is provided for this step.

For example, a water sample tested shows a result of 3–2–1 (3 × 10 mL positive, 2 × 1 mL positive, 1 × 0.1 mL positive) gives an MPN value of 17, i.e. the water sample contains an estimated 17 coliforms per 100 ml

Confirmed test

Requirements:

-One Petri plate of EMB

-One Petri plate of Endo agar

Working Steps:

1. Test tubes showing positive results in the presumptive test by the accumulation of gas in Durham tubes are selected for the confirmed test to determine if the gas-producing bacteria are Gram-negative.
2. A loopful from positive result tubes, is inoculated on both Eosin Methylene Blue agar or Endo agar by streaking to detect the presence of coliforms (*E. coli*) in the respective water samples, as well as to differentiate *E. coli* and other Gram-negative coliform bacteria such as *Enterobater aerogenes* which shows similar morphological and physiological characteristics of that produced by coliforms including the ability to ferment lactose with gas production.
3. Incubate the plates at 37º C for 24hrs.
4. Look for the typical coliform colonies on both media. Record the results.

Note: Both of media (EMB and Endo agar) inhibit the growth of Gram-positive bacteria and cause coliforms colonies to be distinguished from non coliforms.

Results Reading:

EMB agar: contains methylene blue which inhibits Gram-positive bacteria. Gram-negative lactose-fermenters (coliforms) that grow on this medium produce colonies with dark centres (nucleated colonies) and can be differentiated according to the size and the presence of greenish metallic sheen. These bacteria include *E. coli* (small colonies with metallic sheen) and *Enterobacter aerogenes* (large colonies without metallic sheen). *E. coli* is the more reliable sewage indicator since it is not normally present in soil, while *E. aerogenes* has been isolated from soil and grains.

Endo agar: contains a fuchsin sulfite indicator that makes identification of lactose fermenter relatively easy. Coliform colonies and the surrounding medium appear red. Non fermenters of lactose are colorless and do not affect the color of the medium.

The presence of coliform-like colonies confirms the presence of a lactose -fermenting Gram-negative bacteria. If no coliform colonies are present, the water is considered bacteriologically safe to drink.

Completed test

Requirements:

-One nutrient agar slant

-One lactose broth with Durham tube

Working Steps:

1. Samples revealed positive confirmed test (a growth on agar medium) are selected. Single colonies grown on media are inoculated on a nutrient agar slant and in lactose broth 1X (with Durham tube) again for the assurance of gas production after fermentation of lactose.
2. Incubate the plates at 37º C for 24hrs.

Results Reading:

If gas is produced in lactose broth and a Gram staining slide from the agar slant reveals the presence of Gram-negative, non-pore forming short bacilli or coccobacilli which ferments lactose, that means the presence of coliforms in water.

Note: Positive results of the three tests determine the presence of coliforms. However, the coliform present could be *E. coli* (the better sewage indicator) or *Enterobacter aerogenes* (non-sewage origin). IMViC tests should be performed to differentiate between the two bacterial species.

Advantages of MPN:

1. Ease of interpretation, either by observation or gas production.
2. Effective method of analyzing highly turbid samples such as sediments, sludge, mud, etc. that cannot be analyzed by membrane filtration.

Disadvantages of MPN:

1. It takes a long time to get the results.
2. Results are not very accurate.
3. Requires more hardware (glassware) and media.
4. Probability of false positives.

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Classification of Drinking Water in European Countries

The Total Number of Bacteria / 1ml	Class of Drinking Water
Less than 10	Complete purity water
10 -100	Very purified water
100 – 1000	Purified water
1000 -10000	Medium purified water
10000- 100000	Contaminated water
More than 100000	Very contaminated water

Water Classification Depending on The Number of Coliforms

No. of bacteria	Water class
Less than 1	Water is very acceptable
1-2	Acceptable water
3-10	Questionable water
More than 10	Water is unacceptable