8. MPN METHOD OF ENUMERATION OF INDICATOR ORGANISM

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Most Probable Number (MPN) Test

Serial dilution tests measure the concentration of a target microbe in a sample with an estimate called the most probable number (MPN). The MPN is particularly useful for low concentrations of organisms (<100/g), especially in milk and water, and for those foods whose particulate matter may interfere with accurate colony counts. Only viable organisms are enumerated by the MPN determination. The expected result is the number of tubes and the number of tubes with growth at each dilution, will imply an estimate of the original, undiluted concentration of bacteria in the sample.

The MPN is the number which makes the observed outcome most probable. It is the solution for λ, concentration, in the following equation

$$\sum_{j=1}^{k} \frac{g_j m_j}{1-\exp(-\lambda m_j)} = \sum_{j=1}^{k} t_j m_j$$

where exp(x) means ex, and

K denotes the number of dilutions,

gj denotes the number of positive (or growth) tubes in the *j*th dilution,

mj denotes the amount of the original sample put in each tube in the *j*th dilution,

tj denotes the number of tubes in the *j*th dilution.

The 95 percent confidence intervals in the tables have the following meaning:

Before the tubes are inoculated, the chance is at least 95 percent that the confidence interval associated with the eventual result will enclose the actual concentration. MPN is used to estimate the presence of viable coliforms group of bacteria in a replicate liquid broth (ten-fold dilutions). It is commonly used in estimating microbial populations in fish, waters and ice samples. MPN is most commonly used for checking the quality of water whether it's *safe or not.* Coliform is a group of bacteria belongs to the enterobacteriaceae and assessing at three levels viz., Total coliforms, fecal coliforms and *E. coli*. The presence of fecal coliforms clearly indicates the fecal contamination and its presence in large numbers would indicate a possibility of containing the disease- producing coliforms.

Coliform bacteria are rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria which fermentlactose with the production of acid and gas at 37^{o}C. The presence of

coliform group of bacteria is commonly used as an indicator of sanitary quality of foods and water. Coliforms can be found in the soil, vegetation and aquatic environment and they are normally present in large numbers in the feces of warm-blooded animals

Coliform group of bacteria belongs to the genera such as

- *Citrobacter*
- *Enterobacter* not of fecal origin
- *Klebsiella*
- *Escherichia* - originate from feces

***Escherichia coli* (*E. coli*)**, a rod-shaped member of the coliforms group, can be distinguished from most other coliforms by its ability to ferment lactose at 44°C in the fecal coliform test. Confirmed on an eosin methylene blue (EMB) plate (metallic green colonies on a dark purple media). *E. coli* are mainly of fecal sources of animal and human. Other coliform bacteria will appear as thick, slimy colonies, with non-fermenters being colorless, and weak fermenters being pink.

A **fecal coliform** is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulatingbacterium and is mainly from the intestines of warm-blooded animals. Fecal coli forms are capable of growth in the presence of bile salts, oxidase negative and utilize produce lactose(produce acid and gas) at 44 ± 0.5°C within 48 hours. Because of its growth at 44 ± 0.5°C called as "thermotolerant coliform".

Principle

Sample is diluted serially and inoculated in lactose broth and the lactose is utilized by the coliforms group of bacteria and leads to produce acid and gas. The presence of acid is indicated by color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted durham tube. The number of total coliforms is determined by counting the number of positive reaction *(color change and gas production) in the* tubes and checking the number of positive tubes at each dilution with standard MPN tables.

MPN test is carried out in 3 steps

1. Presumptive test – Presumptive coliforms
2. Confirmatory test- Total Fecal coliforms
3. Completed test- *E. coli*

Step 1: Presumptive test: Screening test for the coliform group of organisms.

Requirements:

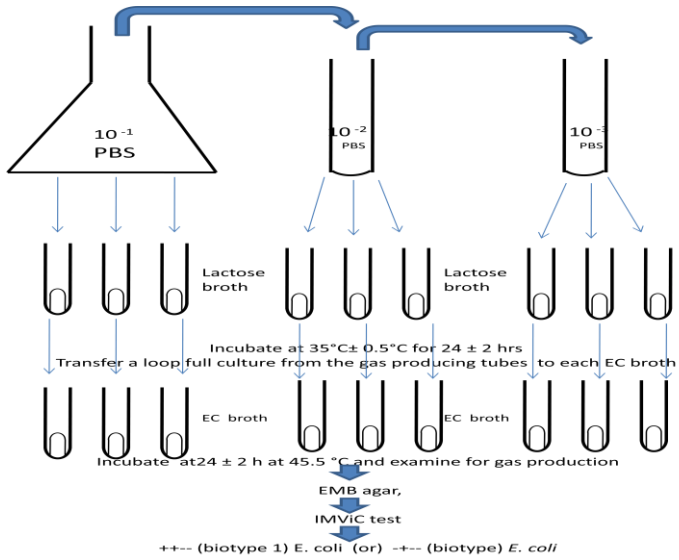
Medium: Lactose broth or Mac Conkey Broth

Glasswares: Test tubes of various capacities (20ml, 10ml, 5ml), Durham tubes

Plasticwares: Sterile pipettes and tips

Others: Discard box, Spirit .

Enumeration on E. coli by MPN method



Preparation of the Medium

Prepare the medium (Mac Conkey or Lactose broth) in single and double strength concentration. Dispense the double strength medium and single strength medium either 5 ml or 10 ml (5 tubes for solid/ semi solid samples and 10 tubes for water and ice) in each tube and put durham tube in inverted position without air bubbles. Sterilize the medium by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

MPN Testing of samples

1. Take 5 tubes of double strength and 10 tubes of single strength.
2. Add 10 ml of the samples to 5 tubes containing 10 ml double strength medium.
3. Add 1 ml of sample to 5 tubes containing 10 ml double strength strength medium and 0.1 ml water to remaining 5 tubes containing 10 ml double strength medium.
4. Incubate all the tubes at 37°C for 24 hrs.
5. Observe at 24 hrs, If no tubes shows positive for growth and gas production, re-incubate up to 48 hrs.
6. Note the number of tubes for positives from each sets and compare the number of tubes giving positive
7. reaction to the 5tubes MPN standard chart and record it.
8. The result is the total number of bacteria present in the sample as MPN values.
9. *For example: 5–4–3 (5 × 10 ml positive, 4 × 1 ml positive, 3 × 0.1 ml positive) = the MPN value is280. So sample contains an estimated 280 coliforms per 100 gram*

StepII – (For confirmed total coliforms)

Requirements :- BGLB 2% broth.

Inoculate one loopful of culture from the +ive tubes of step I to BGLB 2% broth. Incubate at 370C for 24 hrs. Note growth and gas production. Results are noted as +ives if there are growth and gas production. Compare with 3 tube MPN table.

Step III – (For faecal coliforms and *E.coli*)

From the +ive tubes of StepII, inoculate one loopful each to EC broth and Tryptone broth. (indole medium). Incubate at 44.50 °C for 24 hrs.

EC broth:- Growth and gas production.

Tryptone broth:- Test for indole produces by adding 4 drops of Kovac's indole reagent. A pink or red color at the top layer indicates a +ive test for indole.

Coliforms bacteria which products gas in EC broth and indole in tryptone broth of 44.50 °C are *E.coli*.

A loopful of sample from each tube showing positive test *(color change with gas)* is streaked onto two selective medium like Eosin Methylene Blue agar or Endo's medium. One plate each is incubated at 37°C and another at 44.5± 0.2°C for 24 hours.

High temperature incubation (44.5 ±0.2) is for detection of thermo tolerant *E.coli.*