

Enumeration of Bacteria Most probable number (MPN)

Most probable number (MPN) is a method used to estimate the number of viable microorganisms in a test sample. It is based upon the application of probability to the number of observed positive growth responses to a series of standard dilutions.

MPN is commonly used in heterogeneous samples such as soil, water, and agricultural products in which exact cell numbers of individual microorganisms may be impossible to determine.

The MPN technique involves using a decimal (ten-fold) dilution series of a sample and a typical test uses a minimum of three dilutions and five tubes per dilution, then inoculating one ml of each dilution into a separate tube containing a broth medium. The pattern of positive and negative tubes is then noted after the incubation and results checked against a standardized MPN table to determine the most probable number of microorganisms per unit volume of the original sample.

Using the Most Probable Number Technique to Count Coliform in Water

The Most Probable Number method is used to check water (if the water is safe enough to be drinking water). In water only a few coliform per liter can represent a potential health hazard.

Coliforms are Gram negative bacilli that have the ability to ferment lactose with the production of acid and gas. Fecal coliforms are those coliforms that are normally found in the feces of warm blooded animals (including humans). MPN method thus enumerates the fecal coliforms in water samples. *E. coli* is used as an indicator organism.

MPN test is performed in 3 steps

1. **The Presumptive test:** The samples are first inoculated into lactose broth containing a Durham tube. The culture considered positive if acid and gas produced, a positive presumptive test is a very good indication of the presence of coliform.
2. **The Confirmed test:** All positive lactose broth cultures are subjected to the confirmed test: a loopful of each culture is streaked onto Eosine Methylene Blue agar (EMB) and inoculated into Brilliant Green Lactose Bile Broth (BGLBB). A confirmed test is positive when colonies with a green metallic sheen form on EMB and gas occurs in BGLBB.
3. **The Completed test:** Gram stain will do for cell from isolated colony on EMB.

Advantages of the MPN technique include:

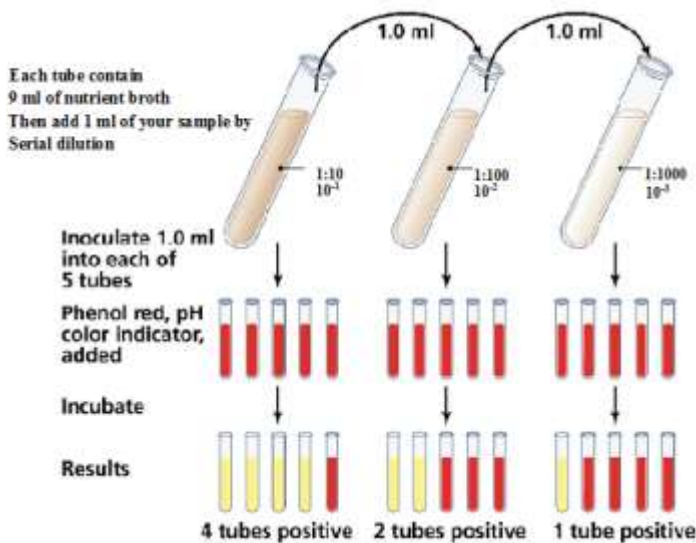
1. Ease of interpretation, either by observation or gas emission
2. Effective method of analyzing several samples such as sediments, sludge, mud, etc.

Disadvantages include:

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

Procedure

1. Prepare at least 50 tubes with 10 ml of nutrient broth and sterilize them.
2. Arrange these tubes and label them as 10^0 , 10^{-1} , 10^{-2} (five tubes for each dilution).
3. Arrange another set of tubes labeled as 10^{-3} , 10^{-4} , 10^{-5} (five tubes for each dilutions).
4. Dilute the given sample serially 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and label them appropriately.
5. Take 1 ml aliquots from each dilution of samples separately and inoculate into the respective tubes containing growth medium.
6. Incubate the tubes at 37°C for 24 hrs.
7. Note the number of tubes showing growth in each dilution.
8. Determine the number of positive tubes in the first three successive tenfold dilutions (10^0 , 10^{-1} , 10^{-2}) and refer to the MPN Table.
9. If all the tubes in the range exhibited growth, determine the number of positive tubes in the next successive tenfold dilutions (10^{-3} , 10^{-4} , 10^{-5}) and refer the corresponding MPN number from the table considering 10^{-3} , 10^{-4} , 10^{-5} dilutions relating to 10^0 , 10^{-1} , 10^{-2} dilutions respectively. The number of viable cells obtained should be multiplied by 10^3 as dilution factor.
10. If you find still all tubes exhibited growth, dilute the sample further to 10^{-6} , 10^{-7} , 10^{-8} and conduct the experiment. Refer the MPN table considering these dilution relating to 10^0 , 10^{-1} , 10^{-2} dilutions respectively. Here number of cells obtained should be multiplied by 10^6 as dilution factor



Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	9	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360

MPN table