MAPÚA UNIVERSITY

SCHOOL OF CIVIL, ENVIRONMENTAL, AND GEOLOGICAL ENGINEERING

# Experiment No. 6: MOST PROBABLE NUMBER (MPN)
## IN WATER QUALITY TESTING

**ESE141P-2 / A6**

(MICROBIOLOGY AND PARASITOLOGY FOR ENVIRONMENTAL AND SANITARY ENGINEERS)

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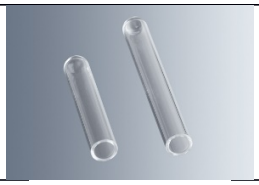
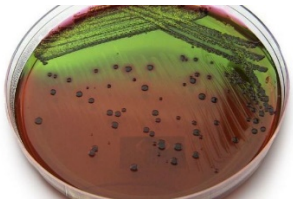
# Background or Rationale of the experiment.

Most Probable Number (MPN) *is a method used to estimate the coliform concentration of microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions.* MPN is a qualitative method were estimates are provided instead of absolute counts, and observations are drawn from the change in color and bubble and acid productions in lactose broth tubes. Fecal coliforms are indicators fecal contamination in water sample which could lead to serious and harmful diseases to humans. The presence of very low fecal coliform would mean that the water may not contain disease causing organisms, while having large amounts of fecal coliforms would indicate a high probability that the water contains disease-causing organisms.

In presumptive test, the water is diluted in certain amounts in a lactose broth. The resulting number of test tubes with significant change in color and presence of bubbles and gases collected in the Durham tube would be compared and assessed with the use of standard statistical table. When samples are tested positive from the presumptive test, confirmatory and completed tests are required. Otherwise, samples that are tested negative would no longer require further testing. Confirm test are used to confirm the positive samples from presumptive tests by examining gas productions and incubating the samples further, using agar slants, and microscopic inspections. Since some results from confirmed tests may be false, completed tests is done with the use of EMB agar to cultivate gram – negative microbes and inhibit gram -positive microbe growth. When gram-negative, non-spore forming microbes' flourishes on an EMB agar, the test will be concluded positive.

# Materials and Equipment

| PRESUMPTIVE TEST | | |
|---|---|---|
| Lactose broth |  | used in the detection and/or verification of the presence of coliform organisms in samples of water and dairy products |
| Test tubes |  | slender containers that hold small amounts of liquid and are used in scientific experiments |
| Durham tube |  | Used to detect production of gas by microorganisms. These are smaller test tubes inserted upside down in another test tube. |
| Sterile pipettes |  | Used to transport water samples to test tubes filled with lactose broth |
| CONFIRM TEST | | |
| Lactose-broth or brilliant green lactose fermentation tubes |  | used for the confirmation of presumptive positive tubes showing gas production in Lactose Broth |
| Agar slant |  | are used for storing pure cultures for a moderately long term |
| Tryptone water |  | used for the cultivation and maintenance of fastidious aerobic and facultative microorganisms |

| COMPLETED TEST | | |
|---|---|---|
| Eosin Methylene Blue Agar |  | is a both selective and differential culture medium. It is selective culture medium for Gram-negative bacteria (selects against Gram positive bacteria) and is commonly used for the isolation and differentiation of coliforms and fecal coliforms |

# Procedures

## Preliminary Test

1. Take 5 tubes of double strength and 10 tubes of single strength for each water sample to be tested.
2. Using a sterile pipette add 10 ml of water to 5 tubes containing 10 ml double strength medium.
3. Similarly add 1 ml of water 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. If no tubes appear positive re-incubate up to 48 hrs.
5. Compare the number of tubes giving positive reaction to a standard chart and record the number of bacteria present in it.

## Confirmed Test

1. Incubate the inoculated lactose-broth fermentation tubes at 37°C and inspect gas formation after 24 ± 2 hours. If no gas production is seen,further incubate up to maximum of 48 ±3 hours to check gas production.

2. The agar slants should be incubated at 37°C for 24± 2 hours and **Gram-stained preparations** made from the slants should be examined microscopically.
3. The formation of gas in lactose broth and the demonstration of Gram negative, non-spore-forming bacilli in the corresponding agar indicates the presence of **a member of the coliform group** in the sample examined.
4. The absence of gas formation in lactose broth or the failure to demonstrate Gram-negative, non-spore-forming bacilli in the corresponding agar slant constitutes a negative test *(absence of coliforms in the tested sample)*.

## Completed Test

Since some of the positive results from the confirmatory test may be false, it is desirable to do completed tests. For this inoculum from each positive tube of the confirmatory test is streaked on a plate of EMB or Endo agar.

In this process, a loopful of sample from each positive BGLB tubes is streaked onto 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.

# Answers to Questions

1. Why are the coliforms selected as the indicator of water potability?
   Coliforms were selected as indicator of water potability because having presence of these microbes on water samples means that there are fecal matter which may cause mild to serious infections once consumed by humans.

2. Does a positive presumptive test indicate that water is potable?
   No. A positive presumptive test may indicate that the water is not potable.

3. Why is MPN test qualitative rather than quantitative?
   MPN test is qualitative because the methods do not involve manually counting or using quantitative measures to determine the number of coliforms present. Instead, MPNs are determined based on the quality of the lactose in the test tube after placing the water sample. The number of test tubes for each set who has a change in color is the basis used in order to have estimates about the possible number of coliforms in a sample.

4. What is the function of the following MPN test?
   a. Lactose broth-Lactose Broth
      Lactose both is used for cultivating and detecting salmonella and coliform bacteria in food, dairy, and water products in a laboratory. Lactose-fermenting bacteria metabolize lactose which contributes to a low pH and bacteriostatic effect on competing microorganisms. Lactose-broth is a presumptive test.

   b. Levine’s EMB or LES Endo agar
      The function ofLevine’s EMB or Les Endo agar is a selective and differential medium. This tests selects gram-negative bacteria and the dyes used in this method inhibits the growth of gram-positive organisms.

   c. Nutrient agar slant
      The purpose of Nutrient Agar Slant functions as a source of stain to confirm the Gram-Negative, non-spore-forming nature if coliforms. Nutrient Agar is a confirmatory test

   d. Gram stain
      Grams staining helps identify gram negative organisms in the sample. This is done in completed test.

5. What does a metallic green sheen indicate on an EMB plate? Pink to dark red colonies with a metallic surface sheen on LES Endo agar?

A metallic green sheen on an EMB plate indicates the presence of E. Coli. This is due to the rapid fermentation of lactose and production of strong acids, thus resulting in a rapid reduction of pH of the EMB agar.

Meanwhile, pink to dark red colonies with metallic surface sheen on LES Endo agar also indicates the presence of coliform microorganisms,

6. What bacterial diseases can be transmitted by polluted water?
   The common bacterial diseases that can be transmitted through water pollution includes:

   - **Dysentery** – a combination of nausea, abdominal cramps with severes diarrhea

   - **Polio** – acute viral infection which affects the central nervous system. This leads to high fever, headaches, seizures, and paralysis.

   - **Trachoma** - eye infection cause by bacterium Chlamydia Trachomatis found in polluted water. This leads to severe pain in the eyes, lesions in inner surface of the eyelids as well as eventual blindness,

   - **Typhoid Fever –** caused by Salmonellla Typhi Bacterua through consumption of contaminated food and water. The microbial samples passes through intestinal tract which makes it detectable in stool samples.

   - **Schistosomiasis –** diseased caused by worms that are soread by freshwater snails living in polluted water. The worms can penetrate to the skin while bein in contact with a polluted water and causing infections in major organs like liver, intestines, lungs, and bladder,

   - **Cholera –** infection in the small intestine caused by bacterium Vibrio Cholera, The disease is capable of killing someone within hours if immediate treatment is not at hand. Diarrhea, vomiting, abdominal cramps, and headache are the common symptoms of cholera

   - **Diarrhea –** most often caused vt water-borne viruses as well as bacteria and parasites found in contaminated water. Results in loose watery stools that can cause dehydration and death to many young children and infants.

   - **Malaria –** Is a disease cause by parasites and spread by female mosquitoes trough biting a person. It can cause high fever, headache and lead to complications like anemia, coma, and even death.

7. What is the application of this experiment in your future profession as a Sanitary Engineer? Reflect on its use in the ever-changing technological advancement and degradation of the environment.

Sanitary Engineers are people who get themselves involved in determining water-related problems and find solution to make it suitable for consumption. The goals of the experiment aligns with the purpose of sanitary engineers which is to detect coliforms in water sources and therefore providing information and analysis on how to eradicate them in order to convey safe water to civilians every day.
MPN procedures could be considered simple, however, they provide meaningful and crucial information that determines the quality of human life. Though it is does not involve difficult and complicated technological methods, MPN could still be a preferable conventional method in determining coliform count despite the possibility of developing other methods to determine such microorganisms in water samples. The possibility of MPN method becoming obsolete and replaced by faster, easier, more sustainable methods that provides absolute values instead of estimates is inevitable in the future. Nonetheless, MPN would serve as a cornerstone for formulating new methods in coliform related issues in the future to solve degradation issues in the environment.

# Reflection, Conclusion, Realization,
## and Application in your studies or future profession.

Most Probable Method (MPN) is a method used to provide estimation on the coliform concentration in water sample with the use of lactose broth in ten-fold dilutions. MPN involves three important steps: Presumptive test, Confirm Test, and Completed Test.

A water sample that has been concluded negative in the Presumptive Test will no longer undergo further testing. While a sample that produced acid and gases in any tube of a certain sample will then be subjected to Confirmatory test. Some results from the Confirmatory Test may be false, which is why Completed Test is done.

The MPN method, though providing only estimates of the coliform count, has been useful in providing safe and consumable water to many people every day. The main use of MPN is found in water quality testing. The idea behind MPN methods can be considered a competent approach using science to determine coliform presence. Providing a controlled environment for bacterias to foster and inhibit growth by placing samples on a lactose broth and EMB agars respectively, microbiologists and engineers were able to take advantage of that environment to determine and confirm coliform presence. With that, the intelligent use of such science has been serving and saving humans for plenty of years from contagious diseases that may be sourced from contaminated water.

The application of this method will be significant for Sanitary Engineers to deliver their objectives by determining microbial contaminants and in providing solutions for safe water consumption. The estimated data gathered from MPN method will serve as a crucial information in raising concerns about water quality as well as enforcing warnings to people about possible harmful water sources at certain vicinities.

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