

Water Quality: Standard Coliform Most Probable Number (MPN) Test

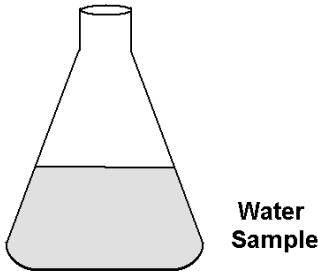
The number of total coliforms (*Enterobacter*, *Klebsiella*, *Citrobacter* and *Escherichia*) in a water sample can be determined by a statistical estimation called the **most probable number (MPN)** test. This test involves a multiple series of Durham fermentation tubes and is divided into three parts: the **presumptive**, **confirmed** and **completed** tests.

In the presumptive test, different volumes from the water sample are added to lactose or lauryl tryptose broth fermentation tubes. (We will use lactose broth tubes). After 24 to 48 hours of incubation at 35 °C, one looks for bacteria capable of fermenting lactose with gas production, presumably coliforms. (The lauryl tryptose broth is selective for gram-negative bacterial due to the presence of lauryl sulfate).

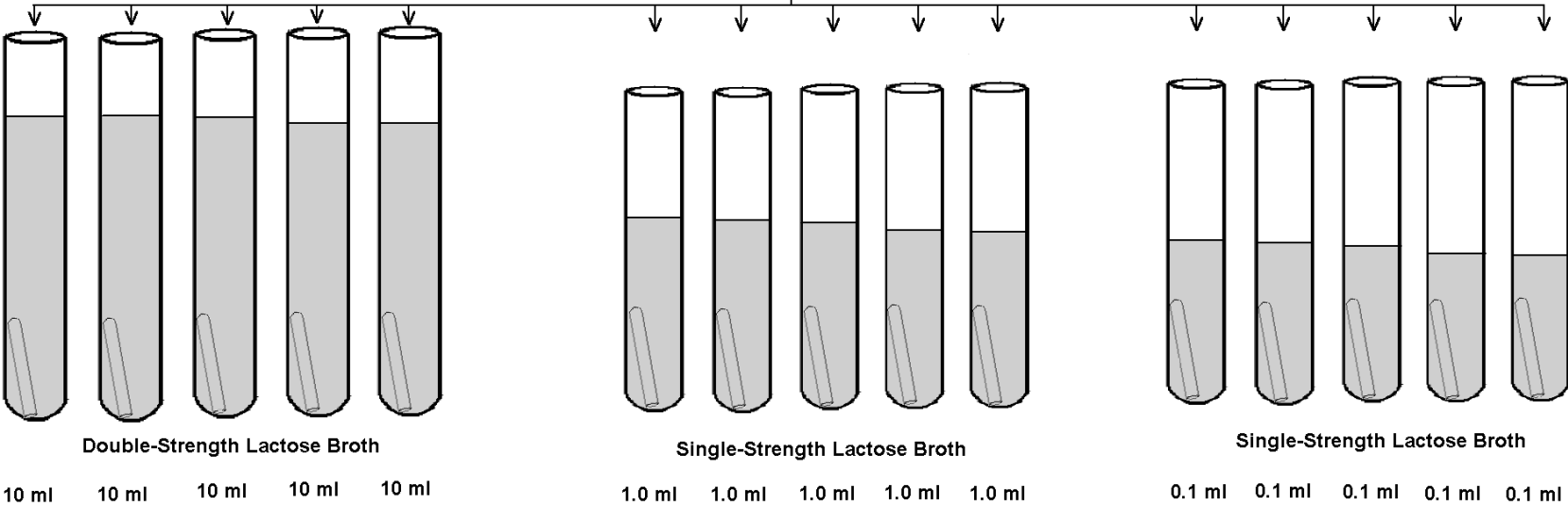
In the confirmed test, one transfers material from the highest dilution (lowest volume inoculated) of those lactose broth tubes that showed growth and gas production into brilliant green lactose bile broth, which is selective and differential for coliforms. The tubes are incubated for 48 +/- 2 hours at 35 °C. Gas formation in the Durham tube is a confirmed test for total coliforms.

In the completed test a sample from a positive brilliant green lactose bile broth tube is streaked onto Levine's EMB (Eosin Methylene Blue) agar or LES Endo agar and incubated for 18-24 hours at 35 °C. On EMB agar, coliforms produced small colonies with dark centers. Also *Escherichia coli*, one of the members of the coliform group, forms shiny metallic green colonies on EMB agar. On LES Endo agar, coliforms produce reddish colonies. Individual colonies are then inoculated into brilliant green lactose bile both and onto a nutrient agar slant. These tubes are incubated for 24 hours at 35 °C. If gas is produced in the brilliant green lactose bile broth, and the isolated bacterium is a gram-negative (based on a gram-stain) nonsporing rod, the completed test is positive for coliforms.

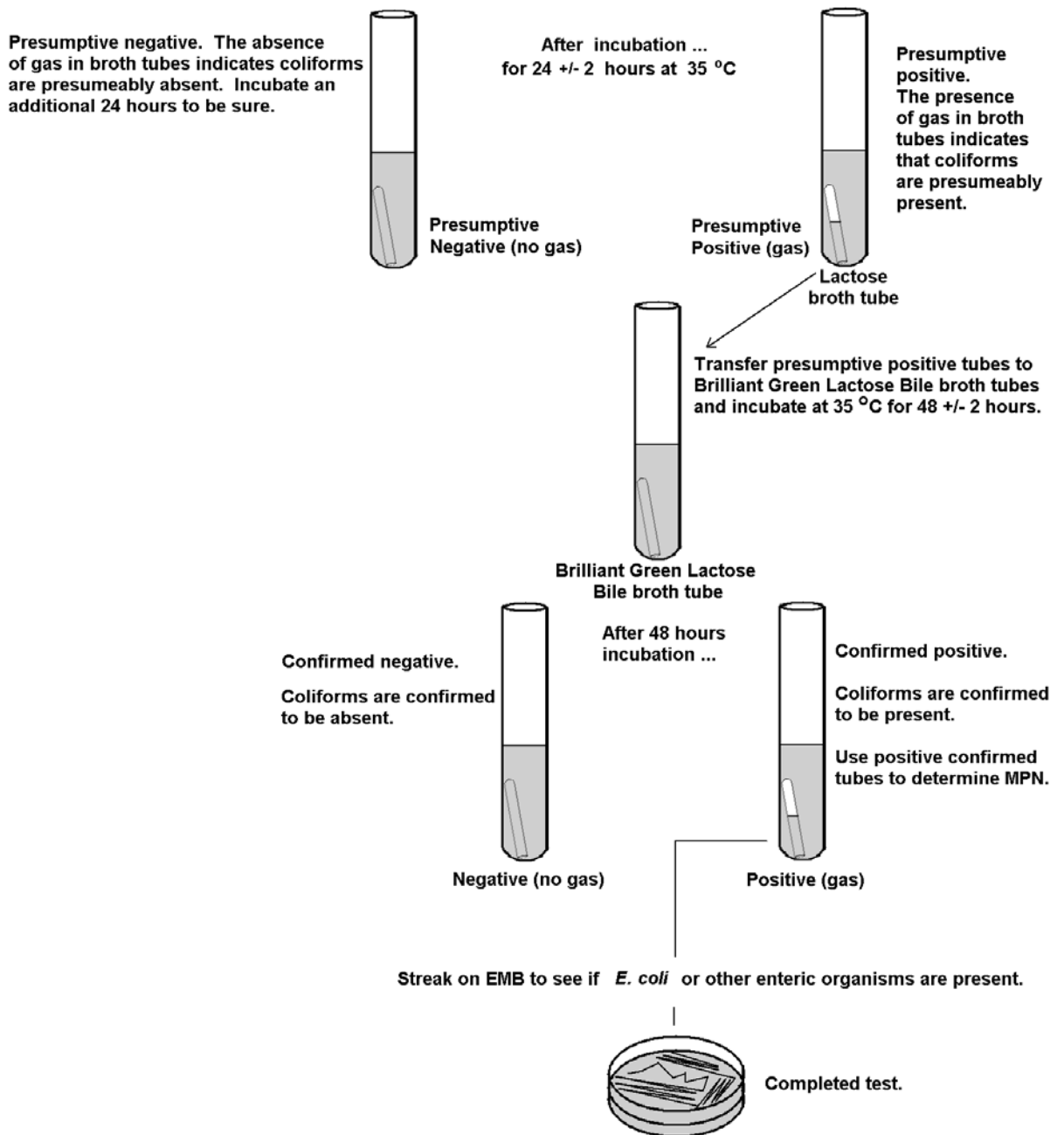
An estimate of the number of coliforms (most probable number) can also be done in the presumptive test. In this procedure 15 lactose broth tubes are inoculated with the water sample. Five tubes of double-strength lactose broth receive 10 ml of water, 5 tubes of single-strength lactose broth receive 1.0 ml of water and 5 tubes of single-strength lactose broth receive 0.1 ml of water. A count of the number of tubes in each volume group is then made, and the numbers are compared to a table developed by the American Public Health Association. The number is the most probable number (MPN) of coliforms per 100 ml of the water sample. (It should be noted that the MPN index usually comes from the presumptive test if raw, untreated sewage water is being tested and comes from confirmed or completed tests for other types of samples).



Inoculate 15 tubes: 5 with 10 ml of sample, 5 with 1.0 ml of sample and 5 with 0.1 ml of sample



Incubate all tubes for 24 +/- 2 hours at 35 °C



E. coli forms shiny metallic green colonies on EMB.

Individual isolated colonies (+/- green sheen) can be Gram-stained and inoculated into either Lactose broth or Brilliant Green Lactose Bile broth (both with Durham tubes) for further characterization.

Gram-negative, nonspore-forming rods that produce gas in lactose broth belong to the coliform group.

More recently a simple and very sensitive alternative to the classical MPN procedure has been developed, the presence-absence (P-A) coliform test. The P-A test is a procedure in which a large water sample (100 ml) is incubated in a single culture bottle containing the ColiLert medium. The ColiLert medium contains growth substrate, nutrients and two test substrates, ONPG and MUG. The ONPG will turn yellow if coliforms are present and the MUG will turn a fluorescent blue if *E. coli* is present. A modification of the ColiLert test using the Quantitray 2000 multiple well tray (with several heat-sealed wells) converts the simple P-A test into an MPN test that can be used to estimate the number of coliforms (or *E. coli*) per 100 mls of water sample. The advantage of both the ColiLert P-A test and the ColiLert MPN test is that the results are obtained in 24 hours, instead of 48-96 hours.