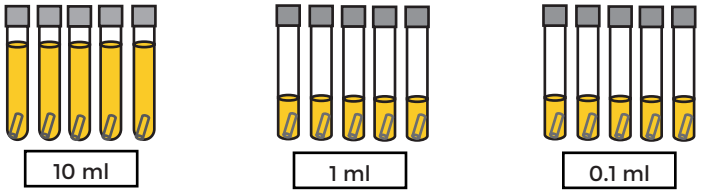
Wastewater Sample

PRESUMPTIVE

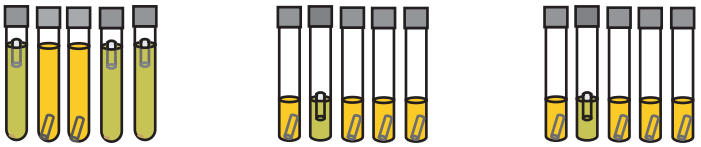
Make required serial dilutions (See Note 1)
Example: Expected coliform value range 5000 – 50000.
First dilution (1:10): 11 ml sample + 99 ml dilution water
Second dilution (1:100): 11 ml of first dilution + 99 ml dilution water
Third dilution (1:1000): 11 ml of second dilution water + 99 ml dilution water

Setup 15 tubes with Lauryl Tryptose Broth and an inverted vial (Durham tube)
5 tubes each for 10 ml, 1 ml and 0.1ml sample



10 ml | 1 ml | 0.1 ml

Incubate 24 hrs – check tubes for positive results marked by : 1) broth turning cloudy, and 2) gas collection in the inverted tube. If no gas collection or media clouding is observed, incubate for another 24 hrs and then check again to see if the tubes are positive



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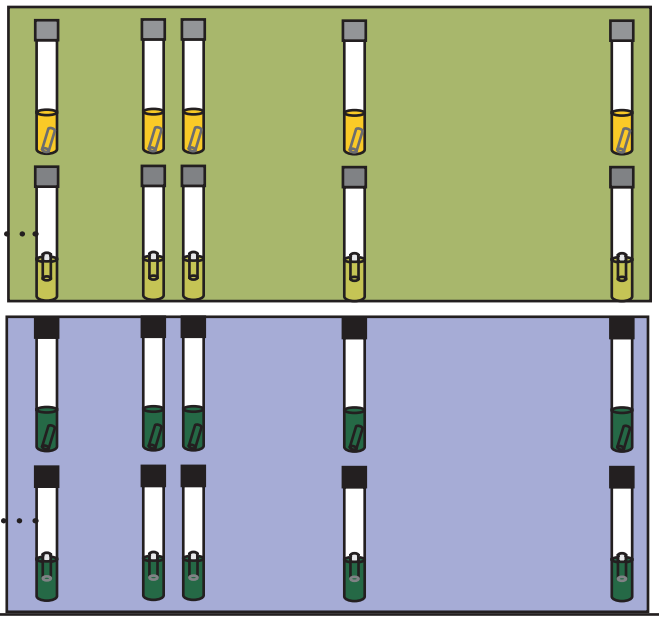
10 ml | 1 ml | 0.1 ml

CONFIRMED

Inoculate each positive into bacteria specific broths – EC broth for fecal coliforms and Brilliant Green Bile (BGB) broth for total coliforms with inverted tubes. Incubate for upto 24 hrs for fecal coliforms and 24 hours for total coliforms and observe for positive results



EC Broth (for fecal coliform}
Note: EC Broth with MUG is used for *E. Coli*

BGB Broth (for total coliform)

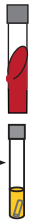
Count number of confirmed positives for each set of the three sample volumes
Example above: 10 ml – 3, 1 ml – 1, 0.1 ml - 1

Establish the MPN using the statistical MPN Table
Example 3-1-1 From Table - MPN = 14 MPN/100 ml. Given 1:1000 dilution, MPN = 14 * 1000 = 14,000 MPN/100 ml

COMPLETED

Streak agar plates & incubate

Use colonies to innoculate an agar slant and nutrient broth



If after 24 hours, from the gram staining of the colonies from the agar slant, nonsporing gram negative bacteria can be identified and the lactose broth shows gas formation, the completed test is deemed positive