

Medium Check

After sterilization and cool down, take one tube and check the pH. The pH should be within +/- 0.2 of recommended pH. Before a new batch of media is put into use, it is checked for selectivity and support of growth using positive and negative cultures. *Escherichia Coli* is used for positive culture and *Pseudomonas Aeruginosa* or *Enterobacter Aerogenes* are used for negative. Tests with a negative result for a positive organism or a positive result for a negative organism would be cause for discarding that batch of media.

Potable Water Test

Shake the dechlorinated sample vigorously 25 times. With a sterile pipette, aliquot 20 ml of sample into 5 test tubes of Triple Strength Laurel Tryptose Broth. Mix test portions by gentle agitation of rack.

Incubate the inoculated tubes at 35 +/- 0.5 C. After 24 hours (+/- 2 hours), swirl and examine each tube for growth, gas, and acidic reaction. If no gas is evident, reincubate and reexamine at the end of 48 hours (+/- 2 hours). Record presence or absence of growth, gas or acidic reaction.

Interpretation of a positive test result is based on the production of gas or an acidic reaction in the inverted vial. Positive results in the Presumptive Test are submitted to the Confirmed Test, Brilliant Green Bile Medium and Fecal/Ecoli Coliform.

Wastewater and Nonpotable Water Test

Shake the dechlorinated sample vigorously 25 times. With a sterile pipette, aliquot 10 ml of sample into 5 test tubes of Double Strength Laurel Tryptose Broth. Mix test portions by gentle agitation of rack. With the same sterile pipette, aliquot 1 ml and 0.1 ml of sample into 5 test tubes each of Single Strength Laurel Tryptose Broth, mix by gentle agitation.

Incubate the inoculated tubes as for potable water. Interpretation and transfers are the same as for potable water.

Confirmed Test Procedure

Gently shake or rotate presumptive tubes showing gas or acidic growth to resuspend the organisms. With a sterile 3.0 mm diameter loop, transfer one loop full of culture from the presumptive to the fermentation tube containing Brilliant Green Bile Broth and/or EC/ECMUG medium.

Incubate the BGB tubes at 35 +/- 0.5 C. Formation of gas in any amount in the inverted vial of the BGB at any time, constitutes a positive confirmed test. Examine the tubes at 24 hours (+/- 2 hours) and again at 48 hours (+/- 2 hours).

Incubate EC/ECMUG in circulating water bath at 44.5 C +/- 0.2C for 24 hours (+/- 2 hours). Formation of gas constitutes a positive test, fluorescence of ECMUG constitutes positive Ecoli.