Multiple Tube Fermentation

Reference: STANDARD METHODS, 19th Edition

Medium Storage

Prior to using any prepared media, check the date of the tubes to make sure that tubes have not exceeded the three-month life span date for refrigerated storage; from the date of preparation, or exceeded the two week life span for non refrigerated storage. Discard all tubes that exceed the life span date.

Medium Preparation

Required Equipment

Triple Beam Balance	Beaker
Hot Plate/Stirrer	Graduated Cylinder
Magnetic Stir Bar	Weigh Boat
Test Tubes	Culture Tubes
Autoclave Tape	Kim-Kaps or screw caps

All media is dated when first opened and is used within the next six months. Information logged: expiration date of dried media, date of prepared media, preparer, media type, lot number, amount used and volume of water used for rehydration. Also recorded at time of sterilization is the time in/time out, psi, total time and temperature.

Media is added to the water, the mixture of water and media is magnetically stirred and heated. It is dispensed into properly cleaned fermentation test tubes with inverted culture vials. Dispense 10 mls into each test tube, loosely cap the tubes.

Sterilization

After rehydration and dispensing of medium, sterilize within two hours. Place in sterilizer along with Prospore vial. Sterilize in autoclave at 121 C for 12 to 15 minutes. When the pressure reaches zero in the autoclave, promptly remove the media from the sterilizer cool quickly in cool water bath to avoid decomposition. Sterilization time must be limited to a total time of 45 minutes. Mark the rack of prepared fermentation tubes with the date of preparation along with the expiration date.

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, Brillian 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 cultue 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.

Turbid Growth In absence of Gas/Acid Production in LTB-Presumptive Phase

If a water sample produces turbid growth in the absence of gas/acid production in the LTB-Presumptive Phase the following procedure is followed:

A confirmed test is conducted on turbid non-gas/acid producing LTB culture(s).
If sample is total coliform positive, MPN is reported. If total coliform negative the sample is invalidated and the site is resampled.

Quality Assurance

Innoculate one EC/ECMUG test tube with known positive negative checks with each presumptive test. If using ECMUG use *Klebsiella* for a positive/negative check.

Interpretation

Use the MPN index for five 20 ml sample portions for drinking water.
Use the MPN index for sample Dilutions.