MUG Brilliant Green Bile Broth

M1038

For detection of *Escherichia coli* in water and food samples by the fluorogenic assay procedure.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	10.000
Lactose	10.000
Oxgall	20.000
Brilliant green	0.0133
4-Methylumbelliferyl ß-D-Glucuronide (MUG)	0.050
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.1 grams in 1000 ml distilled water. Heat if necessary to ensure completely solution. Dispense 10 ml amounts in test tubes containing inverted Durham's tubes. Sterilize by autoclaving at 15 lbs pressure121°C) for 15 minutes.

For testing larger quantities of sample prepare concentrated medium to accommodate volume of the test sample.

Principle And Interpretation

Brilliant Green Bile Broth is one of the most widely used medium for the detection of coliform bacteria in water, wastewater, foods, and milk and dairy products. This medium is formulated as per APHA (1, 2, 3) for the presumptive identification and confirmation of coliform bacteria (4, 5).

Pancreatic digest of gelatin serves as a source of essential nutrients. Lactose is the fermentable carbohydrate. Ox gall inhibits gram-positive bacteria whereas the gram-negative bacteria are inhibited by brilliant green. Production of gas from lactose fermentation is detected by incorporating inverted Durham's tube, which indicates the positive evidence of faecal coliform since non faecal coliforms growing in this medium do not produce gas. Gram-positive spore formers may produce gas if the bile or brilliant green inhibition is weakened by reaction with food material. The fluorogenic compound, MUG (4-Methylumbelliferyl-β-D-glucuronide) in the medium permits the rapid detection of *E.coli* which produces a blue fluorescence when hydrolyzed by the enzyme β-glucuronidase and is observed using a long-wave UV light source.

During examination of water samples, growth from presumptive positive tubes showing gas in Lactose Broth (M026) or Lauryl Tryptose Broth (M080) is inoculated in Brilliant Green Bile Broth 2% (M121). Gas formation within 48 ± 2 hours confirms the presumptive test (1).

Quality Control

Appearance
Light yellow to light green homogeneous free flowing powder

Colour and Clarity of prepared medium
Emerald green coloured clear solution

Reaction
Reaction of 4.01% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M1038: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Gas	Fluorescence (at 366 nm)
Cultural Response				

Escherichia coli ATCC 25922	50-100	luxuriant	Positive	Positive (by adding 0.2N NaOH)
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	Positive	Negative
Enterococcus faecalis ATCC 29212	50-100	none-poor	Negative	Negative
Staphylococcus aureus ATCC 25923	>=10^3	inhibited		

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1.Greenberg A. E., Eaton A. D. and Clesceri L. S., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C.
2.Downes F. P. and Ito K. (Eds.) 2001, Compendium of Methods for the Microbiological Examination of Food. 4th Ed, APHA, Washington, D.C.
3.Richardson G., (Ed.), 1985, Standard Methods for the Examination of Dairy Products, 15th Ed, APHA, Washington, D.C.
4.McCrady and Langerin, 1932, J. Dairy Science, 15:321.
5.McCrady, 1937, Am. J. Publ. Health, 27:1243.

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