MUG Brilliant Green Bile Broth

M1038

Intended Use:

Recommended for detection of *Escherichia coli* in water and food samples by a fluorogenic assay method.

Composition**

Ingredients	Gms / Litre
Gelatin peptone	10.000
Lactose	10.000
Bile □	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

□ Equivalent to Oxgall

Directions

Suspend 40.1 grams in 1000 ml purified/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, 6,7) for the presumptive identification and confirmation of coliform bacteria (4, 5).

Gelatin peptone serves as a source of essential nutrients. Lactose is the fermentable carbohydrate. Bile 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).

Type of specimen

Food and dairysamples; Water samples

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

Organism	Inoculum (CFU)	Growth	Gas	Fluorescence (at 366 nm)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	Positive	Positive (by adding 0.2N NaOH)
#*Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	Positive	Negative
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	Negative	Negative
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited		

Key : *Corresponding WDCM numbers. # Formerly known as *Enterobacter aerogens*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

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3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. McCrady and Langerin, 1932, J. Dairy Science, 15:321.
5. McCrady, 1937, Am. J. Publ. Health, 27:1243.
6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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