Brilliant Green Bile Broth 2%

M121

Intended Use:

Recommended for detection and confirmation of coliform bacteria in water, waste water, food, milk and dairy products.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Lactose	10.000
Bile#	20.000
Brilliant green	0.0133
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Oxgall

Directions

Suspend 40.01 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Distribute in fermentation tubes containing inverted Durhams tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. For preparation of double strength it is recommended to heat the dissolved broth (80.02 grams per litre) at 100°C for 30 minutes.

Principle And Interpretation

Brilliant Green Bile Broth 2% 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). This medium is also recommended by the ISO Committee for enumeration of coliforms by most probable number technique (6).

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. Further gas production in EC broth (M127) at 45°C used as a confirmation of faecal coliform. Gram-positive spore formers may produce gas if the bile or brilliant green inhibition is weakened by reaction with food material.

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 dairy samples; Water samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,2,8).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3)
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. Do not autoclave double-strength broth.
2. Gram-positive sporing organisms may produce gas if the bile/brilliant green inhibition is attenuated by food material.

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

Cream to pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate.

Reaction

Reaction of 4.0% 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 18-48 hours.

Organism	Inoculum (CFU)	Growth	Gas
Bacillus cereus ATCC 10876	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
Enterobacter aerogenes ATCC 13048	50-100	good-luxuriant	positive reaction
Enterococcus faecalis ATCC 29212	50-100	none-poor	negative reaction
Staphylococcus aureus ATCC 25923	>=10^4	inhibited	

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.
6. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.

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