



## Brilliant Green Bile Broth 2%

M121

Brilliant Green Bile Broth 2% is recommended for the detection and confirmation of coliform bacteria in water, wastewater, foods, milk and dairy products.

### Composition\*\*

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Lactose	10.000
Oxgall	20.000
Brilliant green	0.0133
Final pH ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 40.01 grams in 1000 ml 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. Do not autoclave double strength broth.

### 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).

Peptic digest of animal tissue 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. 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  $48 \pm 2$  hours confirms the presumptive test (1).

### 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.

#### Cultural Response

Organism	Inoculum (CFU)	Growth	Gas
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#### Cultural Response

<i>Bacillus cereus</i> ATCC 10876	$\geq 10^3$	inhibited	
<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	positive reaction
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	good-luxuriant	positive reaction
<i>Enterococcus faecalis</i> ATCC 29212	50-100	none-poor	negative reaction
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	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.
6. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.

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