



Brilliant Green Bile Broth

M121I

Brilliant Green Bile Broth is recommended for the confirmation of coliform bacteria present in foods and environmental samples in the area of food production and food handling.

Composition**

| Ingredients | Gms / Litre |
|----------------------------|-------------|
| Enzymatic digest of casein | 10.000 |
| Lactose monohydrate | 10.000 |
| Dehydrated oxbile | 20.000 |
| Brilliant green | 0.0133 |
| Final pH (at 25°C) | 7.2±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.51 grams (the equivalent weight of dehydrated medium per liter) in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense the medium in quantities of 10ml in test tubes of approximately 16mm x 160mm containing Durham tubes. Sterilize in an autoclave set at 121°C for 15 minutes.

Note: The Durham tube shall not contain air bubbles after sterilization.

Principle And Interpretation

Brilliant Green Bile Broth is formulated as per ISO 4831:2006(E) for confirmation of coliform bacteria (1) present in food samples or environmental samples in the area of food handling or food sampling.

Brilliant green and oxgall present in the medium inhibit gram-positive bacteria including lactose fermenting *Clostridia* (2). Production of gas from lactose fermentation is detected by incorporating inverted Durham's tube, indicates a positive evidence of faecal coliforms since nonfaecal coliforms growing in this medium do not produce gas.

During examination of food samples or environmental samples, growth from presumptive positive tubes showing gas in Lauryl Tryptose Broth (M080) is inoculated in Brilliant Green Bile Broth wherein gas formation within 48 ± 2 hours confirms the presumptive test (1). Gram-positive spore-formers may produce gas if the bile or brilliant green inhibition is weakened by food material.

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 3.95% 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 |
|------------------------------------|----------------|----------------|-------------------|
| 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.International Standard, ISO 44831:2006 (E). Microbiology of food and animal feeding stuff- Horizontal method for the detection and enumeration of coliforms- Most Probable number technique.
- 2.McCrady and Langerin, 1932, J. Dairy Science, 15:321.

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