Brilliant Green Bile Broth

M121I

Intended Use:

Recommended for isolation and cultivation of coliform organisms from cream, yogurt and raw milk. The composition and performance criteria of this medium are as per the specifications laid down in ISO 4831:2006, ISO 11133:2014 & Amd.2 :2020 (E).

Composition**

ISO Specifications : BGBLB

Ingredients	g / L
Enzymatic digest of casein	10.000
Lactose	10.000
Dehydrated Ox bile	20.000
Brilliant green	0.0133
Final pH (at 25°C)	7.2±0.2

Brilliant Green Bile Broth **M121I**

Ingredients	g / L
Tryptone$	10.000
Lactose monohydrate	10.000
Dehydrated 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 Enzymatic digest of casein

Directions

Suspend 39.51 gram (the equivalent weight of dehydrated medium per liter) in 1000 ml purified / 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. Cool to 45-50°C. *Note: The Durham tube shall not contain air bubbles after sterilization.*

Principle And Interpretation

Brilliant Green Bile Broth is formulated as per ISO for confirmation of coliform bacteria (1,2) present in food samples or environmental samples in the area of food handling or food sampling.

Brilliant green and dehydrated bile present in the medium inhibit gram-positive bacteria including lactose fermenting *Clostridia* (3). 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 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.

Type of specimen

Food samples

Specimen Collection and Handling:

ISO 4831:2006 (1,2)

Depending on the limit of detection that is required, x ml of the test sample if liquid, or x ml of the initial suspension in the case of other products, is transferred to a tube containing 10 ml of double-strength selective enrichment medium. Incubate at 30°C or 37°C (as agreed) for 24 h ± 2 h, continue incubation for another 24 h ± 2 h for gas formation. Gas formation is considered as positive.

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further biochemical & serological identification is necessary for confirmation.

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 3.95% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Productivity : Cultural characteristics observed after an incubation at 30±1°C for 24±2h to 48±2h.

Selectivity : Cultural characteristics observed after an incubation at 30±1°C for 24±2h to 48±2h.

Organism	Inoculum (CFU)	Growth	Gas
Productivity			
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
Escherichia coli ATCC 8739 (00012*)	50-100	good-luxuriant	positive reaction
Citrobacter freundii ATCC 43864 (00006*)	50-100	good-luxuriant	positive reaction
Selectivity			
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	negative reaction
Enterococcus faecalis ATCC 19433 (00009*)	50-100	none-poor	negative reaction

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 (4,5).

Reference

1. International Standard, ISO 4831:2006 (E). Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique.

2. Microbiology of food, animal feeding stuffs and water- Preparation, production,storage and performance testing of culture media, EN ISO 11133:2014(E) /Amd.: 2020 .

3. McCrady and Langerin, 1932, J. Dairy Science, 15:321.

4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

5. 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.

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