Technical Information

Brilliant Green Bile Agar

Product Code: DM 1059

Application: Brilliant Green Bile Agar is recommended for enumeration of coliform bacteria in water and wastewater.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	8.250
Lactose	1.900
Sodium sulphite	0.205
Ferric chloride	0.0295
Monopotassium phosphate	0.0153
Erioglaucine	0.0649
Basic fuchsin	0.0776
Oxgall	0.00295
Brilliant green	0.0000295
Agar	10. 150
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Brilliant Green Bile Agar was initially formulated by Nobel and Ponney [1] for enumeration of coliform bacteria from materials of sanitary importance. Subsequently APHA approved the medium for the estimation of coliforms in test samples of various materials [2, 3].

The medium contains a combination of brilliant green and oxgall, which is highly selective for coliforms, inhibiting the growth of most of the gram-positive bacteria including lactose fermenting Clostridia [4] and some gram-negative bacteria. Erioglaucine and basic fuchsin together form the indicator system of the medium. When the pH is neutral, colour of the medium is blue while acid production from lactose turns the medium pink and colonies appear pink to deep red depending on the pH change. Colonies of coliform bacteria are deep red surrounded by a pink halo against blue background of the medium. It is recommended that the medium be prepared just prior to use and if the medium has to be stored, it should be kept in dark. Brilliant Green Bile Agar medium is sensitive to light, particularly direct sunlight. Direct exposure may exhibit a decrease in the productivity of the medium and also the colour of the medium may change from deep blue to purple or red.

Methodology

Suspend 20.7 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates. For plating 10 ml quantities of water samples, prepare the medium in double strength.Caution: Basic fuchsin is a potential carcinogen and care should be taken to avoid inhalation of the powdered dye and contamination of the skin.

Quality Control

Physical Appearance

Pinkish purple to light purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

Bluish purple coloured, slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 2.07% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH range

6.70-7.10

Cultural Response/Characteristics

DM1059: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colony of colony
Escherichia coli ATCC 25922	50-100	Good-Luxuriant	>=50%	deep red (may have bile precipitate)
Enterobacter aerogenes ATCC 13048	50-100	Good-Luxuriant	>=50%	Pink
Salmonella Enteritidis ATCC13076	>=10^3	Good-Luxuriant	>=50%	colourless to light pink
Staphylococcus aureus ATCC 25923	50-100	inhibited	0%	

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Noble and Tonney, 1935, J. Am. Waterworks Assoc., 27:108.
2. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
3. 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.
4. McCrady and Langerin, 1932, J. Dairy Science, 15:32 1

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