



## Brilliant Green Bile Agar

M059

### Intended use

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

### Composition\*\*

#### Ingredients

#### Gms / Litre

Peptone	8.250
Lactose	1.900
Sodium sulphite	0.205
Ferric chloride	0.0295
Monopotassium phosphate	0.0153
Erioglaucine	0.0649
Basic fuchsin	0.0776
Bile*	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

### Directions

Suspend 20.7 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. 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 plating 10 ml quantities of water samples, prepare the medium in double strength.

### Principle And Interpretation

Brilliant Green Bile Agar was originally 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 bile, which is highly selective for coliforms, inhibiting 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.

### Type of specimen

Water samples

### Specimen Collection and Handling

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 clinical specimens. Safety guidelines may be referred in individual safety data sheets

## Limitations :

1.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.

## 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

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

6.70-7.10

### Cultural Response

M059: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<b>Cultural Response</b>				
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	good-luxuriant	≥50%	deep red (may have bile precipitate)
<i>Enterobacter aerogenes</i> ATCC 13048 (00175*)	50-100	good-luxuriant	≥50%	pink
<i>Salmonella Enteritidis</i> ATCC 13076 (00030*)	50-100	good-luxuriant	≥50%	colourless to light pink
<i>Staphylococcus aureus</i> ATCC 25923 (00034*)	≥10 <sup>3</sup>	inhibited	0%	

Key : \*Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. Use before expiry date on the label.

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

## Reference

1. Noble and Tonney, 1935, J. Am. Waterworks Assoc., 27:108.
2. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
3. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
4. McCrady and Langerin, 1932, J. Dairy Science, 15:321
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2<sup>nd</sup> Edition.
6. 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.

**Disclaimer :**

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