BRILLIANT GREEN BILE AGAR (BRILLIANT GREEN LACTOSE BILE AGAR) TM 048

For enumeration of coliform bacteria in water and foods

Composition

Ingredients	Gms/Ltr.
Agar	10.15
Peptic digest of animal tissue	8.25
Lactose	1.90
Sodium sulphite	0.205
Ferric chloride	0.0295
Monopotassium phosphate	0.0153
Erioglaucine	0.0649
Basic fuchsin	0.0776
Brilliant green	0.0295
Oxbile	0.00295

* Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight.

Instructions for Use

Dissolve 20.70gms in 1000ml distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Allow to stand for 5-10 minutes so that the agar hydrates correctly. Sterilize by autoclaving at 15 psi (121°C) for 10 minutes. Cool to 45 - 50°C and pour into sterile Petri plates.

Appearance: Blue to purple colour, clear slightly opalescent gel
pH at 25°C: 6.9 ± 0.2

Principle

BRILLIANT GREEN BILE AGAR is used for enumeration of coliform bacteria in water and foods. It uses Basic fuchsin to differentiate between positive lactose-fermenting and lactose-non-fermenting bacteria. The Peptic digest of animal tissue provides the essential nutrients for growth: nitrogen, vitamins, minerals and amino acids. Lactose is the fermentable carbohydrate providing carbon and energy. Ox bile and Brilliant green inhibit Gram-positive bacteria and most Gram-negative bacteria except Coliforms. Acetaldehyde production by lactose-fermenting organisms, such as *E.coli,* produce characteristic red colonies with a pink surrounding area, marked by its reaction with Sodium sulphite in the presence of Basic fuchsin, a pH indicator. Lactose non-fermenters form colorless, transparent colonies. For the enumeration of coliform bacteria employ sample dilutions, which yield between 10 - 50 colonies per plate using the pour plate method. Therefore, several dilutions should be made in the melted medium, poured and once gelled, incubated at 35 ± 2°C for 18 - 24 hours. The coliform colonies have an intensely red center zone surrounded by a pink halo sharply outlined against the uniformly blue background of the medium. *Salmonella* spp. which do not ferment Lactose, produce colorless to pale pink colonies. The medium is sensitive to light, which reduces its effectiveness and changes its color from strong blue to purple or pink. The medium should be prepared immediately before use and, if necessary, stored in the dark for as little time as possible.

Interpretation

Cultural characteristics observed after inoculation (10^3CFU / ml), on incubation at temperature of 35ºC ± 2ºC for 18 - 24 hours.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth	Appearance of colony
Escherichia coli	25922	10^3	Luxuriant	Deep red with bile precipitate
Salmonella enteritidis	13076	10^3	Luxuriant	Colorless to pale pink
Enterobacter aerogenes	13048	10^3	Luxuriant	Pink
Staphylococcus aureus	25923	10^3	Marked to complete Inhibition	-

References

1. Methods for the Examination of Water and Wastewater, 10th Ed APHA, Inc. New York. (1958).
2. Recommended Methods for the Microbiological Examination of Foods, APHA, Inc. New York. (1958).