Brilliant Green Agar Medium

MU016

Brilliant Green Agar Medium is used for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces, foods, dairy products etc. in accordance with United States Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Tryptone	5.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	20.000
pH after sterilization (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58.09 grams in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Brilliant Green Agar medium is recommended as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et al as medium for differentiation of paratyphoid B from other Gram negative enteric bacteria (1). Kauffmann further modified it for isolation of *Salmonella* from stool samples (2). Brilliant green agar is also recommended by APHA (3,4) FDA (5) and is in accordance with United States Pharmacopoeia (6). This medium is employed in testing clinical specimens. Heavy inocula and heavily contaminated samples can be analyzed due to the outstanding selectivity of this medium. Brilliant Green Agar is used in the microbial limits test and with novobiocin for testing food and pharmaceutical products.

Combination of peptone, tryptone and yeast extract makes the medium highly nutritious and supplies amino acids and long chains of peptides. Sodium chloride maintains the osmotic equilibrium. Lactose and sucrose are the fermentable carbohydrate sources. Phenol red serves as an acid base indicator giving yellow colour to lactose and or sucrose fermenting bacteria. This medium also contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive, bacteria. *Salmonella* Typhi, *Shigella* species, *Escherichia coli*, *Proteus* species, *Pseudomonas* species, *Staphylococcus aureus* are mostly inhibited.

However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite or Tetrathionate Broth are plated on Brilliant Green Agar along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar. Non-lactose fermenting bacteria develop white to pinkish red colonies within 18-24 hours of incubation. *Salmonella* typhi and *Shigella* species may not grow on this medium, moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish brown clear to slightly opalescent gel forms in Petri plates

pH
6.70-7.10

Growth Promotion Test
Growth Promotion was observed in accordance with USP.

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation temperature
Growth Promotion Test						
Salmonella Typhimurium ATCC 14028	50 -100	good-luxuriant	25 -100	>=50 %	pinkish white	24 -48 hrs
Salmonella Abony NCTC 6017	50 -100	good-luxuriant	25 -100	>=50 %	pinkish white	24 -48 hrs
Additional Microbiological testing						
Salmonella Enteritidis ATCC 13076	50 -100	Good-luxuriant	25 -100	>=50 %	pinkish white	24 -48 hrs
Salmonella Typhi ATCC 6539	50 -100	poor-good	15 -40	30 -40 %	reddish pink	24 -48 hrs
Escherichia coli ATCC 25922	50 -100	none-poor	0 -10	0 -10 %	yellowish green	24 -48 hrs
Escherichia coli ATCC 8739	50 -100	none-poor	0 -10	0 -10 %	yellowish green	24 -48 hrs
Escherichia coli NCTC 9002	50 -100	none-poor	0 -10	0 -10 %	yellowish green	24 -48 hrs
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0	0%		24 -48 hrs
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0	0%		24 -48 hrs

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium between 2-8°C. Use before expiry date on the label.

Reference
1. Kristensen M., Lester V, and Jurgens A., 1925, Brit.J.Exp.Pathol.,6:291.
2. Kauffman F., 1935, Seit F. Hyg. 177:26
3. Downes F P and Ito K. (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
4. Standard Methods for the Microbiological Examination of Dairy Products, 1995, 19th Ed, APHA, Washington, D.C.
5. Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
6. The United States Pharmacopoeia, 2009. USP Conv. Rockville, MD.

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