



Medium 16. Brilliant green Agar

MM016

Medium 16.Brilliant Green Agar is used for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces, foods, dairy products etc. in accordance with Indian Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	12.000
pH after sterilization	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.09 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 or as per validated cycle. AVOID OVERHEATING. Cool to 50°C. Mix well before pouring 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 Indian 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 medium is used in the microbial limits test and with novobiocin for testing food and pharmaceutical products.

Peptone 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.

Being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. Cultures are enriched in Selenite F-Broth or Tetrathionate Bile Brilliant green broth and plated on atleast two of the following selective media Brilliant Green Agar, Bismuth Sulphite Agar, Xylose Lysine Deoxycholate Agar and Deoxycholate Citrate Agar. *Salmonella* typhi and *Shigella* species may not grow on this medium, moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens.

Quality Control

Appearance

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% agar gel.

Colour and Clarity of prepared medium

Greenish brown coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.01% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural response

Cultural response was carried out as per IP and was observed after an incubation at 30-35°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	Lot value (CFU)	Recovery	Colour of Colony
<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	good-luxuriant	25 -100	≥50 %	pinkish white
<i>Salmonella Abony</i> NCTC 6017	50 -100	good-luxuriant	25 -100	≥50 %	pinkish white
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	luxuriant	25 -100	≥50 %	pinkish white
<i>Salmonella Typhi</i> ATCC 6539	50 -100	fair-good	15 -40	30 -40 %	reddish pink
<i>Escherichia coli</i> ATCC 25922	50 -100	none-poor	0 -10	0 -10 %	yellowish green
<i>Escherichia coli</i> ATCC 8739	50 -100	none-poor	0 -10	0 -10 %	yellowish green
<i>Escherichia coli</i> NCTC 9002	50 -100	none-poor	0 -10	0 -10 %	yellowish green
<i>Staphylococcus aureus</i> ATCC 25923	≥10 ³	inhibited	0	0%	
<i>Staphylococcus aureus</i> ATCC 6538	≥10 ³	inhibited	0	0%	

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

- Kristensen M., Lester V, and Jurgens A., 1925, Brit.J.Exp. Pathol.,6:291.
- Kauffman F., 1935, Seit F. Hyg. 177:26
- Downes F P and Ito K. (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
- Standard Methods for the Microbiological Examination of Dairy Products, 1995, 19th Ed, APHA, Washington, D.C.
- Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
- The Indian Pharmacopoeia 1985, Government of India , Ministry of Health and Family Welfare, New Delhi.

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