

Brilliant Green Agar Medium 16 (In accordance with IP 2007)

MM016

Intended Use:

Recommended for selective isolation of Salmonellae other than *Salmonella typhi* from faeces, food, dairy products etc. in accordance with IP 2007.

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 45-50°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 carbon and nitrogen compounds, 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 Nutrient Broth (MM244) and further transferred to Detective Broth in Selenite F-Broth (MM052) or Tetrathionate Bile Brilliant green broth (MM1255) and plated on atleast two of the following selective media Brilliant Green Agar (MM016), Bismuth Sulphite Agar (MM027), Xylose Lysine Deoxycholate Agar (MM031) and Deoxycholate Citrate Agar (MM065). *Salmonella typhi* and *Shigella* species may not grow on this medium, moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens.

Type of specimen

Foodstuffs & dairy samples; Water samples; Pharmaceutical samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (7). 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. Though this medium is selective for *Salmonella* other species of *Enterobacteriaceae* may grow.
2. *Salmonella* Typhi and Shigella species may not grow on this medium.
3. Moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.
4. Further confirmation has to be carried out on presumptive *Salmonella* isolates.

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

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

pH

6.70-7.10

Cultural response

Cultural response was carried out as per IP and was observed after an incubation at 36-38°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of Colony
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	good-luxuriant	25 -100	>=50 %	pinkish white
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	good-luxuriant	25 -100	>=50 %	pinkish white
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	25 -100	>=50 %	pinkish white
<i>Salmonella</i> Typhi ATCC 6539	50 -100	fair-good	15 -40	30 -40 %	reddish pink
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	none-poor	0 -10	0 -10 %	yellowish green
<i>Escherichia coli</i> ATCC 8739 (00012*)	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> subsp. <i>aureus</i> ATCC 25923 (00034*)	>=10 ³	inhibited	0	0%	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	>=10 ³	inhibited	0	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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. 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 (8,9).

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. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, 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 Indian Pharmacopoeia 2007 Government of India , Ministry of Health and Family Welfare, New Delhi.
7. 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.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. 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.

Revision : 04/2023

Disclaimer :

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