Brilliant Green Agar Base, Modified

M016

Intended Use:

Brilliant Green Agar (Modified) is used for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces and other materials.

Composition**

Ingredients	Gms / Litre
Proteose 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	20.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29 grams in 500 ml 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. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (FD068). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Salmonella species cause many types of infections, from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days.Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et. al. (1) and further modified by Kauffmann (2). Brilliant Green Agar is also recommended by APHA (3,4) FDA (5) and described in EP, BP and IP (6,7,8).

This medium contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria. *Salmonella* Typhi, *Shigella* species *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus* are mostly inhibited. Clinical specimens can be directly plated on this medium. 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 is plated on Brilliant Green Agar along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar.

The medium contains proteose peptone and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. The two sugars namely lactose and sucrose serve as energy sources. Fermentation of lactose and/or sucrose in the medium results in the formation of acidic pH which is detected by phenol red indicator. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further supplemented with sulphacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with Salmonella species.

Non-lactose fermenting bacteria develop white to pinkish red colonies within 18 - 24 hours of incubation.

Type of specimen

Clinical : faeces ; Foodstuffs ; Water samples ; Pharmaceutical samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (12,13).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelin,es (3,9,11).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(10).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. 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

Performace 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 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish brown clear to slightly opalescent gel forms in Petriplates

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural response was carried out 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	Recovery	Colour of Colony
Cultural Response				
Escherichia coli ATCC 25922	50 -100	none-poor	0 -10 %	yellowish green
Escherichia coli ATCC 8739	50 -100	none-poor	0 -10 %	yellowish green
Escherichia coli NCTC 9002	50 -100	none-poor	0 -10 %	yellowish green
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0%	
Salmonella Typhi ATCC 6539	50 -100	fair-good	30 -40 %	reddish pink
Salmonella Typhimurium ATCC 14028	50-100	good-luxuriant	>=50 %	pinkish white
Salmonella Enteritidis ATCC 13076	50 -100	luxuriant	>=50 %	pinkish white
Salmonella Abony NCTC 6017	50-100	good-luxuriant	>=50 %	pinkish white

Storage and Shelf Life

Store below 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 (12,13)

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. (Ed), 2001, Compendium of Methods for 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, 5th Ed, 1978, AOAC, Washington D.C.
6. The European Pharmacopoeia, 2008, Council or Europe, Strasbourg.
7. The British Pharmacopoeia, 2007 vol. II, London.
8. Indian Pharmacopoeia, 2007, Ministry of Health and Family Welfare, Govt., of India,
9. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
11. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
12. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.
13. 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.

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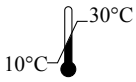
IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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