HARMONIZED HIMEDIA®

Technical Data

Brilliant Green, Phenol Red, Lactose Monohydrate, Sucrose Agar (Agar medium L)

M016B

Intended use

Brilliant Green, Phenol Red, Lactose Monohydrate, Sucrose Agar is used for selective isolation of *Salmonellae* other than *Salmonella Typhi* from faeces, foods, dairy products etc in accordance with British Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
HMC peptone ~	10.000
Yeast extract	3.000
Lactose monohydrate	10.000
Sucrose	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	20.000
pH after sterilization	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

~ - Equivalent to Peptone (meat or casein)

Directions

Suspend 57.59 grams (the equivalent weight of dehydrated medium per litre) 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.

Principle And Interpretation

The composition of medium is as per British Pharmacopoeia and is cited as Agar medium L (1). Brilliant Green, Phenol Red, Lactose monohydrate, Sucrose Agar is used 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 (2). It was further modified by Kauffmann for isolation of *Salmonella* from stool samples (3). Brilliant green agar is also recommended by APHA (4,5) FDA (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 samples.

HMC peptone and yeast extract supplies essential amino acids and long chains of peptides for enhanced growth. Sodium chloride maintains the osmotic equilibrium. Lactose monohydrate 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, and *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.

Type of specimen

Food and dairy samples ; Pharmaceutical samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5,6). For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1-*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.
2. Further biochemical tests must be carried out for confirmation.

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 2.0% 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
Growth Promotion Test
Growth Promotion is carried out in accordance with BP. Cultural response was observed after an incubation at 35-37°C for 18-72 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
Growth Promotion Test					
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	pinkish white
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	25 -100	>=50 %	pinkish white
Additional Microbiological testing					
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	25 -100	>=50 %	pinkish white
Salmonella Typhi ATCC 6539	50 -100	fair-good	15 -40	30 -40 %	reddish pink
Escherichia coli ATCC 8739 (00012*)	50 -100	none to poor	0 -10	0 -10 %	yellowish green
Escherichia coli ATCC 25922 (00013*)	50 -100	none-poor	0 -10	0 -10 %	yellowish green
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^3	inhibited	0	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=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 (7,8).

Reference

1.The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.

2.Kristensen M., Lester V, and Jurgens A., 1925, Brit.J. Exp.Pathol.,6:291.

3.Kauffman F., 1935, Seit F. Hyg. 177:26.

4.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.

5.Wehr H M and Frank J H., 2004, Standard Methods for the Examination of Dairy Products, 17th ed., APHA Inc., Washington, D.C.

6.FDA Bacteriological Analytical Manual, 2005, 18th ed., AOAC, Washington, DC.

7.Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 2nd Edition.

8.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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