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

ME016

Intended use

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

Composition**

Ingredients	g / L
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(at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters
~ - Equivalent to Peptones (meat and 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 15lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

The composition of medium is as per European Pharmacopoeia and is cited as Agar medium L (1). It can also be used for food samples (2) and clinical samples (3,4). 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 (5) It was further modified by Kauffmann for isolation of *Salmonella* from stool samples (6). Brilliant green agar is also recommended by APHA (7,8) FDA (2). 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.

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.

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, and 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.

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 (2,7,8). 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.

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 clear to slightly opalescent gel forms in Petri plates

Reaction

After sterilization,reaction of 5.8% w/v aqueous solution. pH : 6.9±0.2

pH

6.70-7.10

Growth Promotion Test

Growth Promotion was observed in accordance with EP, 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

Cultural characteristics observed after incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Growth Promoting				
Salmonella Typhimurium ATCC 14028	50 -100	good-luxuriant	>=50 %	pinkish white
Salmonella Abony NCTC 6017	50 -100	good-luxuriant	>=50 %	pinkish white
Additional Microbiological testing				
Salmonella Enteritidis ATCC 13076	50 -100	luxuriant	>=50 %	pinkish white
Salmonella Typhi ATCC 6539	50 -100	fair-good	30 -40 %	reddish pink
Escherichia coli ATCC 25922	50 -100	none-poor	0 -10 %	yellowish green
Escherichia coli ATCC 8739	50 -100	none-poor	0 -10 %	yellowish green
Staphylococcus aureus ATCC 25923	>=10^4	inhibited	0%	
Staphylococcus aureus ATCC 6538	>=10^4	inhibited	0%	

Storage and Shelf Life

Store between 10-30°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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
2. FDA Bacteriological Analytical Manual, 2005, 18th ed., AOAC, Washington, DC.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. 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.
5. Kristensen M., Lester V, and Jurgens A., 1925, Brit.J.Exp.Pathol.,6:291.
6. Kauffman F., 1935, Seit F. Hyg. 177:26.
7. 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.
8. Wehr H M and Frank J H., 2004, Standard Methods for the Examination of Dairy Products, 17th ed., APHA Inc., Washington, D.C.

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