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

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**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMC peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>10.000</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.080</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.0125</td>
</tr>
<tr>
<td>Agar</td>
<td>20.000</td>
</tr>
</tbody>
</table>

After sterilization, pH is 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.

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 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**

Clinical samples - Blood; Food and dairy samples; Pharmaceutical samples

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).
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:
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:
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 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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Lot value (CFU)</th>
<th>Recovery</th>
<th>Colour of Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Promotion Test Salmonella Typhimurium ATCC 14028 (00031*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>pinkish white</td>
</tr>
<tr>
<td>Salmonella Abony NCTC 6017 (00029*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>pinkish white</td>
</tr>
<tr>
<td>Additional Microbiological testing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella Enteritidis ATCC50 -100 13076 (00030*)</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>pinkish white</td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 6539</td>
<td>50 -100</td>
<td>fair-good</td>
<td>15 -40</td>
<td>30 -40 %</td>
<td>reddish pink</td>
</tr>
<tr>
<td>Escherichia coli ATCC 8739 50 -100 (00012*)</td>
<td>none to poor</td>
<td>0 -10</td>
<td>0 -10 %</td>
<td>yellowish green</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>none-poor</td>
<td>0 -10</td>
<td>0 -10 %</td>
<td>yellowish green</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0</td>
<td>0 %</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0</td>
<td>0 %</td>
<td></td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

Please refer disclaimer Overleaf.
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 inorder 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 (7,8).

Reference

Revision : 02/ 2018

In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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