**Bromothymol Lactose Blue Agar**

**Intended Use:**
It is a selective medium used for the isolation of Gram-negative bacteria from urine & faeces.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM extract#</td>
<td>3.000</td>
</tr>
<tr>
<td>Fish peptone</td>
<td>3.000</td>
</tr>
<tr>
<td>Peptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>7.500</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium lauryl sulphate (SLS)</td>
<td>0.150</td>
</tr>
<tr>
<td>Lactose</td>
<td>19.000</td>
</tr>
<tr>
<td>Bromo thymol blue</td>
<td>0.083</td>
</tr>
<tr>
<td>Agar</td>
<td>19.000</td>
</tr>
</tbody>
</table>

Final pH (at 25°C) 7.4±0.2

**Directions**

Suspend 73.73 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Reactions with lactose are of great practical importance for the primary isolation of Enterobacteria from clinical specimens. The specimens e.g. faeces is usually plated on a lactose-containing medium on which lactose fermenters and lactose non fermenters form coloured and pale colonies respectively due to the dye incorporated. This procedure makes an immediate presumptive distinction between colonies of the true intestinal pathogens possible. Salmonella and Shigella, do not ferment lactose while the common intestinal commensals, Escherichia and Klebsiella, which do ferment lactose (1). Bromothymol Lactose Blue Agar is used for differentiating lactose fermenting and non-fermenting bacteria belonging to the family Enterobacteriaceae.

HM extract, fish peptone and peptone provides carbon, nitrogen compounds, long chain amino acids, vitamins and other essential nutrients for bacterial metabolism. Lactose provides a fermentable carbohydrate source for the enteric bacteria. Bromo thymol blue is the pH indicator for indicating acid production due to carbohydrate fermentation. The dye turns yellow at acidic pH and imparts yellow colour to the colony. Alkalinization produces a blue coloration. Sodium Lauryl sulphate inhibits gram positive organisms. Sodium chloride maintains osmotic balance.

**Type of specimen**
Clinical samples - Faeces, urine.

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3). 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/protection 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.

Please refer disclaimer Overleaf.
Limitations
1. Further biochemical and serological tests must be carried out for complete identification.

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
Cream to greenish yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.9% Agar gel

Colour and Clarity of prepared medium
Greenish blue coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 7.27% w/v aqueous solution at 25°C. pH : 7.4±0.2

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>yellow</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 25923</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em> ATCC 6539</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>blue/colourless</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td>-</td>
</tr>
</tbody>
</table>

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. 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 (2,3).

Reference
Disclaimer:

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.