Litmus Lactose Bile Salt Agar (LLBSA)

**Internal Use:**
Recommended for selective isolation of enteric bacteria on the basis of lactose fermentation from clinical and non-clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Sodium taurocholate</td>
<td>5.000</td>
</tr>
<tr>
<td>HM peptone B #</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>20.000</td>
</tr>
<tr>
<td>Litmus</td>
<td>0.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 70.5 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**
Numerous plating media are in use today for the differentiation of lactose-fermenters and lactose non-fermenters. Some of these are selective, whereas others are differential. Some lactose fermenting, gram-negative enteric bacteria can tolerate the inhibitory substances present in the media. These bacteria can be recognized readily by their appearance on selective plates. Litmus Lactose Bile Salt Agar is a modification of Litmus Lactose Agar formulated by Wurtz (4) and is used for the isolation of enteric bacteria. It can be successfully used in place of MacConkey Agar.

LLBSA Medium contains sodium taurocholate, which inhibits the growth of gram-positive microorganisms. Lactose is the fermentable sugar utilized by coliform enteric bacteria leading to production of acid. Peptone and HM peptone B supply the essential nutrients like nitrogen compounds for the growth of enteric bacteria. Sodium chloride maintains the osmotic balance of the medium.

**Type of specimen**
Clinical samples- Faeces, Water samples

**Specimen Collection and Handling:**
For clinical samples, follow appropriate techniques for sample collection, processing as per guidelines.(2,3)
For water 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. 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. This medium is general purpose medium and may not support the growth of fastidious organisms.

**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 purple to greyish yellow homogeneous free flowing may contain minute to small particles.

Gelling
Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Light purple coloured slightly opalescent gel forms in Petri plates, may have black particles

Reaction
Reaction of 7.05 w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>red</td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 6539</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>deep blue to violet</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853 (00025*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)</td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis ATCC 25933</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>blue to violet</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212 (00087*)</td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</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. 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
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition

Revision : 01 / 2020

Please refer disclaimer Overleaf.
Disclaimer:

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