Tryptose Blood Agar Base

Intended Use:
Recommended for the isolation of fastidious organisms and determining the haemolytic reactions.

Composition

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>10.000</td>
</tr>
<tr>
<td>HM peptone B #</td>
<td>3.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH ( at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 33 grams in 950 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 the autoclaved medium to 45 - 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix thoroughly, avoiding air bubbles and pour into sterile Petri plates.

Principle And Interpretation
Tryptose Blood Agar Base is a tryptose based medium that can be used for the cultivation of fastidious organisms (2,3), on supplementation with blood. This medium is devoid of dextrose and therefore useful in determining the haemolytic reactions. Tryptose Blood Agar Base is recommended by FDA (4) and APHA (1). Tryptose Blood Agar Base can be used as a general-purpose medium without supplementation of blood. These media can be used to determine the haemolytic reactions of fastidious organisms. The four different types of haemolysis observed are as follows:

a) Alpha haemolysis: partial lysis of the erythrocytes surrounding colony, causing a grey green or brownish discolouration in the media.
b) Beta haemolysis: complete lysis of the red blood cells surrounding a colony, causing a clearing of blood from the medium.
c) Gamma haemolysis: no haemolysis and consequently, no colour change of the medium surrounding a colony. Organisms showing no haemolysis are generally termed non-haemolytic rather than gamma haemolytic.
d) Alpha-prime or wide zone alpha: a small zone of intact erythrocytes immediately adjacent to the colony, with a zone of complete red cell haemolysis surrounding the zone of intact erythrocytes. This type of haemolysis may be confused with beta haemolysis (7).

Tryptose and HM peptone B provide nitrogenous and carbonaceous compounds, sulphur, vitamin B complex and trace elements essential for bacterial metabolism. Blood provides additional nutrients and serves as a base to study haemolytic reactions. This medium not only keeps the blood cells in a good state but also help in forming distinct haemolysis. Perform biochemical test for further identification (8).

Type of specimen
Clinical samples: Blood, CSF.

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). 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 clinical specimens. Safety guidelines may be referred to in individual safety data sheets.

Please refer disclaimer Overleaf.
Limitations
1. Some fastidious organisms may not grow.
2. Inoculum density affects inhibition zone. Heavy inoculum may result in smaller zones while scanty growth may result in enlarged zones.

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 yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium : Yellow coloured clear to slightly opalescent gel forms in Petri plates. After addition of 5% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

Reaction
Reaction of 3.3% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed with added 5% v/v sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth w/o blood</th>
<th>Recovery w/o blood</th>
<th>Growth w/ blood</th>
<th>Haemolysis</th>
<th>Recovery w/ blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Neisseria meningitidis</em> ATCC 50-100</td>
<td>13090</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>luxuriant</td>
<td>none</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus</em> ATCC 25923</td>
<td>171x438 (00034*)</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>luxuriant</td>
<td>beta/gamma</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em> ATCC 12228 (00036*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=70%</td>
<td>luxuriant</td>
<td>gamma</td>
<td>&gt;=70%</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> ATCC 6303</td>
<td>50-100</td>
<td>fair-good</td>
<td>40-50%</td>
<td>good</td>
<td>alpha</td>
<td>50-70%</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> ATCC 19615</td>
<td>50-100</td>
<td>fair-good</td>
<td>40-50%</td>
<td>good</td>
<td>beta</td>
<td>50-70%</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 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).
Reference


Disclaimer:

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