Bile Esclulin Azide Agar, Modified

Intended Use
Recommended for rapid, selective detection and enumeration of Enterococci and Group D Streptococci.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>17.000</td>
</tr>
<tr>
<td>Peptone</td>
<td>3.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Bile #</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Esclulin</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>0.250</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Agar</td>
<td>13.500</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.1±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 56.25 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
Bile Esclulin Azide Agar, Modified was formulated by Isenberg et. al. (2) which is based on the original formula of Bile Esclulin Azide Agar formulated by Swan (8). Isenberg et. al. modified Bile Esclulin Azide Agar by reducing bile concentration from 40 to 10gm/l and added sodium azide.
Bile Esclulin Azide Agar, Modified is a highly nutritious media because of presence of casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract which serve as a source of carbon, nitrogen and essential nutrients. Sodium azide inhibits growth of gram-negative organisms and permits the cultivation of Enterococci and group D Streptococci. OXgall inhibits gram-positive bacteria other than Enterococci. Sodium citrate acts as a buffering agent. Esclulin is hydrolysed by Enterococci and group D streptococci to esculetin which reacts with ferric ammonium citrate to form dark brown or black complex (5). Bile tolerance of group D Streptococci permits its isolation and identification in 24-48 hours.

Type of specimen
Food samples; Water samples

Specimen Collection and Handling
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).
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.

Limitations
1. Excess inoculation may decreases the ability of the ox bile to inhibit growth of other gram-positive organisms that may hydrolyze esclulin.
2. Some streptococci that do not hydrolyze esculin but will grow in the presence of bile.

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.

Please refer disclaimer Overleaf.
Quality Control

Appearance
Light yellow to brownish yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.35% Agar gel

Colour and Clarity of prepared medium
Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates.

Reaction
Reaction of 5.62% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH
6.90-7.30

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>Esculin Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td></td>
<td>positive reaction, blackening of medium around the colony</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>negative reaction</td>
</tr>
<tr>
<td>Streptococcus pyogenes ATCC 19615</td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td>negative reaction</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>&gt;=10^4</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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

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.