Bile Esculin Agar Slant in Tubes

**Intended use**
Recommended for differential isolation and presumptive identification of group D Streptococci in food and pharmaceutical products.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>HM peptone B #</td>
<td>3.000</td>
</tr>
<tr>
<td>Bile □</td>
<td>40.000</td>
</tr>
<tr>
<td>Esculin</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>6.6±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

# Equivalent to Beef extract
□ Equivalent to Oxgall

**Directions**

Streak the test inoculum aseptically into the slant and incubate at appropriate conditions. Incubate the slants at 30-35°C for 18-24 hours.

**Principle And Interpretation**

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (8). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (10). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (9). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (12). Bile Esculin Agar was originally formulated by Swan (4) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (2,5) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non-Group D Streptococci.

Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera (11) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (3).

The medium is highly nutritious. Peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile inhibits most of the other accompanying bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridians Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (6). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/L horse serum (9). Inoculate and incubate the test sample in Todd Hewitt Broth (M313). After 24 hours incubation add two drops of the culture onto the surface of slant or plate media (3, 9).

**Type of specimen**

Food samples
**Specimen Collection and Handling**
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,13,14). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**
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**
Sterile Bile Esculin Agar slant in tubes.

**Colour medium**
Amber coloured medium with a bluish tinge

**Quantity of medium**
4ml of medium in glass tube

**Reaction**
6.40-6.80

**Sterility Test**
Passes release criteria

**Cultural Response**
Cultural characteristics observed in an increased atmosphere of Carbon dioxide after an incubation at 35-37°C for 18-24 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><strong>Enterococcus faecalis ATCC 50-100</strong> 29212 (00087*)</td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>positive reaction, blackening of medium around the colony negative reaction</td>
</tr>
<tr>
<td><strong>Proteus mirabilis ATCC 25933</strong></td>
<td>50-100</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>negative reaction</td>
</tr>
<tr>
<td><strong>Streptococcus pyogenes ATCC 19615</strong></td>
<td>50-100</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td>negative reaction</td>
</tr>
</tbody>
</table>

Key : *Corresponding WDCM numbers.

**Storage and Shelf Life**
On receipt store between 2-8°C 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 (6,7).

*Please refer disclaimer Overleaf.*
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