Edwards Medium Base, Modified is a selective medium for the rapid isolation of *Streptococcus agalactiae* and other streptococci associated with mastitis and also from other clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>10.000</td>
</tr>
<tr>
<td>Beef extract</td>
<td>10.000</td>
</tr>
<tr>
<td>Esculin</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.0013</td>
</tr>
<tr>
<td>Thallous sulphate</td>
<td>0.330</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

**Final pH (at 25°C)** 7.4±0.2

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

**Directions**

Suspend 41.33 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at D 115°C for 20 minutes. Cool to 50°C and aseptically add 5 to 7% v/v sterile sheep blood. Mix well and pour into sterile Petri plates.

(D corresponds to 10lbs pressure)

**Principle And Interpretation**

Streptococci are gram-positive facultatively anaerobic bacteria, which constitute normal commensal flora of mouth, skin, intestine and upper respiratory tract of humans. Group B Streptococci are an important cause of systemic infections in infants and occasionally of bacterial endocarditis (1). Mastitis is a disease of cattle caused by the organisms *Streptococcus agalactiae*. It belongs to the Lancefield group B Streptococci.

The most common selective agents used for selective isolation of Streptococci are crystal violet and thallium salts. A selective medium containing crystal violet was used by Haxthausen to isolate skin Streptococci (2). Subsequently it was observed that Streptococci from milk were able to grow on Gentian Violet Blood Agar whereas the other saprophytic milk bacteria were inhibited on this medium (3). An Esulin Blood Agar containing crystal violet was used by Edwards to isolate the causative agent of mastitis (4). A similar medium containing thallous acetate was also used to isolate the causative agent of mastitis (5).

Peptic digest of animal tissue and beef extract serve as sources of carbon, nitrogen and other essential nutrients. Esculin helps to differentiate esculin-positive (group D Streptococci) organisms from esculin-negative (*S. agalactiae*) organisms. Sodium chloride helps to maintain the osmotic equilibrium of the medium. Crystal violet and thallous sulphate serve as the selective agents for Streptococci. Supplementation with blood provides additional nutrients in addition to serving as an indicator of haemolysis. Mastitis Streptococci show alpha, beta or gamma type of haemolysis. Esulin differentiates esculin-positive group D Streptococci (black colonies) from esculin-negative *Streptococcus agalactiae* (blue to colourless colonies).

Centrifuged test milk sample is directly inoculated on the surface of the medium plate. Esulin-negative (blue to colourless) *S. agalactiae* organisms are further subcultured for identification tests.

**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: Amber coloured, clear to slightly opalescent gel. After addition of 5-7% v/v sterile defibrinated sheep blood: Cherry red coloured opaque gel forms in Petri plates

**Reaction**
Reaction of 4.13% w/v aqueous solution at 25°C. pH: 7.4±0.2

**pH**
7.20-7.60

**Cultural Response**
Cultural characteristics observed with added 5-7% v/v sterile defibrinated sheep blood after an incubation at 35-37°C for 24-48 hours.

**Cultural Response**

<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>Enterococcus faecalis ATCC 29212</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Streptococcus agalactiae ATCC 13813</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless, w/ haemolysis</td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

**Reference**

Revision: 1 / 2011

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