Edward's Medium Base, Modified (DM938)

Intended Use
Edward's Medium Base, Modified (DM938) is recommended for selective and rapid isolation of *Streptococcus agalactiae* and other *Streptococci* associated with bovine mastitis.

Product Summary and Explanation
*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 persistent, inflammatory reaction of the udder tissue and is the most common disease in dairy cattle. Crystal violet and thallium salts are most commonly used selective agents for selective isolation of *Streptococci*. Haxthausen\(^2\) employed a selective crystal violet medium for the isolation of skin streptococci. Bryan\(^3\) using gentian violet blood agar, found that the growth of saprophytic milk bacteria was prevented whilst that of streptococci was unaffected. Edwards\(^4\) employed a crystal violet aesculin blood agar for the cultural diagnosis of bovine mastitis, whilst McKenzie\(^5\) used a medium containing thallium acetate for the same purpose. Hauge et al.\(^6\) described a composite medium containing all the components of modified Edwards Medium. Edward's Medium Base, Modified is a selective medium for the rapid isolation of *Streptococcus agalactiae* and other streptococci involved in bovine mastitis.

Principles of the Procedure
Edward’s Medium Base, Modified contains peptic digest of animal tissue and beef extract which provides carbonaceous, nitrogenous substances and other essential nutrients for growth of the organisms. Esculin helps to differentiates the negative *Streptococcus agalactiae* (blue to colourless colonies) from aesculin-positive Group D streptococci (black colonies). 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.

Formula / Liter

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>10.00</td>
</tr>
<tr>
<td>Beef extract</td>
<td>10.00</td>
</tr>
<tr>
<td>Esculin</td>
<td>1.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.0013</td>
</tr>
<tr>
<td>Thallous sulphate</td>
<td>0.33</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 41.33 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Distribute into tubes or flasks.
4. Autoclave at D 115°C for 20 minutes / validated cycle. (D corresponds to 10lbs pressure)
5. Cool to 50°C and aseptically add 5 to 7% v/v sterile sheep blood.
6. Mix well and pour into sterile Petri plates.
PRODUCT SPECIFICATION SHEET

Quality Control Specifications

<table>
<thead>
<tr>
<th>Dehydrated Appearance</th>
<th>Cream to yellow homogeneous free flowing powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepared Medium</td>
<td>Basal medium : Amber coloured, clear to slightly opalescent gel</td>
</tr>
<tr>
<td></td>
<td>After addition of 5-7% v/v sterile defibrinated sheep blood : Cherry red coloured opaque gel forms in Petri plates</td>
</tr>
<tr>
<td>Reaction of 4.13% Solution</td>
<td>pH : 7.4 ± 0.2 at 25°C</td>
</tr>
<tr>
<td>Gel Strength</td>
<td>Firm, comparable with 1.5% Agar gel</td>
</tr>
</tbody>
</table>

Expected 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.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Organisms</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Enterococcus faecalis ATCC 29212</em></td>
<td>50 - 100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>black</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli ATCC 25922</em></td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Staphylococcus aureus ATCC 25923</em></td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Streptococcus agalactiae ATCC 13813</em></td>
<td>50 - 100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless, w/ haemolysis</td>
</tr>
</tbody>
</table>

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure
Centrifuged test milk sample is directly inoculated on the surface of the medium plate. Look for pale blue colonies which should then be sub-cultured for further identification tests. Refer appropriate references for specific test procedures.

Results
Refer appropriate references and test procedures for interpretation of results.

Storage
Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Edward’s Medium Base, Modified
Product Code : DM938
Available Pack sizes : 100gm

References
Further Information

For further information please contact your local MICROMASTER Representative.

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