Bile Esculin HiVeg Agar w/ Kanamycin

Bile Esulin HiVeg Agar w/ Kanamycin is recommended for the selective isolation and presumptive identification of *Bacteroides fragilis* group of bacteria from mixed flora.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg peptone No. 2</td>
<td>17.000</td>
</tr>
<tr>
<td>HiVeg extract</td>
<td>6.000</td>
</tr>
<tr>
<td>Synthetic detergent</td>
<td>5.000</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Esclulin</td>
<td>1.000</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>0.100</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>0.010</td>
</tr>
<tr>
<td>Vitamin K1</td>
<td>0.010</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 44.6 grams in 1000 ml 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 and pour into sterile Petri plates. DO NOT OVERHEAT.

**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 (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (6) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (7, 8) 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 (9) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (5).

Bile Esulin Agar with Kanamycin is recommended for the selective isolation and presumptive identification of *Bacteroides fragilis* group of bacteria from mixed flora. This medium is a modification of the original formulation of Swan (6). In this medium kanamycin is added to an enriched Bile Esculin Agar, enriched with hemin and vitamin K1. Hemin and vitamin K1 enriches and enhances the growth of *Bacteroides* species. Kanamycin selectively promotes the growth of *Bacteroides fragilis* while inhibiting the growth of facultative anaerobic and aerobic gram-negative bacilli. Anaerobes that are incapable of hydrolyzing esculin do not form brown or black pigmented colonies on this medium. The plates should be reduced by keeping in anaerobic jar for 18-24 hours, just before incubation (10).

HiVeg peptone no. 2 and HiVeg extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Synthetic detergent and kanamycin inhibits most of the other accompanying bacteria. Esclulin 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. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (11). To enhance the growth of Enterococci, Bile Esulin Agar can be supplemented with 50ml/l horse serum (3).

Please refer disclaimer Overleaf.
The test specimens can be directly streaked on the surface of the plate. The inoculated plates should be immediately incubated under anaerobic conditions at 35-37°C. Incubation should be carried out for up to 7 days.

**Quality Control**

**Appearance**
Cream to yellow coloured homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Medium amber coloured clear to slightly opalescent gel forms in Petri plates

**Reaction**
Reaction of 4.46% w/v aqueous solution at 25°C. pH: 7.1±0.2

**pH**
6.90-7.30

**Cultural Response**
MV1035: Cultural characteristics observed under anaerobic condition, after an incubation at 35-37°C for 18-24 hours (in case of no growth, incubation continued up to 7 days).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Esulin Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides fragilis</em> ATCC 25285</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>≥50%</td>
<td>Positive reaction, blackening of medium around the colony</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
<td>none-poor</td>
<td>≤10%</td>
<td>Negative reaction</td>
</tr>
<tr>
<td><em>Fusobacterium necrophorum</em> ATCC 25286</td>
<td>50-100</td>
<td>none-poor</td>
<td>≤10%</td>
<td>Negative reaction</td>
</tr>
</tbody>
</table>

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

**Reference**