Violet Red Bile Glucose HiVeg™ Agar w/o Lactose

Intended Use
Recommended to be used for detection and enumeration of *Enterobacteriaceae* in raw foods and clinical samples.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg™ peptone</td>
<td>7.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Synthetic detergent No. 1</td>
<td>1.500</td>
</tr>
<tr>
<td>Glucose (Dextrose)</td>
<td>10.000</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.030</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.002</td>
</tr>
<tr>
<td>Agar</td>
<td>12.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 38.53 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation
Violet Red Bile Agar, a modification of MacConkeys original formulation (1) is used for the enumeration of coli-aerogens bacterial group. VRBGA, HiVeg™ is prepared by completely replacing animal peptone with vegetable peptones, to make the media BSE/TSE risks free. Violet Red Bile Glucose HiVeg™ Agar w/o Lactose, is a modification of VRBA (MV049), formulated by Mossel et al (2,6,7) where glucose is added to serve the same purpose of enumeration of *Enterobacteriaceae* (2). It employs the selective inhibitory components crystals violet and bile salts and the indicator system glucose and neutral red. Sought bacteria will dissimilate glucose and produce purple zones around the colonies (3). ISO committee has also recommended this medium (4). Selectivity of VRBGA can be increased by incubation under anaerobic conditions and/or at elevated temperature, i.e. equal to or above 42°C (5-7).

HiVeg™ peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Glucose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (8).

Type of specimen
Clinical samples - Blood; Food samples

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (11).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:
In Vitro diagnostic Use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:
1. Further Biochemical testing is required for identification of microorganisms.
2. Some organisms may show poor growth due to nutritional variations.
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
Light yellow to pinkish beige homogeneous free flowing powder

Gelling
Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium
Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

\[
\begin{array}{cccccc}
\text{Organism} & \text{Inoculum (CFU)} & \text{Growth} & \text{Observed Lot value (CFU)} & \text{Recovery} & \text{Incubation temperature} \\
\hline
\text{Escherichia coli ATCC 8739} & 50 - 100 & \text{luxuriant} & 25 - 100 & \geq 50\% & \text{pink-red with bile precipitate} \\
\text{Pseudomonas aeruginosa ATCC 9027} & 50 - 100 & \text{luxuriant} & 25 - 100 & \geq 50\% & \text{pink to red} \\
\text{Escherichia coli NCTC 9002} & 50 - 100 & \text{good-luxuriant} & 25 - 100 & \geq 50\% & \text{pink-red with bile precipitate} \\
\text{Escherichia coli ATCC 25922} & 50 - 100 & \text{good-luxuriant} & 25 - 100 & \geq 50\% & \text{pink-red with bile precipitate} \\
\text{Salmonella Enteritidis ATCC 13076} & \geq 10^3 & \text{inhibited} & 0 & \text{0\%} & \text{light pink} \\
\text{Enterobacter aerogenes ATCC 13048} & 50 - 100 & \text{good-luxuriant} & 25 - 100 & \geq 50\% & \text{pink-red} \\
\text{Staphylococcus aureus ATCC 25923} & \geq 10^3 & \text{inhibited} & 0 & \text{0\%} & \geq 24\text{ hrs} \\
\text{Staphylococcus aureus ATCC 6538} & \geq 10^3 & \text{inhibited} & 0 & \text{0\%} & \geq 24\text{ hrs} \\
\end{array}
\]

Key: *Corresponding WDCM numbers.

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).
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