Violet Red Bile Glucose Agar

Intended use
Recommended for detection and enumeration of *Enterobacteriaceae* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Gelatin peptone #</td>
<td>7.000</td>
</tr>
<tr>
<td>Bile salts</td>
<td>1.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>10.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Neutral red</td>
<td>0.030</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>0.002</td>
</tr>
<tr>
<td>pH after heating ( at 25°C)</td>
<td>7.4±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

# Pancreatic digest of gelatin

Directions
Violet Red Bile Glucose Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized, hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium in tubes as butts/slants or in plates as desired and allow to solidify. If on plate, either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically.

Principle And Interpretation

Violet Red Bile Glucose Agar is a selective medium recommended for detection and enumeration of *Enterobacteriaceae* especially the bile tolerant gram negative bacteria in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (7,1,2,3,4) from non-sterile products and pharmaceutical preparations.

Gelatin peptone and yeast extract provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other nutrients essential for bacterial metabolism. This media is selective due to presence of the inhibitors; bile salts positive organisms especially Staphylococci. Neutral red indicator helps to detect glucose fermentation. Glucose fermenting and crystal violet. Crystal violet inhibits gram-strains produce red colonies with pink-red halos in the presence of neutral red. Sodium chloride maintains the osmotic equilibrium in the medium. The red colour is due to absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8.

Type of specimen
Pharmaceutical samples, Clinical samples- urine, faeces, blood

Specimen Collection and Handling
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (7,1,2,3,4).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:
In Vitro diagnostic use. 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. Though the medium is for selective isolation of *Enterobacteriaceae*, further biochemical and serological testing must be carried out for further confirmation.
2. Over incubation may result in reverting of reaction.

**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 Violet Red Bile Glucose Agar in glass bottle.

**Colour medium**
Reddish purple coloured

**Quantity of medium**
500 ml of medium in glass bottle.

**Reaction**
7.20-7.60

**Sterility Test**
Passes release criteria

**Growth Promotion Test**
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP. Cultural response was observed after an incubation at 30-35°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

**Growth promoting properties**
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu (at 30-35°C for <=18 hours).

**Indicative properties**
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100 cfu (at 30-35°C for 18-24 hours).

**Cultural Response**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Observed Lot value (CFU)</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 8739 (00012*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>pink-red with bile precipitate</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 9027 (00026*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>pink to red</td>
</tr>
</tbody>
</table>

**Additional Microbiological Testing**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Observed Lot value (CFU)</th>
<th>Recovery</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> NCTC 9002 50 -100</td>
<td>good-luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>pink-red with bile precipitate</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>50 -100</td>
<td>good-luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>pink-red with bile precipitate</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC50 -100 13076 (00030*)</td>
<td>good-luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>light pink</td>
<td></td>
</tr>
<tr>
<td># <em>Klebsiella aerogenes</em> ATCC 13048 (00175*)</td>
<td>50 -100</td>
<td>good-luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>pink-red</td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp. aureus</em> ATCC 25923 (00034*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus subsp. aureus</em> ATCC 6538 (00032*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

**Key:** (-#) Formerly known as *Enterobacter aerogenes* (* Corresponding WDCM numbers

---

Please refer disclaimer Overleaf.
Storage and Shelf Life
Store between 15-25°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference
2. European Pharmacopoeia, 2018, European Dept. for the quality of Medicines.
4. Indian Pharmacopoeia, 2018 Ministry of Health and Family Welfare, Govt. of India.

Disclaimer:
User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.