Violet Red Bile Glucose Agar Plate

**Intended use**
Recommended for the selection and subculture of bile tolerant organisms in accordance with the 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>

**Directions**
Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

**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 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 (7).

**Type of specimen**
Pharmaceutical samples

**Specimen Collection and Handling**
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (8,1,2,3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**
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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user’s unique requirement.

3. It is recommended to store the plates at 24-30°C to avoid minimum condensation.

4. 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 90 mm disposable Petri plate.

**Colour**
Red with purplish tinge coloured medium

**Quantity of Medium**
25ml of medium in 90mm plate.

**pH**
7.20 - 7.60

**Sterility Test**
Passes release criteria

**Cultural Response**
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP. Cultural response was observed after 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 18-24hours).

<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>
<th>Incubation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Promoting + Indicative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>
<td>18-24 hrs</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>
<td>18-24 hrs</td>
</tr>
<tr>
<td><strong>Additional Microbiological Testing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> NCTC 9002 50-100</td>
<td>good-luxuriant</td>
<td>25-100</td>
<td>&gt;=50%</td>
<td></td>
<td></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>
<td>18-24 hrs</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em> ATCC 50-100</td>
<td>good-luxuriant</td>
<td>25-100</td>
<td>&gt;=50%</td>
<td></td>
<td>pink-red with bile precipitate</td>
<td>18-24 hrs</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>light pink</td>
<td>18-24 hrs</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 25923 (00034*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
<td>pink-red</td>
<td>18-24 hrs</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp. aureus ATCC 6538 (00032*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td>0</td>
<td>0%</td>
<td>pink-red</td>
<td>18-24 hrs</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
Key: (†) Formerly known as Enterobacter aerogenes  (*) Corresponding WDCM numbers

Storage and Shelf Life
On receipt store between 20-30°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference
2. European Pharmacopoeia, 2019, European Dept. for the quality of Medicines.
4. Indian Pharmacopoeia, 2018 Ministry of Health and Family Welfare, Govt. of India.

Revision : 00/ 2020

Disclaimer :
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