Violet Red Bile Glucose Agar

For selective isolation, detection and enumeration of coli-aerogenes bacteria in water, milk and other dairy food products.

**Directions**

Place the bottle in boiling water bath to melt the medium. DO NOT AUTOCLAVE. When the entire medium in the bottle is in molten form only then pour into sterile Petri plates. On solidification, inoculate the medium with 50-100 cfu of the organisms or alternatively streak the organisms and incubate at required time and temperature.

**Principle And Interpretation**

The coliform group consists of several genera of bacteria belonging to the family *Enterobacteriaceae*. The historical definition of this group has been based on the method used for detection i.e. lactose fermentation. This group is defined as all aerobic and facultative anaerobic, gram-negative, non-spore-forming rod shaped bacteria that ferment lactose with gas and acid formation within 48 hour at 35°C (1, 2). Examination of foods, ingredients and raw materials, for the presence of marker groups such as coliforms is the one of the common tests.

Violet Red Bile Agar, a modification of MacConkey's original formulation (3) is used for the enumeration of coli-aerogenes bacterial group. It relies on the use of the selective inhibitory components crystals violet and bile salts and the indicator system lactose, and neutral red. Thus, the growth of many unwanted organisms is suppressed, while tentative identification of sought bacteria can be made. Organisms, which rapidly attack lactose, produce purple colonies surrounded by purple halos. Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones (4). VRBA is recommended by APHA (2, 5).

Selectivity of VRBA can be increased by incubation under anaerobic conditions and/or at elevated temperature, i.e. equal to or above 42°C (6-8).

Peptic digest of animal tissue and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Lactose 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. Violet Red Bile Agar is not completely specific for enteric; other accompanying bacteria may give the same reaction. Further biochemical tests are necessary for positive identification (7).

**Quality Control**

**Appearance**
Sterile glass bottle containing slightly opalescent Violet Red Bile Glucose Agar.

**Colour**
Reddish purple coloured medium

**Quantity of medium**
100ml of medium in glass bottle

**Reaction**
7.20 - 7.60

**Sterility test**
Passes release criteria

**Cultural response**
Cultural characteristics after melting the medium and pouring into sterile petri plates. The plates are inoculated with following test organisms and incubated at 35 - 37°C for 18-24 hours.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter aerogenes ATCC 13048</td>
<td>Luxuriant</td>
<td>Pink</td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922</td>
<td>Luxuriant</td>
<td>Pinkish red with bile precipitate</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>Inhibited</td>
<td></td>
</tr>
<tr>
<td>S. serotype Enteritidis ATCC 13076</td>
<td>Luxuriant</td>
<td>Pinkish red</td>
</tr>
</tbody>
</table>

**Storage and Shelf Life**

Store between 15-25°C. Use before expiry date on the label.

**Reference**


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