EC Blue Broth

Intended Use:
Recommended for detection and confirmation of *Escherichia coli* and total coliforms from water samples, using a combination of chromogenic and fluorogenic substrates.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>1.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium lauryl sulphate</td>
<td>0.100</td>
</tr>
<tr>
<td>Isopropyl-β-D-1-thiogalactopyranoside (IPTG)</td>
<td>0.100</td>
</tr>
<tr>
<td>X-Gal</td>
<td>0.100</td>
</tr>
<tr>
<td>4-Methylumbelliferyl β-D-Glucuronide (MUG)</td>
<td>0.100</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.1±0.2</td>
</tr>
</tbody>
</table>

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

Directions
Suspend 17.40 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

Principle And Interpretation
EC Blue Broth was designed for detection and confirmation of *Escherichia coli* and other coliforms. *Escherichia coli* can be distinguished from other coliforms by its unique ability to fluoresce in the presence of fluorogenic substrate (2,3). The fluorogenic substrate, MUG is split by enzyme β-glucuronidase especially present in *Escherichia coli*. The reaction is indicated by the development of a blue fluorescence under UV light. The presence of total coliforms is indicated by blue-green colourations due to the cleavage of the chromogenic substrate (X-Gal). IPTG amplifies enzyme synthesis and increases the activity of β-galactosidase.

Peptone provides essential growth nutrients and is useful for the simultaneous detection of indole production. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium chloride helps to maintain the osmotic balance. Sodium lauryl sulphate makes the medium selective by inhibiting accompanying microflora, especially the gram-positive organisms.

Type of specimen
Clinical samples - faeces, urine; Food samples.

Specimen Collection and Handling:
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,7). 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:
1. This medium is general purpose medium and may not support the growth of fastidious organisms.

Please refer disclaimer Overleaf.
2. Further biochemical and serological tests must be carried out for further identification.
3. Some organism may show poor growth due to nutritional variation.

**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
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Cream coloured clear solution having slight precipitate in tubes

Reaction
Reaction of 1.74 % w/v aqueous solution at 25°C. pH : 7.1±0.2
pH
6.90-7.30

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Colour change in medium</th>
<th>Fluorescence under UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
<td>luxuriant</td>
<td>Bluish green</td>
<td>positive reaction</td>
</tr>
<tr>
<td>#Klebsiella pneumoniae ATCC 13883</td>
<td>50-100</td>
<td>luxuriant</td>
<td>blue</td>
<td>negative reaction</td>
</tr>
<tr>
<td>#Klebsiella aerogenes ATCC 13048</td>
<td>50-100</td>
<td>luxuriant</td>
<td>blue</td>
<td>negative reaction</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em> ATCC 8090</td>
<td>50-100</td>
<td>luxuriant</td>
<td>Bluish green</td>
<td>negative reaction</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028</td>
<td>50-100</td>
<td>luxuriant</td>
<td>colourless</td>
<td>negative reaction</td>
</tr>
</tbody>
</table>

Key : (*) Corresponding WDCM numbers.
(一号) Formerly known as *Enterobacter aerogenes*

**Storage and Shelf Life**
Store dehydrated 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. 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 (4,5).

**Reference**

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.