**EC Broth, Granulated**

**Intended Use:**
EC Broth, Granulated is recommended for the selective enumeration of presumptive *Escherichia coli* by MPN technique from water samples and from clinical samples.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.000</td>
</tr>
<tr>
<td>Bile salts mixture</td>
<td>1.500</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>1.500</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.9±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 37 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in test tubes containing inverted Durhams tube. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Adjust the concentration of medium in accordance with sample size.

**Principle And Interpretation**
EC Medium is used for detection of coliforms during bacteriological examination of water, milk and foods. It was originally described by Hajna and Perry (1). This medium was later used by Fishbein and Surkiewicz to carry out *Escherichia coli* confirmatory tests (2). It is also used in MPN methods (3) and is often used for confirmation of coliforms. The procedure employing EC Medium provides information regarding the source of the coliform group (fecal or non-fecal) when used as a confirmatory test (4). EC Broth should not be used for the direct isolation of coliforms since prior enrichment in a presumptive medium for optimal recovery of fecal coliforms is required.

Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and essential growth nutrients. Lactose is the fermentable sugar. Bile salts mixture inhibit gram-positive bacteria especially bacilli and faecal Streptococci. Phosphates control the pH during fermentation of lactose. Gas production in a fermentation tube within 24 hours or less is a presumptive evidence of the presence of coliform bacteria. This medium can be used at 37°C for the detection of coliform organisms or at 44.5°C for the isolation of *Escherichia coli* from water and shellfish) or 45.5°C for foods.

When using sample more than 10 ml, the medium must be reconstituted at a concentration equivalent to that specified on the directions, once the sample is added, the working procedure is as follows:

Transfer a loopful of culture from all the tubes of Lauryl Sulphate Broth (M080) showing gas formation within 24 hours and from all the tubes showing xbaacterial growth within 48 hours to EC Broth tubes. Within 30 minutes from the inoculum, place the tubes in a water bath and incubate at 44°C for 24 hours. Consider the growth showing gas production as positive.

Calculate the density of the faecal coliform organisms by using MPN tables. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents (5).

Gas formation at 44.5°C or 45.5°C (and 37°C) *Escherichia coli*, possibly also other coliforms.

Gas formation at 37°C Coliform bacteria without *Escherichia coli*
Type of specimen
Clinical - faeces; Food samples; Water sample

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,6,10).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3,5)
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. For identification, organisms must be in pure culture.
2. Morphological, biochemical and/or serological tests should be performed for final identification.

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 granular media.

Colour and Clarity of prepared medium
Yellow coloured, clear solution without any precipitate

Reaction
Reaction of 3.7% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed after an incubation at 44.5°C ± 0.2 for 24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae ATCC 13883 (00097*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853 (00025*)</td>
<td>50-100</td>
<td>fair to good</td>
<td>negative reaction</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 29212 (00087*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>positive reaction</td>
</tr>
<tr>
<td># Klebsiella aerogenes ATCC 13048 (00175*)</td>
<td>&gt;=10³</td>
<td>inhibited</td>
<td></td>
</tr>
</tbody>
</table>

Key * - Corresponding WDCM Numbers ; # - Formerly known as Enterobacter aerogenes

Please refer disclaimer Overleaf.
**Storage and Shelf Life**

Store below 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. 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 (7,8).

**Reference**


**Revision : 02 / 2016**
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.