**Modified Buffered Peptone Water Broth**

**Intended Use:**
Recommended for isolation of Enterohaemorrhagic coli (EHEC)

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>6.000</td>
</tr>
<tr>
<td>Acicase™ #</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodiumchloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>3.600</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>1.500</td>
</tr>
<tr>
<td>Sodiumpyruvate</td>
<td>1.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 42.1 grams in 1000 ml of purified /distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Principle And Interpretation**

*Escherichia coli* O157:H7 belongs to the Enterohemorrhagic *Escherichia coli* (EHEC) group and it predominates as a food borne pathogen. *E.coli* O157: H7 was first recognized as a human pathogen in 1982 when two outbreaks of hemorrhagic colitis were associated with consumption of undercooked ground beef that has been contaminated with this organism (3).

Enterohemorrhagic infections are usually food or water borne and have been implicated in undercooked beef, lunch meat, raw milk, water, sprouts and vegetables (5).

Enterohaemorragic *E.coli* strains are also termed as verocytotoxin-producing *E.coli* (VTEC/EHEC). Although many different serotypes of *Escherichia coli* are known to produce verocytotoxin (4) those of *Escherichia coli* O157:H7 and O157:H are so far the common types causing human infections. O157 VTEC strains have several unusual biochemical characters that are exploited in methods for their laboratory identification. They belong to the minority of *E.coli* that are β-glucuronidase negative and do not ferment sorbitol or rhamnose within 24 hours.

Modified Buffered Peptone Water Broth is recommended for the detection of EHEC. Acicase™ and Tryptone provide nitrogen, long chain amino acids, yeast extract provides essential vitamins and minerals to the organisms. Lactose provides energy. Sodium chloride in the medium maintains osmotic balance. Sodium pyruvate stimulates growth. Disodium hydrogen phosphate and potassium phosphate act as buffering agents.

**Type of specimen**

Food samples

**Specimen Collection and Handling**

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3). 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. Some organisms may show poor growth due to nutritional variations.

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
Light yellow to amber coloured clear solution without any precipitate

Reaction
Reaction of 4.21% w/v aqueous solution at 25°C. pH: 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 36-38°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>50-100</td>
</tr>
<tr>
<td>(00013*)</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli O157:H7</em> ATCC 35150</td>
<td>50-100</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC 14028</td>
<td>50-100</td>
</tr>
<tr>
<td>(00031*)</td>
<td></td>
</tr>
</tbody>
</table>

Key: (*): Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

5. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/Bacteriological Analytical Manual BAM.

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.