Buffered Peptone Water w/ Pyruvate

**Intended Use:**
Recommended for the isolation of Enterohemorrhagic *E. 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>Sodium chloride</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>Sodium pyruvate</td>
<td>1.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2±0.2</td>
</tr>
</tbody>
</table>

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

# - Equivalent to Casein acid hydrolysate

**Directions**

Suspend 21.05 grams in 500 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add the rehydrated contents of one vial of Acriflavin-Cefsulodin-Vancomycin Supplement (ACV Supplement, FD284).

**Principle And Interpretation**

Enterohaemorrhagic *E. coli* (EHEC) can cause severe foodborne disease. EHEC is the primary cause of hemorrhagic colitis. This infection can also lead to hemolytic uremic syndrome (4). It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Its significance as a public health problem was recognized in 1982, following an outbreak in the United States of America. EHEC produces toxins, known as verotoxins or Shiga-like toxins because of their similarity to the toxins produced by *Shigella dysenteriae* (5).

The media contains Tryptone, Acicase™ and Yeast extract as carbon and nitrogen sources, long chain amino acids, vitamins and sources for bacterial growth. Phosphates buffer the medium. The phosphate buffer system prevents bacterial damage due to changes in the pH of the medium. Sodium chloride maintains the osmotic balance. Lactose serves as a carbon source.

Antibiotic supplement contains Acriflavin, Cefsulodin, and Vancomycin which effectively suppress the normal flora while allow the growth of *Escherichia coli O157:H7* (4).

After the sample has been prepared, material will be placed in Buffered Peptone Water w/Pyruvate and incubated at 37 ± 1 °C for 5 hours. Next add rehydrated contents of one vial of Acriflavin-Cefsulodin-Vancomycin Supplement (ACV Supplement, FD284) and incubate at 42± 1 °C for 18-24 hours.

**Type of specimen**

Clinical samples -faeces; Food samples.

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). 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
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. This medium is general purpose medium and may not support the growth of fastidious organisms.
2. Further biochemical and serological tests must be carried out for complete 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
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 with an added Acriflavin-Cefsulodin-Vancomycin Supplement (ACV Supplement, FD284) after an incubation at 42± 1 °C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli O157:H7</em></td>
<td>50-100</td>
<td>good</td>
<td>40-50%</td>
</tr>
<tr>
<td>(NCTC 12900) (00014*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> subsp.</td>
<td>&gt;=10^4</td>
<td>inhibited</td>
<td>0%</td>
</tr>
<tr>
<td><em>aureus 25923</em> (00034*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella Typhimurium</em> ATCC</td>
<td>50-100</td>
<td>good</td>
<td>40-50%</td>
</tr>
<tr>
<td>14028 (00031*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC</td>
<td>&gt;=10^4</td>
<td>inhibited</td>
<td>0%</td>
</tr>
<tr>
<td>29212 (00087*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>&gt;=10^4</td>
<td>inhibited</td>
<td>0%</td>
</tr>
<tr>
<td>(00013*)</td>
<td></td>
<td></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 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 (1,2).
Reference

4. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.html
5. www.who.int/mediacentre/factsheets/fs125/en/

Revision: 02/2019

In vitro diagnostic medical device
CE Marking
Storage temperature
10°C
30°C

Do not use if package is damaged

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