Buffered Peptone Water

Intended use
Buffered Peptone Water is a pre-enrichment medium used for increasing the recovery of injured Salmonella species from foods prior to selective enrichment and isolation and from clinical samples.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Disodium phosphate, anhydrous</td>
<td>3.500</td>
</tr>
<tr>
<td>Potassium hydrogen phosphate</td>
<td>1.500</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

Directions
Suspend 20.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in 50 ml amounts into tubes or flasks or as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. If desired aseptically add rehydrated contents of one vial of EC O157:H7 Selective Supplement (FD247) to 1000 ml of medium for enrichment of Escherichia coli O157:H7.

Principle And Interpretation
Buffered Peptone Water is a pre-enrichment medium designed to help recovery of sub-lethally damaged Salmonellae before transfer to a selective medium. This pre-enrichment medium is free from inhibitors and is well buffered and provides conditions for resuscitation of the cells that have been injured by processes of food preservation. It was noted by Edel and Kampelmacher (3) that sub-lethal injury to Salmonella may occur due to food preservation techniques involving heat, desiccation, high osmotic pressure, preservatives or pH changes. Buffered Peptone Water during the pre-enrichment period helps in recovery of injured cells that may be sensitive to low pH (7). This is particularly important for vegetable specimens, which have low buffering capacity. This medium can be used for testing dry poultry feed (6). In a survey involving isolation of Salmonellae from meat that had been artificially contaminated with sub-lethally injured organisms, pre-enrichment in Buffered Peptone Water at 37°C for 18 hours before selection in Tetrathionate Brilliant Green Bile Broth (M1255) showed superior results compared with direct selection method. Lactose Broth is frequently used as a pre-enrichment medium but it may be detrimental to recovery of Salmonellae (2).

The media contain proteose peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance and phosphates buffer the medium. The broth is rich in nutrients and produces high resuscitation rates for sub lethally injured bacteria and supports intense growth. The phosphate buffer system prevents bacterial damage due to changes in the pH of the medium.

Inoculate 10 grams specimen in 50 ml of these media and incubate at 35-37°C for 18 hours. Transfer 10 ml from this medium to 100 ml of Tetrathionate Broth (M032) and incubate at 43°C for 24 - 48 hours and then subculture on selective plating media. Examine the plates for characteristic Salmonella colonies.

Type of specimen
Clinical samples - stool samples for primary enrichment, Food and dairy 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 (1,8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Please refer disclaimer Overleaf.
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. Due to nutritional variations some strains may show poor growth.

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 coloured, clear solution without any precipitate

Reaction
Reaction of 2.0% 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 35-37°C for 18-24 hours. (Recovery is carried out using XLD Agar, M031).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella Enteritidis ATCC 13076 (00030*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 6539</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
<tr>
<td>Escherichia coli 0157:H7 NCTC 12900 (00014*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
</tr>
</tbody>
</table>

[Recovery on Tryptone soya Agar (M290)]

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 inorder 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

Please refer disclaimer Overleaf.

Disclaimer:
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