Buffered Peptone Water with NaCl

Intended use
Buffered Peptone Water with NaCl is recommended as a diluent for carrying microbial limit test from clinical and non clinical specimens.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>1.000</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>3.560</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>7.230</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.300</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters

Directions
Suspend 16.09 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Add 0.1 to 1% w/v polysorbate 20 or 80 if desired. Dispense in tube or flasks as desired. and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

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 (1) 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 (2). This is particularly important for vegetable specimens, which have low buffering capacity. These media can be used for testing dry poultry feed (3). 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 (4). The composition of Buffered Peptone Water with NaCl medium is as per IP and EP specifications recommended to dilute the sample for microbial examination (5, 6). Depending on the amount of fat in the sample to examine the kind and quantity of emulsifying agent to be used.

These pre-enrichment media contain 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 subletally 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 - Blood ; Food and dairy samples

Specimen Collection and Handling
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). 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:
This medium contains low nutrients and hence is not recommended for the growth of organisms.

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
White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium
Colourless to pale yellow clear solution without any precipitate

pH
6.80-7.20

Cultural response
Cultural characteristics observed after recovery on Soybean Casein Digest Agar after an incubation at 30-35°C for 18-24 hours for bacteria and Sabouraud Dextrose Agar at 30-35°C for 24-48 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Recovery within 2 hours of incubation</th>
<th>Recovery within 4 hours of incubation</th>
<th>Recovery within 24 hours of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli ATCC 8739</em> (00012*)</td>
<td>50 -100</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count (stored at 2-8°C)</td>
</tr>
<tr>
<td><em>Escherichia coli ATCC 25922</em> (00013*)</td>
<td>50 -100</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count (stored at 2-8°C)</td>
</tr>
<tr>
<td><em>Escherichia coli NCTC 9002</em></td>
<td>50 -100</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count (stored at 2-8°C)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus ATCC 6538</em> (00032)</td>
<td>50 -100</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count (stored at 2-8°C)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus ATCC 25923</em> (00034*)</td>
<td>50 -100</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count (stored at 2-8°C)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa ATCC 9027</em> (00026*)</td>
<td>50 -100</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count (stored at 2-8°C)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa ATCC 27853</em> (00025*)</td>
<td>50 -100</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count (stored at 2-8°C)</td>
</tr>
<tr>
<td><em>Salmonella Typhimurium ATCC 14028</em> (00031*)</td>
<td>50 -100</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count</td>
<td>no decrease in colony count (stored at 2-8°C)</td>
</tr>
</tbody>
</table>
Salmonella Ahony NCTC 6017 (00029*) 50 -100 no decrease in colony count no decrease in colony count no decrease in colony count (stored at 2-8°C)

Bacillus subtilis subsp. spizizenni ATCC 6633 (00003*) 50 -100 no decrease in colony count no decrease in colony count no decrease in colony count (stored at 2-8°C)

Micrococcus luteus ATCC 9341 50 -100 no decrease in colony count no decrease in colony count no decrease in colony count (stored at 2-8°C)

Candida albicans ATCC 10231 50 -100 no decrease in colony count no decrease in colony count no decrease in colony count (stored at 2-8°C)

Candida albicans ATCC 2091 50 -100 no decrease in colony count no decrease in colony count no decrease in colony count (stored at 2-8°C)

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 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 (10,11).

Reference
6. European Pharmacopoeia, 2008, European Directorate For The Quality of Medicine

* - Corresponding WDCM Numbers
In vitro diagnostic medical device

CE Marking

Storage temperature

10°C

30°C

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
B /4-6 , MIDC, Dindori, Nashik MH

www.himedialabs.com

CE Partner 4U ,Esdoornlaan 13, 3951
DB Maarn The Netherlands,
www.cepartner 4u.eu

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