



Buffered Peptone Water

M614

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.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Sodium chloride	5.000
Disodium phosphate, anhydrous	3.500
Monopotassium phosphate	1.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.00 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, aseptically add rehydrated contents of 1 vial of EC O157 : H7 Selective Supplement (FD247) for isolation of *Escherichia coli* O157 from foods to previously molten and cooled to 45-50°C medium. Mix well and dispense into sterile tubes or flasks as desired.

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 sublethally 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

Food and dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,8,;). 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 :

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).

Organism	Inoculum (CFU)	Growth	Recovery
<i>Salmonella Enteritidis</i> ATCC 50-100 13076 (00030*)		good-luxuriant	≥50%
<i>Salmonella Typhi</i> ATCC 6539	50-100	good-luxuriant	≥50%
<i>Salmonella Typhimurium</i> ATCC 14028 (00031*)	50-100	good-luxuriant	≥50%
<i>Escherichia coli</i> 0157:H7 NCTC 12900 (00014*)	50-100	good-luxuriant [Recovery on Tryptone soya Agar(M290)]	≥50%

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

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5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

6. Juven, Cox, Bailey, Thomson, Charles and Schutze, 1984, J. Food Prot., 47:299.
7. Sadovski, 1977, J. Food Technol., 12:85.
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9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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