Buffered Peptone Water with NaCl

M1275

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

Composition**

Ingredients	Gms / Litre
Peptone	1.000
Potassium dihydrogen phosphate	3.560
Disodium hydrogen phosphate	7.230
Sodium chloride	4.300
Final pH (at 25°C)	7.0±0.2

**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 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 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* .

Quality Control

Appearance
White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium
Colourless 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 .

Organism	Inoculum (CFU)	Recovery within 2 hours of incubation	Recovery within 4 hours of incubation	Recovery within 24 hours of incubation
Escherichia coli ATCC 8739	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Escherichia coli ATCC 25922	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Escherichia coli NCTC 9002	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Staphylococcus aureus ATCC 6538	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Staphylococcus aureus ATCC 25923	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Pseudomonas aeruginosa ATCC 9027	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Pseudomonas aeruginosa ATCC 27853	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Salmonella Typhimurium ATCC 14028	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Salmonella Abony NCTC 6017	50 -100	no decrease in colony count	no decrease in colony count	no decrease in colony count (stored at 2-8°C)
Bacillus subtilis ATCC 6633	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 below 30°C in tightly closed container and the prepared medium between 2 8°C. Use before expiry date on the label.

Reference

1. Edel and Kampelmacher, 1973, Bull. W.H.O., 48:167.
2. Sadovski, 1977, J. Food Technol., 12:85.
3. Juven, Cox, Bailey, Thomson, Charles and Schutze, 1984, J. Food Prot., 47:299.
4. Angelotti, 1963, Academic Press, New York, N.Y.
5. Indian Pharmacopoeia, 1997, Ministry of Health and Family Welfare,Govt. of India, Vol. 2.
6. European Pharmacopoeia, 2008, European Directorate For The Quality of Medicine

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