# Buffered Peptone Water

M614

## Intended use

Recommended as a pre-enrichment medium used for increasing the recovery of injured *Salmonella* species from food prior to selective enrichment and isolation and also from samples.

## Composition**

| Ingredients | g / L |
|---|---|
| Proteose peptone | 10.000 |
| Sodium chloride | 5.000 |
| Disodium hydrogen phosphate | 3.500 |
| Potassium hydrogen phosphate | 1.500 |
| Final pH ( at 25°C) | 7.2±0.2 |

**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 CCV 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 (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. This medium 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 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

Food and dairy samples

## Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7). 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

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further enrichment and isolation must be carried out for confirmation.

## 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 media.

**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 |
|---|---|---|---|
| *Salmonella* Enteritidis ATCC 13076 (00030*) | 50-100 | good-luxuriant | >=50% |
| *Salmonella* Typhi ATCC 6539 | 50-100 | good-luxuriant | >=50% |
| *Salmonella* Typhimurium ATCC 14028 (00031*) | 50-100 | good-luxuriant | >=50% |
| *Escherichia coli 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 15-30°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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

## Reference

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4.Angelotti, 1963, Academic Press, New York, N.Y.

5.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

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