



Buffered Peptone Water

M1494I

Intended use

Buffered Peptone Water is used as pre-enrichment medium for increasing the recovery of injured *Salmonella* species from foods prior to selective enrichment and isolation. The composition and performance criteria of this medium are as per the applications laid down in ISO 6579-2017, ISO 6887 and ISO 21528-2017.

Composition**

Ingredients	Gms / Litre
Tryptone #	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate.12H ₂ O	9.000
Potassium dihydrogen phosphate	1.500
FinalpH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Enzymatic digest of casein

Directions

Suspend 20.07 grams (equivalent weight of dehydrated medium) in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Microorganisms that are subjected to environmental stresses may become structurally or metabolically damaged or injured. These microorganisms are unable to replicate in selective environments. Therefore these injured organisms must be resuscitated or permitted to repair the damage by incubation in an appropriate, non-selective environment (1). Edel and Kampelmacher (2) noted that sublethal injury to *Salmonellae* may occur in many food preservation processes. Enriching injured cells in Lactose Broth (pH 6.9) may be further detrimental to their recovery (3). Pre-enrichment in Buffered Peptone Water (M1494I) at 35°C for 18-24 hours results in repair of injured cells (4). The buffering system prevents bacterial damage due to change in the pH of the medium. Recently ISO committee has also recommended this pre-enrichment medium for the detection of *Salmonella* (5) and *Enterobacteriaceae* (6) from food stuffs and other materials. It is also recommended as diluent for all enumerations of microorganisms (7-10) and *Listeria* (11) as recommended by ISO.

ISO 6579 : Inoculate 25 grams specimen in 225 ml of Buffered Peptone Water (M1494I) and incubate at 34-38°C for 16-20 hours. Transfer 1 ml from this medium to 10 ml of Mueller Kauffman Tetrathionate Novobiocin Broth Base (M1496I) and incubate at 36-38°C for 24±3 hours. Simultaneously inoculate 0.1 ml to 10 ml of Rappaport Vassiliadis Soya Broth (RVS Broth) (M1448I) or MSR/V agar (M1428) and incubate at 41.5±1° C for 24±3 hours. After selective enrichment, subculture on selective media like XLD Agar, Modified (M031I) and either Brilliant Green Agar Base, Modified (M016) or Bismuth Sulphite Agar (M027). Examine the plates for colonies of *Salmonella* species. Further biochemical and serological testing must be carried out for confirmation.

ISO 21528 : Inoculate x grams/ml specimen in 9x ml of Buffered Peptone Water (M1494I) and incubate at 37°C or 30°C for 16-20 hours. The enriched sample is then isolated on Violet Red Bile Agar (M581) and incubate at 36-38°C for 24±2 hours. The colonies are then isolated on non-selective medium, Nutrient Agar (M087I). Further biochemical testing must be carried out for confirmation.

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 (1,2,5,6, 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 :

The 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

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 : 7.0±0.2

pH

6.80-7.20

Cultural Response

ISO 6579 & ISO 21528:

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Organism	Inoculum (CFU)	Growth
<i>Salmonella Enteritidis</i> ATCC 13076 (00030*)	50-100	luxuriant
<i>Salmonella Typhimurium</i> ATCC 14028 (00031*)	50-100	luxuriant
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant
<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	luxuriant
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	luxuriant
<i>Cronobacter sakazakii</i> ATCC 29544 (00214*)	50-100	luxuriant

ISO 6887

Recovery of ±30% of the original count (recovered on Tryptone Soya Agar , M290), when the inoculated sample holding time is 45 minutes to 1 hour at 20-25°C. The plates are incubated at 37 ± 2°C for 18 ± 2 hours.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery
<i>Staphylococcus aureus</i> <i>subsp.aureus</i> ATCC 25923 (00034*)	50-100	15-130	30-130 %
<i>Staphylococcus aureus</i> <i>subsp.aureus</i> ATCC 6538 (00032*)	50-100	15-130	30-130 %
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	15-130	30-130 %

<i>Escherichia coli</i> ATCC 8739 (00012*)	50-100	15-130	30-130 %
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Key :* Corresponding WDCM numbers

ISO 11290-1

Recovery of $\pm 30\%$ of the original count (recovered on Tryptone Soya Agar , M290), when the inoculated sample holding time is 1 hour \pm 5 minutes at 20 ± 2 °C. The plates are incubated at 37 ± 2 °C for 18 ± 2 hours.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery
<i>Listeria monocytogenes</i> 4b ATCC 13932 (00021*)	50-100	15-130	30-130%
<i>Listeria monocytogenes</i> 1/2a ATCC 35152 (00109*)	50-100	15-130	30-130%
<i>Listeria innocua</i> ATCC 33090 (00017*)	50-100	15-130	30-130%

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 (6,7).

Reference

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11. International Organization for Standardization ISO 11290-2, 2017, Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. —Part 2: Enumeration method
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