Buffered Peptone Water

M1494I

Intended use

Recommended as a pre-enrichment medium of *Enterobacteriaceae* organisms such as *Salmonella* and *Cronobacterium* species from food and animal feeding stuffs, water, milk, milk products and other products. The composition and performance criteria of this medium are as per the specifications laid down in ISO 6579-1:2017, ISO 6887-1:2017, ISO 21528-1:2017, ISO 22964:2017, ISO19250:2010, ISO 11133:2014 & A1:2018.

Composition**

ISO 6579-1:2017, ISO 6887-1:2017 (E), ISO 21528-1:2017, ISO 22964:2017, Specification - Buffered peptone water (BPW)

Ingredients	g/ L
Enzymatic digest of casein	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate, dodecahydrate,($Na_2HPO_4.12H_2O$)	9.000
Potassium dihydrogen phosphate (KH_2PO_4)	1.500
Final pH (at 25°C)	7.0±0.2

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**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 purified/ 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. This medium is also recommended by APHA & FDA BAM for pre-enrichment of *Salmonella, Cronobacter* and *Listeria* (1,2). Edel and Kampelmacher (3) noted that sub-lethal injury to Salmonellae may occur in many food preservation processes. 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. ISO committee has also recommended this pre-enrichment medium for the detection of *Enterobacteriaceae* (5), *Salmonella* (6,7) *Cronobacter* (8) and *Listeria* species (9) species from food stuffs, water and other materials. It is also recommended as a diluent for enumerations of all microorganisms (10).

Type of specimen

ISO 6579-1:2017/ ISO 6887-1:2017/ ISO 11290-1:2017/ ISO 21528-1:2017/ ISO 22964:2017

Food samples including milk and milk products, in animal feed, in animal faeces, water and in environmental samples from the primary production stage.

Specimen Collection and Handling:

Processesing : ISO 6887-1:2017 (10) / ISO 11290-1:2017 (9)

Dilution samples : Preparation of test samples, initial suspension and decimal dilutions for microbiological examination

Processesing : ISO 6579-1:2017 (6)

Pre-enrichment : Samples (25 grams in 225 ml) are preenriched in Buffered Peptone Water (M1494I) and incubated at 34° C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre- enriched sample is inoculated in 10 ml RVS Broth (M1448I) or MSRV Agar (M1428) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTTn broth (M1496I) and incubated at 37 ± 1°C for 24 ± 3 hours.

Isolation : The culture thus obtained is then plated on XLD Agar, Modified (M031I) and incubated at 37± 1°C for 24 ± 3 hours . Simultaneously plating on second isolation agar is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

Processesing : ISO 21528-1:2017 (5)

Pre-enrichment : Samples (10 grams in 90 ml) are preenriched in Buffered Peptone Water (M1494I) and incubated at 37 ± 1°C for 18 h ± 2 hours.

Isolation : The culture thus obtained is then plated on Violet red bile glucose (VRBG) agar (M1684) and incubated at 37 ± 1 °C for 24±2hours.

Confirmation : Biochemical and serological tests are performed for confirmation.

Processesing : ISO 22964:2017 (8)

Pre-enrichment : Samples (10 grams in 90 ml) are preenriched in Buffered Peptone Water (M1494I) and incubated at 34° C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre- enriched sample is inoculated in 10 ml Cronobacter Selective Broth (M1786I) and incubated at 41.5 ± 1°C for 24±2hours.

Isolation : The culture thus obtained is then plated on HiCrome™ Cronobacter Isolation Agar(CCI Agar)(M2062I) and incubated at 41.5 ± 1°C for 24±2hours.

Confirmation : Biochemical and serological tests are performed for confirmation.

Processesing : ISO 19250:2010(E) (7)

Non-selective pre-enrichment: Inoculate 50 ml of BPW (M1494I) at room temperature with the sample or dilutions thereof and incubate at (36 ± 2) °C for (18 ± 2) h

Selective enrichment: Transfer 0.1 ml of the culture obtained enrichment broth to a tube containing 10 ml of the RVS Broth (M1448I) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTTn broth (M1496I) and incubated at 36± 2°C for 24 ± 3 hours.

Confirmation: : The culture thus obtained is then plated on XLD Agar, Modified (M031I) and incubated at 36± 2°C for 24 ± 3 hours . Simultaneously plating on second isolation agar is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

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 in 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.

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

Organism	Inoculum (CFU)	Recovery

ISO 6887-1:2017

Dilution : 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 at 37°±2°C for 18 h ± 2 hours.

Escherichia coli ATCC 8739 (00012*)*	50-100	±30% of the original count
Escherichia coli ATCC 25922 (00013*)	50-100	±30% of the original count
Staphylococcus aureus ATCC 6538 (00032*)*	50-100	±30% of the original count
Staphylococcus aureus ATCC 25923 (00034*)*	50-100	±30% of the original count

ISO 6887-1:2017
Dilution : Recovery of ± 30% of the original count (recovered on Tryptone Soya Agar, M290), when the inoculated sample holding time is 1 hour ± 5 minutes at 20 ± 2°C. The plates are incubated at 37°±2°C for 18 h ± 2 hours.
Recovery of

Listeria monocytogenes ATCC 13932 (00021*)	50-100	±30% of the original count
Listeria monocytogenes ATCC 35152 (00109*)	50-100	±30% of the original count

ISO 6579-1:2017 & ISO 21528-1:2017
Productivity
Cultural characteristics observed after an incubation at 34°C to 38°C for 18 h ± 2 hours.

Organism	**Inoculum CFU)**	**Growth**
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant
Escherichia coli ATCC 8739 (00012*)	50-100	good-luxuriant
Escherichia coli ATCC 25922 (00013*)*	50-100	good-luxuriant

ISO 22964:2017
Productivity
Cultural characteristics observed after an incubation at 34°C to 38°C for 18 h ± 2 hours.

Cronobacter sakazakii ATCC 29544 (00214*)	50-100	good-luxuriant
Cronobacter muytjensii ATCC 51329 (00213*)	50-100	good-luxuriant

ISO 19250:2010 (E)
Productivity
Cultural characteristics observed after an incubation at 36± 2°C for 24 ± 3 hours

Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant

Key :* Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (12,13).

Reference

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