Saline Peptone Water w/6% NaCl

M2087I

Intended use

Recommended for performing salt tolerance test for *Vibrio* species from food, animal feeding stuff and environmental samples in the area of food production and food handling. The composition and performance criteria of this medium are as per the specifications laid down in ISO 21872-1: 2017

Composition**

ISO 21872-1 Specification - Saline peptone water

Ingredients	g / L
Peptone	10.000
Sodium chloride	60.000
Final pH after sterilization (at 25°C)	7.5±0.2

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**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 70.0 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Vibrio's are fairly easy to isolate from both food, animal feed and environmental samples in the area of food production and food handling. This medium is recommended for selective enrichment and differentiation of *V.parahaemolyticus*, *V.vulnificus* and *V.alginolyticus* from other *Vibrio species* based on its salt tolerance. This medium is recommended by ISO (1).

Vibrio furnissii is a non-halophilic *Vibrio,* which cannot grow in media with a concentration of sodium chloride greater than 5-6% and is able to grow in media lacking NaCl (1). All other *Vibrio* species except *Vibrio furnissii and Vibrio mimicus* is able to grow in this medium. Peptone provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium and serves as a selective and inhibitory agent to other microorganisms.

Type of specimen

Food and animal feeding stuffs, environmental samples from areas in food production and food handling

Specimen Collection and Handling:

For food and animal feeds, environmental samples in area of food production and food handling, follow appropriate techniques for sample collection and processing as per guidelines (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Processesing : ISO 21872-1:2017 (E)

Primary Selective enrichment : 25gm or 25ml of test portion in 225ml ASPW, temperature depends upon the target Vibrio species and state of product like deep frozen or fresh for 6 h ± 1 hour (For *V. parahaemolyticus* & *V.furnissii* at 41.5± 1°C in fresh foods and 37± 1°C for deep frozen dried or salted products, For *V.vulnificus* at 37± 1°C for all product states).

Secondary Selective enrichment : Transfer 1 ml of culture fromprimary enrichment broth to 10ml of ASPW (sample is not agitated before taking the aliquot). Incubate the ASPW at 41,5 °C ± 1 °C and/or 37 °C ± 1 °C for 18 h ± 1 hour. Isolation and identification : The cultures obtained in the ASPW are transfered on TCBS Agar (M189), incubate at 37 °C ± 1 °C for 24 h ± 3 hour, for development of well-isolated colonies. For the second selective medium, examine for the presence of colonies, which, according to their characteristics, may be considered as possible isolates of *V. parahaemolyticus, V. vulnificus* and/or *V.furnissii*

Confirmation : By molecular PCR and/or biochemical approaches. For biochemical testing, inoculate the colonies selected onto the surface of plates of Saline Nutrient Agar (M2086I) . Incubate at 37 °C ± 1 °C for 24 h ± 3 hour. From these isolated colonies are inoculated in Arginine Dihydrolase Saline Broth (M1644I) and/or L- Lysine Decarboxylase Saline Broth (LDC)

(M1778I), incubate at 37 °C ± 1 °C for 24 h ± 3 hour and/or Saline Peptone Water w/6% NaCl (M2087I).

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. Further biochemical test to be required to fully distinguish these species from each other and from non-pathogenic *Vibrio* spp.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. 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

Colourless to pale yellow coloured clear to solution in tubes.

Reaction

Reaction of 7.0 % w/v aqueous solution at 25°C. pH : 7.5±0.2

pH

7.30 -7.70

Cultural Response

Cultural characteristics observed after an incubation at 37 ± 1°C for 24 ± 3 hours.

Organism	Inoculum (CFU)	Growth
Specificity		
Vibrio parahaemolyticus NCTC 10885 (00185*)	50-100	good- luxuriant
Vibrio vulnificus ATCC27562 (00139*)	50-100	good- luxuriant
Vibrio alginolyticus ATCC 17749	50-100	good- luxuriant
Selectivity		
Vibrio mimicus ATCC 33653	>=10^4	inhibited
Vibrio furnissii NCTC 11218 (00186*)	>=10^4	inhibited

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 (2,3).

Reference

1.Microbiology of food and animal feeding stuffs - Horizontal method for detection of potentially enteropathogenic *Vibrio* spp.- Part 1: International Organization for Standardization (ISO), 21872-1:2017

2.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

3.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.

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