



Arginine Dihydrolase Saline Broth

M1644I

Intended use

Recommended as one of the medium for confirmation of *Vibrio* species from food, animal feeds and environmental samples in area of food production and food handling. The composition and performance of this medium is as per the composition laid down in ISO 21872-1:2017.

Composition**

ISO 21872-1 Specification - Arginine dihydrolase saline medium (ADH)

Ingredients	g / L
Arginine, monohydrochloride	5.000
Yeast extract	3.000
Glucose	1.000
Sodium chloride	10.000
Bromocresol purple	0.015
Final pH after sterilization (at 25°C)	6.8±0.2

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Ingredients	g / L
Arginine, monohydrochloride	5.000
Yeast extract	3.000
Glucose (Dextrose)	1.000
Sodium chloride	10.000
Bromocresol purple	0.015
Final pH after sterilization (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 19.01 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely and distribute in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in an upright position.

Principle And Interpretation

Arginine Dihydrolase Saline Broth is one of the medium recommended for the confirmation of *Vibrio* species from food, animal feeds, environmental samples in area of food production and food handling in accordance with ISO specifications (1). Decarboxylase Media used for the detection of arginine dihydrolase and lysine and ornithine decarboxylase was first introduced by Moeller (2, 3, 4). Bacteria can be differentiated on the basis of their decarboxylating activity towards the amino acid. Bacteria producing arginine dihydrolase enzyme in this medium decarboxylate arginine to putrescine. The production of amine, putrescine elevates the pH of medium which is detected by the indicator, bromocresol purple which forms purple colour in alkaline condition. Colour change from purple to yellow (Dextrose utilization) and then back to purple is positive reaction (Arginine hydrolysis). Yeast extract provide the necessary nutrients to the organisms while L-arginine stimulates the arginine dihydrolase synthesis and glucose provides the energy source. Sodium chloride maintains osmotic balance and supports growth of *Vibrio* species.

As per the ISO specifications (1) the sample under test is enriched in Alkaline saline peptone water. This enriched medium is then plated on TCBS Agar. Typical colonies are then plated on Saline Nutrient Agar. The typical colonies obtained after incubation are confirmed by performing the biochemical tests. Arginine Dihydrolase saline Broth (M1644I) is one of the biochemical medium used. Arginine reaction is strictly anaerobic; therefore the broth tubes must be over layed with mineral oil. In differentiation of *Vibrio* species, control tubes without arginine must be used. If the tubes gives purple reaction the test is considered as negative.

Type of specimen

Food and animal feeds, environmental samples in area of 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.

Processing : 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. cholerae* 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 from primary 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 transferred 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. cholerae*.

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), incubate at 37 °C ± 1 °C for 24 h ± 3 hour.

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. This medium is suited for Arginine hydrolysis of *Vibrio* species.
2. It may not be suited for the detection of arginine hydrolysis of other species.
3. Additional phenotypic tests to be required to fully distinguish these species from each other and from non-pathogenic *Vibrio* spp.

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**

Light yellow to grey homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured clear solution in tubes

Reaction

Reaction of 1.9% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Detection of L-Arginine dihydrolase : Cultural response was observed after an incubation at 37±1°C for 24 ± 3 hours by overlaying with about 1 ml of sterile mineral oil.

Organism	Growth	Arginine dihydrolase
<i>Vibrio parahaemolyticus</i> NCTC 10885 (00185*)	good-luxuriant	negative reaction, yellow colour
<i>Vibrio parahaemolyticus</i> ATCC 17802 (00037*)	good-luxuriant	negative reaction, yellow colour
<i>Vibrio fluvialis</i> ATCC 33809 (00137*)	good-luxuriant	positive reaction, turbidity with violet/ purple colour

Key : (#) corresponding WDCM

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Microbiology of the food chain- Horizontal method for the determination of *Vibrio* spp.- Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*. International Organization for Standardization Draft ISO/TS 21872-1:2017/ ISO 11133:2014/Amd.2:2020 (E).
2. Moeller, 1954, Acta Pathol. Microbiol. Scand., 34:102.
3. Moeller, 1954, Acta Pathol. Microbiol. Scand., 34:259.
4. Moeller, 1955, Acta Pathol. Microbiol. Scand., 36:158.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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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