



L- Lysine Decarboxylase Saline Broth (LDC)

M1778I

Intended use

Recommended for confirmation of *Vibrio* species on the basis of lysine decarboxylation from food and animal feeding stuff, and environmental samples in areas of food manufacturing and food handling. The composition and performance of this medium is as per the specification laid down in ISO 21872-1:2017.7

Composition**

ISO 21872-1 Specification - L-lysine decarboxylase saline medium (LDC)

Ingredients	g / L
L-Lysine monohydrochloride	5.000
Yeast Extract	3.000
Glucose (Dextrose)	1.000
Sodium chloride	10.000
Bromocresol purple	0.015
pH after sterilization (at 25°C)	6.80±0.2

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L-Lysine 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. Dispense the medium in quantities of approximately 2ml to 5ml into narrow test tube. Sterilize by autoclaving at 15 lbs pressure (121° C) for 15 minutes.

Principle And Interpretation

Vibrio parahaemolyticus is a halophilic estuarine organism. This organism can be isolated from a variety of sea food product and marine environments. The organism, when isolated from fresh sea food, is usually found in low number and is sensitive to refrigeration and heat.

This medium is recommended by ISO (1) for biochemical identification of *Vibrio parahaemolyticus*, *Vibrio furnissii* and *Vibrio vulnificus* on the basis of lysine decarboxylation from food and animal feeding stuff, and environmental samples in areas of food manufacturing and food handling.

Yeast extract provide nitrogen and carbon compounds, growth factors essential for the growth of *Vibrio species*. Sodium chloride maintains osmotic balance and facilitate easy growth of *Vibrio species*. Glucose (Dextrose) is the fermentable carbohydrate and bromocresol purple is the pH indicator.

During the initial stages of incubation, fermentation of glucose by the organisms, with acid production results in a colour change of indicator to yellow. On further incubation, if L-Lysine is decarboxylate to cadaverine, there will be an alkaline reaction and indicator colour will then change to purple. If colour remains yellow, the decarboxylase reaction is negative.

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 feeding stuff, 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. 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 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. 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 L- Lysine Decarboxylase Saline Broth (M1778I), 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 greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured clear solution forms in tubes

Reaction

Reaction of 1.9% w/v aqueous solution after sterilization at 25°C. pH : 6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 37°C± 1°C for 24 ± 4 hours.(Inoculated tubes are overlaid with sterile mineral oil).

Organism	Lysine decarboxylation
Specificity	
<i>Vibrio parahaemolyticus</i> NCTC 10885 (00185*)	Positive (Purple colour with turbidity)
<i>Vibrio furnissii</i> NCTC 11218 (00186*)	Positive (Purple colour with turbidity)
<i>Vibrio vulnificus</i> ATCC 27562 (00129*)	Positive (Purple colour with turbidity)
<i>Vibrio mimicus</i> ATCC 33653	Positive (Purple colour with turbidity)
<i>Vibrio alginolyticus</i> ATCC 17749	Positive (Purple colour with turbidity)

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 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
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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Disclaimer :

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