Dihydrolase Broth Base

M915

Intended Use:

Recommended for studying dihydrolase reaction of *Vibrio parahaemolyticus.*

Composition**

Ingredients	g / L
Peptone	5.000
Yeast extract	6.000
Dextrose (Glucose)	2.000
Sodium chloride	30.000
Bromo cresol purple	0.032
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.03 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Divide in 2 parts. Add 0.5% L-Arginine to first portion and use second portion as basal broth control. Dissolve completely and dispense 3.0 ml into 13 mm X 100 mm screw cap tubes. Sterilize by autoclaving at Δ115°C for 15 minutes.

Δ *corresponds to 10 lbs pressure.*

Principle And Interpretation

Vibrios are fairly easy to isolate from both clinical and environmental material, though some species may require growth factors and /or vitamins. *Vibrio parahaemolyticus* is the leading cause of bacterial diarrhoea associated with the consumption of contaminated food products (1). Dihydrolase Broth Base is formulated as per APHA (2) and is used for studying dihydrolase reaction of *V. parahaemolyticus*.

Dextrose is utilized by *Vibrio* species where there is drop in pH indicated by Bromocresol purple resulting in yellow colour. The medium is supplemented with L-Arginine as a substrate for dihydrolase reaction (3,4). L-Arginine is converted to putrescine by the dihydrolase enzyme; however putrescine is also formed from arginine by the decarboxylase system as well. In the decarboxylase system, L-Arginine undergoes decarboxylation to yield agmatine. Agmatine is then catabolized by the enzyme agmatine dihydrolase to putrescine, CO_2 and ammonia by way of an intermediate compound monocarbaminyl putrescine (5). Thus, because of production of amine like putrescine in the medium the pH is elevated (6) changing the colour of the indicator from yellow to purple. Bromocresol purple is the pH indicator in the medium, which turns purple from yellow at alkaline pH. For confirmation, it is suggested to inoculate a basal medium tube, which does not contain L-Arginine. Alkalinization of the surface of the medium may be caused by exposure to air, so a dihydrolase negative organism may be misidentified as positive. It is therefore recommended to protect the inoculated tubes from air with overlay of sterile mineral oil.

Peptone and yeast extract provide nitrogenous nutrients to support bacterial growth. Dextrose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium.

Type of specimen

Pure isolates from clinical samples and food samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (9).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. It is recommended to protect the inoculated tubes from air with overlay of sterile mineral oil.

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 without any precipitate

Reaction

Reaction of 4.3% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed with added 0.5% L-Arginine after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Growth	Arginine dihydrolase
#*Klebsiella aerogenes* ATCC 13048 (00175*)	good-luxuriant	negative reaction,yellow colour
Vibrio cholerae ATCC 15748	good-luxuriant	negative reaction,yellow colour
Vibrio parahaemolyticus ATCC 17802 (00037*)	good-luxuriant	negative reaction, yellow colour

Key : (*) Corresponding WDCM numbers, (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

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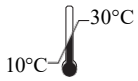
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

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