Ornithine Decarboxylase Broth

M1223

Intended Use

Recommended for detection of the ability of microorganisms to decarboxylate ornithine. The medium is also recommended by ISO 10273:2003 for *Yersinia enterocolitica.*

Composition**

ISO 10273:2003 Specification - Ornithine Decarboxylase medium

Ingredients	g / L
L-Ornithine monohydrochloride	5.000
Yeast extract	3.000
Glucose (Dextrose)	1.000
Bromo cresol purple	0.015
Final pH (at 25°C)	6.8±0.2

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**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 9.01 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense in test tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. After inoculation, overlay the tubes with 2-3 ml sterile mineral oil.

Principle And Interpretation

Decarboxylation is the process in which bacteria that possess specific decarboxylase enzyme attack amino acids at their carboxyl end (-COOH) to yield an amine or a diamine and carbon dioxide (1). The amino acid L ornithine is decarboxylated by the enzyme ornithine decarboxylase to yield the diamine putrescine and carbon dioxide (2,3). Ornithine Decarboxylase Broth is based on the Taylors modification (4). It is recommended by the ISO Committee (5) for the detection of ornithine decarboxylation by *Yersinia enterocolitica.*

Yeast extract in the medium provides nitrogen and other nutrients necessary to support bacterial growth. The amino acid ornithine is added to detect the production of ornithine decarboxylase. Glucose is the fermentable carbohydrate, which during the initial stages of incubation, is fermented by the organisms with acid production, which results in colour change of the pH indicator (BCP) to yellow. The acidic condition also stimulates decarboxylase activity. If the organism produces the appropriate enzyme, i.e. decarboxylase, the amino acid (ornithine) in the medium is degraded, yielding a corresponding amine. Decarboxylation of ornithine yields putrescine. The production of this amine elevates the pH of the medium towards alkalinity, changing the color of the indicator from yellow to purple or violet. If the organism does not produce the appropriate enzyme, the medium remains acidic or yellow in colour.

Type of specimen

Isolated Microorganism from food and animal feeding stuff

Specimen Collection and Handling:

Processing (5)

Enrichment : For the first initial suspension place the sample (x) in known volume of the PSB broth (M941I), to give a dilution of 1/10 dilution (by mass/volume or volume/volume). Homogenize the suspension using a peristaltic blender for 2 min. Incubate at 22°C to 25°C for 2 to 3 days with or 5 days without agitation.

For the second initial suspension in the same way with the ITC broth (M1220) so as to obtain a test portion/enrichment medium dilution of 1/100 (mass/volume or volume/volume). Incubate at 25°C for 48 hours.

Isolation : 1. Inoculate the culture obtain from PSB culture on the surface of CIN agar plate (M843) and incubate at 30°C for 24 to 48 hours.

2.Alkaline treatment : Using sterile pipette transfer 0.5ml of the PSB culture into 4.5 ml of KOH solution and mix for 20 seconds only. Immediately inoculate on CIN agar plate. Incubate at 30°C for 24 to 48 hours.

3.Using ITC culture inoculate the surface of SSDC agar plate (M1703). Incubate at 30°C for 24 to 48 hours.

Purification : Streak the selected colonies on the surface of Nutrient Agar (M561A). Incubate at 30°C for 24 hours.
Confirmation : Inoculate just below the surface of the liquid medium. If the tubes are not full of medium and airtight, cover the surface with molten (heated then just cooled so that it remains still liquid) vaseline oil or sterile liquid paraffin.
Incubate at 30°C for 24 hours.
A violet color after incubation indicates a positive reaction.
A yellow color indicates a negative reaction.

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 on 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

Light yellow to light green homogeneous free flowing powder

Colour and Clarity of prepared medium

Dark purple coloured clear solution without any precipitae

Reaction

Reaction of 0.9% aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours. Inoculated tubes are overlayed with mineral oil.

Organism	Inoculum (CFU)	Ornithine Decarboxylation
Escherichia coli ATCC 25922 (00013*)	50-100	variable reaction
#*Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	positive reaction, purple colour
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	negative reaction, yellow colour
Proteus mirabilis ATCC 25933	50-100	negative reaction, yellow colour
$ *Proteus hauseri* ATCC 13315	50-100	positive reaction, purple colour
Salmonella Paratyphi A ATCC 9150	50-100	positive reaction, purple colour
Salmonella Typhi ATCC 6539	50-100	negative reaction, yellow colour

Shigella flexneri ATCC 12022 (00126*)	50-100	negative reaction, yellow colour
Shigella sonnei ATCC 25931	50-100	positive reaction, purple colour
Yersinia enterocolitica ATCC 27729	50-100	positive reaction, purple colour

Key : *Corresponding WDCM numbers.

(#) Formerly known as *Enterobacter aerogenes* $ Formerly known as *Proteus vulgaris*

Storage and Shelf Life

Store **b**etween 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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 (6,7).

Reference

1. Smith D.T., Coant N.F., Willett H.P., Zinssers Microbiology, 14th Ed., New York: Appleton-Century-Crofts, 1968:118-119.
2. MacFaddin J.F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
3. Moeller V., 1955, Acta Pathol. Microbiol. Scand. 36 (2): 158-172.
4. Taylor W.I., 1961, Appl. Microbiol., 9:487.
5. International Organization for Standardization (ISO), 1994, Draft ISO/DIS 10273:2003 : Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica.*
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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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