

Technical Data

L. D. Agar

M742

Intended Use:

Recommended for cultivation and identification of fastidious anaerobic bacteria.

Composition**

Ingredients	g / L
Tryptone	5.000
Yeast extract	5.000
Sodium chloride	2.500
Sodium sulphite	0.100
L-Cystine	0.400
L-Tryptophan	0.200
Vitamin K1	0.010
Hemin	0.010
Agar	20.000
Final pH (at 25°C)	$7.4{\pm}0.2$

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.22 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Organisms that grow in the absence of oxygen are termed as anaerobes. Depending upon their ability to tolerate oxygen, they are classified as either facultative or obligate anaerobes. The anaerobic gram-negative bacteria are part of the normal flora of the upper respiratory tract, mouth, intestinal tract and urinogenital tract of human and animals. The bile-resistant *Bacteroides fragilis* group is the most commonly recovered anaerobe in clinical specimens and is more resistant to antimicrobial agents than any other anaerobe. *Fusobacterium necrophorum* is a very virulent anaerobe that may cause severe infections, usually in children or young adults (1).

L.D. Medium or Lombard-Dowell Medium was developed by Dowell and Lombard (2) for the cultivation andidentification of fastidious anaerobic bacteria. L. D. Agar is used to evaluate the degree of growth of anaerobes and also to assess indole and catalase production by Bacteroides and Fusobacterium species. L. D. Agar is essentially a casein digest agar, enriched with hemin, vitamin K1, L-cystine and yeast extract (3). This medium contains various nutritious substances, which can promote the growth of fastidious anaerobic bacteria. Tryptone and yeast extract provide the necessary nitrogenous nutrients while hemin and vitamin K1 supply additional growth factors. L-cystine and L-tryptophan serve as the amino acid sources. Sodium sulphite is an antioxidant. Sodium chloride maintains osmotic balance of the medium. Catalase-positive reaction may not be evident uptill 30 seconds to 1 minute after application of 3% hydrogen peroxide (4,5).

Type of specimen

Clinical samples - faeces, wounds, etc

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. 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. Catalase-positive reaction may not be evident uptill 30 seconds to 1 minute after application of 3% hydrogen peroxide (4,5).

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

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.32% w/v aqueous solution at 25°C. pH : 7.4±0.2

pН

7.20-7.60

Cultural Response

Cultural characteristics observed under anaerobic condition, after an incubation at 35-37°C for 40-48 hours .

Organism	Growth	Indole production	Catalase
Bacteroides fragilis ATCC	good-luxuriant	negative	positive
25285		reaction	reaction
Bacteroides corrodens	fair-good	negative	negative
		reaction	reaction
Fusobacterium necrophorum	good-luxuriant	positive	negative
ATCC 25286		reaction	reaction
Fusobacterium nucleatum	fair to good	positive	negative
ATCC 25586		reaction	reaction

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 (6,7).

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

2. Dowell V. and Lombard G., June 1977, U.S., DHEW, Center for Disease Control (CDC), Atlanta. Ga.

3. Finegold S. M., Baron E. J., Bailey and Scotts Diagnostic Microbiology, 8th Ed., 1990, The C.V. Mosby Company

4.Koneman E., Allen S., Dowell V. and Sommers H., 1979, Colour Atlas and Textbook of Diagnostic Microbiology, J. B. Lippincott Co., Philadelphia.

5.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

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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In vitro diagnostic

medical device

IVD



-30°C Storage temperature

> Do not use if package is damaged

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