

L. D. Agar

M742

L. D. Agar is used for cultivation and identification of fastidious anaerobic bacteria.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	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±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.22 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. 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 (5).

L. D. Medium or Lombard-Dowell Medium was developed by Dowell and Lombard (1) for the cultivation and identification 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. Casein enzymic hydrolysate 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 until

30 seconds to 1 minute after application of 3% hydrogen peroxide (2, 4).

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

pH

7.20-7.60

Cultural Response

M742: Cultural characteristics observed under anaerobic condition, after an incubation at 35-37°C for 40-48 hours .

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

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Dowell V. and Lombard G., June 1977, U.S., DHEW, Center for Disease Control (CDC), Atlanta. Ga.
2. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore
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. Murray P. R., Baron J. H., Pfaffer M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

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