L. D. Esculin Agar

M743

L. D. Esculin Agar is used for identification of anaerobic bacteria especially *Bacteroides* species on the basis of esculin hydrolysis.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	5.000
Sodium chloride	2.500
L-Tryptophan	0.200
Vitamin K1	0.010
L-Cystine	0.400
Hemin	0.010
Esculin	1.000
Ferric citrate	0.500
Agar	20.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.62 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 (3).

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. Esculin Agar is used to determine esculin hydrolysis, hydrogen sulfide and catalase production of *Bacteroides* and *Fusobacterium* species.

L. D. Agar is essentially a casein digest agar enriched with hemin, vitamin K1, L-cystine and yeast extract (2). 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. Esculin is hydrolyzed by the organisms to form esculetin and dextrose. The esculetin reacts with the iron salt of ferric citrate to produce a dark brown to black complex. Also L-cystine is a sulphur-containing amino acid and hence H2S production in combination with ferric citrate gives black colouration to the colonies. Vitamin K1 and hemin are the additional growth factors. Black colour of H2S positive colonies is rapidly lost after exposure to air; hence plates should be observed in anaerobic glove box or immediately upon exposure to air (2). Catalase-positive reaction may not be evident uptill 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 3.46% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M743: Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	H2S produciton	Catalase	Esculin hydrolysis
Bacteroides saccharolyticus ATCC 25260	50-100	luxuriant	negative reaction	negative reaction	negative reaction
Bacteroides fragilis ATCC 25285	50-100	luxuriant	negative reaction	positive reaction, effervescence seen on addition of 3% hydrogen peroxide	positive reaction, brown black precipitate around the colonies
Fusobacterium mortiferum ATCC 9817	50-100	luxuriant	positive reaction, blackening of colonies	negative reaction	positive reaction, brown black precipitate around the colonies

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. Finegold S. M., Baron E. J., Bailey and Scotts Diagnostic Microbiology, 8th Ed., 1990, The C.V. Mosby Company.
3. 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.
4. Koneman E., Allen S., Dowell V. and Sommers H., 1979, Colour Atlas and Textbook of Diagnostic Microbiology, J. B. Lippincott Co., Philadelphia.

Revision : 1 / 2011