L. D. Egg Yolk Agar Base

M744

L. D. Egg Yolk Agar with Egg Yolk Supplement is used for detection of lecithinase activity of anaerobic microorganisms.

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
Dextrose	2.000
Disodium phosphate	5.000
Magnesium sulphate	0.010
Hemin	0.010
Vitamin K1	0.010
Agar	20.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.23 grams in 900 ml 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 and aseptically add 100 ml sterile Egg Yolk Emulsion (FD045). 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 (4).

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. Egg Yolk Agar Base, along with egg yolk emulsion is used for the detection of lipase; lecithinase and proteolytic activity of both spore forming and non-spore-forming obligate anaerobes (2).

L. D. Agar is essentially a casein digest agar enriched with hemin, vitamin K1, L-cystine and yeast extract (3). This medium contain 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.

L. D. Egg Yolk Agar with Egg Yolk Supplement contains egg yolk, which is the source of lecithin in the medium. Magnesium sulphate helps in sporulation. Disodium phosphate buffers the medium.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium
Basal medium : Medium amber coloured clear to slightly opalescent gel. After addition of sterile egg yolk emulsion : Yellow coloured opaque gel forms in Petri plates

Reaction
Reaction of 4.02% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M744: Cultural characteristics observed under anaerobic condition,with added Egg Yolk Emulsion (FD045), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Lecithinase/ Halos	Lipase	Proteolysis
Clostridium perfringens ATCC 12924	50 - 100	luxuriant	positive reaction, opaque zone of insoluble precipitate	negative reaction	negative reaction
Clostridium sporogenes ATCC 11437	50 - 100	luxuriant	negative reaction	positive reaction, irridescent sheen on the colony surface and medium	positive reaction, clear zone surrounding colonies
Fusobacterium necrophorum ATCC 25286	50 - 100	luxuriant	negative reaction	positive reaction, irridescent sheen on the colony surface and medium	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. 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.

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