M-Endo Agar LES

M1106

M-Endo Agar LES is used for enumeration of coliforms in water using a two step membrane filter method.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	3.700
Peptic digest of animal tissue	3.700
Tryptose	7.500
Yeast extract	1.200
Lactose	9.400
Dipotassium phosphate	3.300
Monopotassium phosphate	1.000
Sodium chloride	3.700
Sodium deoxycholate	0.100
Sodium lauryl sulphate	0.050
Sodium sulphite	1.600
Basic fuchsin	0.800
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.05 grams in 980 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45°C and aseptically add 20 ml of 95% ethanol. Mix and dispense 4 ml amounts into 60 mm Petri plates. In large plates, use sufficient medium to give a 1.5 mm depth. DO NOT EXPOSE PLATES TO DIRECT SUNLIGHT.

Caution : Basic fuchsin is a potential carcinogen and care must be taken to avoid inhalation and contamination of the skin.

Principle And Interpretation

It is possible to remove bacteria from fluids by passing them through filters with such small pore size that bacteria are arrested. This filtration technique enables fairly large volumes of water to pass rapidly under pressure, but prevents the passage of any bacteria present. These nutrients are retained on the surface of the membrane which is then brought into contact with suitable liquid nutrients. These diffuse upwards through the pores thereby inducing the organisms to grow as surface colonies which can be counted (1).

Endo Medium was first developed by Endo to differentiate between lactose-fermenters and non-fermenters (2). This medium employed sodium sulphite and basic fuchsin instead of bile salts to achieve inhibition of gram-positive bacteria (2). M-Endo Agar, LES is a modification of the original medium and is formulated as per McCarthy et al of Lawrence Experimental Station (LES) (3) for testing coliforms in water using a two-step membrane filter procedure, wherein Lauryl Sulphate Broth (M080) is used as the primary enrichment medium. This medium is recommended by APHA for testing coliforms in drinking and in bottled water (4, 5). Presumptive coliform bacteria will form red colonies with metallic sheen after an incubation at 35-37°C for 24 hours.

Casein enzymic hydrolysate, tryptose, peptic digest of animal tissue and yeast extract provide essential nutrients especially nitrogenous for the coliforms. Lactose is the fermentable carbohydrate. Sodium sulphite, sodium deoxycholate and basic fuchsin inhibit the growth of gram-positive organisms. Phosphates buffer the medium. Coliforms ferment lactose and the resulting acetaldehyde reacts with sodium sulphite and basic fuchsin to form red colonies and similar colouration of the medium. Lactose non-fermenters form colourless colonies.

In the first step of enrichment, cotton absorbent pad is impregnated with Lauryl Sulphate Broth (M080). Membrane filter through which water sample is passed is aseptically placed on it and incubated without inverting for 2 hours at 35°C in a humid

atmosphere. After incubation, the membrane filter is aseptically transferred to the M-Endo Agar LES plate and incubated at 35°C for 24 hours. Alternatively membrane filter pad can be placed inside the lid of Petri plate of M-Endo Agar LES and then impregnated with 2 ml Lauryl Sulphate Broth (M080) and incubated for 1 - 1½ hours at 35°C. In the second step, the prepared membrane filter is kept directly on the agar surface and incubated as described above. Presumptive coliforms produce golden green colonies with metallic sheen within 24 hours of incubation.

Coliform density calculation : Note the coliform density in terms of total coliforms/100 ml. Extrapolate the count using membrane filters with 20-80 coliform colonies but not more than 200 of all types per membrane.

The formula for calculating the count is as follows:

Total coliform colonies/100 ml = coliform colonies /ml of sample filtered x 100

Quality Control

Appearance
Light pink to purple homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Red coloured slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.1% w/v aqueous solution with 2% v/v alcohol at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M1106: Cultural characteristics observed after an incubation at 35-37°C for 20 - 24 hours.

Organism	Inoculum (CFU)	Growth	Colour of colony (on membrane filter)
Escherichia coli ATCC 25922	50-100	good-luxuriant	pink with metallic sheen
Enterobacter aerogenes ATCC 13048	50-100	good-luxuriant	pink to red (may have sheen)
Salmonella Typhi ATCC 6539	50-100	luxuriant	colourless to very light pink
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	
Klebsiella pneumoniae ATCC 13883	50-100	good-luxuriant	pink to red
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	colourless to very light pink

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Reference

1.Cruickshank R., Duguid J. P., Marmion B. P., Swain R. H. A., (Eds.), Medical Microbiology, 1975, 12th Ed. Vol. II, Churchill Livingstone
2.Endo S., 1904, Zentralbl. Bakteriol., Abt. 1, Orig.35:109-110.
3.McCarthy J. A., Delaney J. E. and Grasso R., 1961, Water and Sewage Works, 108:238.
4.Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
5.Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.

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