M-Endo Agar LES

Intended Use
M-Endo Agar LES is a medium used for estimation of coliforms in water using a membrane filter technique.

Summary
The filtration technique enables fairly large volumes of water to pass rapidly under pressure, but prevents the passage of any bacteria present. These bacteria are retained on the surface of the membrane which is then brought into contact with suitable liquid nutrients. These nutrients diffuse upwards through the pores thereby inducing the organisms to grow as surface colonies which can be counted. Endo Medium was first developed by Endo to differentiate between lactose-fermenters and non-fermenters. This medium employed sodium sulphate and basic fuchsin instead of bile salts to achieve inhibition of Gram-positive bacteria. M-Endo Agar, LES is a modification of the original medium and is formulated as per McCarthy *et al*., of Lawrence Experimental Station (LES) for testing coliforms in water using a two-step membrane filter procedure, wherein Lauryl Sulphate Broth is used as the primary enrichment medium. This medium is recommended by APHA for testing coliforms in drinking and in bottled water.

Principle
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 sulphate, 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 sulphate and basic fuchsin to form red colonies and similar colouration of the medium. Lactose non-fermenters form colourless colonies.

Formula*

Ingredients	**g/L**
Casein Enzymic Hydrolysate	3.7
Peptic Digest of Animal Tissue	3.7
Tryptose	7.5
Yeast Extract	1.2
Lactose	9.4
Dipotassium Phosphate	3.3
Monopotassium Phosphate	1.0
Sodium Chloride	3.7
Sodium Deoxycholate	0.1
Sodium Lauryl Sulphate	0.05
Sodium Sulphate	1.6
Basic Fuchsin	0.8
Agar	15.0
Final pH (at 25°C)	7.2 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Water samples

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 51.05 g of the powder in 980 mL purified / distilled water.
2. Heat to boiling to dissolve the powder completely.
3. DO NOT AUTOCLAVE.
4. Cool to 45°C and aseptically add 20 mL of 95% ethanol.
5. Mix and dispense 4 mL amounts into 60 mm petriplates. In large plates, use sufficient medium to give a 1.5 mm depth. DO NOT EXPOSE PLATES TO DIRECT SUNLIGHT.

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

Quality Control

Dehydrated Appearance: Light pink to purple coloured, homogenous free flowing powder.
Prepared Appearance: Rose pink to red coloured, slightly opalescent gel forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 20-24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 20 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤ 100 cfu of appropriate microorganism.
Inhibitory Properties: No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating >100 cfu of the appropriate microorganism.

Organism (ATCC)	**Growth**	**Colour of Colony**
Escherichia coli (25922)	Good	Pink with metallic sheen
Klebsiella aerogenes (13048)	Good	Pink
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Light pink
Klebsiella pneumoniae subsp. *pneumoniae* (10031)	Good	Pink
Staphylococcus aureus subsp. *aureus* (25923)	Inhibited	-

Note: For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201130050500	Dehydrated Culture Media	500 g
205130890100	Ready Prepared Plate	(90 mm) 100 Plates