M1106

# M-Endo Agar LES

## Intended Use:

Recommended for enumeration of coliforms in water using a two step membrane filtration technique.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Tryptone | 3.700 |
| Peptone | 3.700 |
| Tryptose | 7.500 |
| Yeast extract | 1.200 |
| Lactose | 9.400 |
| Dipotassium hydrogen phosphate | 3.300 |
| Potassium dihydrogen phosphate | 1.000 |
| Sodium chloride | 3.700 |
| Sodium deoxycholate | 0.100 |
| Sodium lauryl sulphate (SLS) | 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 purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°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.

Tryptone, tryptose, Peptone 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

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| *Staphylococcus aureus* *ATCC 25923* | >=$10^4$ | inhibited | |
| *Klebsiella pneumoniae* *ATCC 13883* | 50-100 | good-luxuriant | pink to red |
| *Salmonella* Typhimurium *ATCC 14028* | 50-100 | luxuriant | colourless to very light pink |

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(#) Formerly known as *Enterobacter aerogenes*

## Reference

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