TM 174 - M-ENDO AGAR LES

INTENDED USE

For enumeration of coliforms in water using a twostep membrane filter technique.

PRODUCT SUMMARY AND EXPLANATION

The filtration technique play role in removing bacteria from fluids when passed through small pore size filters and bacteria are arrested. It enables to pass large volumes of water rapidly under pressure without passing bacteria. The retained nutrients on the surface of the membrane is incubated with suitable liquid nutrients and diffusion occurs in upward direction 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.

M-Endo Agar, LES is formulated as per McCarthy et al of Lawrence Experimental Station (LES) and is a modification of the original medium for testing coliforms in water using a two-step membrane filter procedure, wherein primary enrichment is used as Lauryl Sulphate Broth. APHA recommended this medium for testing coliforms in drinking and in bottled water. Presumptive coliform bacteria will form red colonies with metallic sheen after an incubation at 35-37°Cfor 24 hours.

COMPOSITION

Ingredients	Gms / Ltr
Peptone	3.700
Tryptone	3.700
Lactose	9.400
Yeast extract	1.200
Tryptose	7.500
Basic fuchsin	0.800
Sodium sulphite	1.600
Sodium deoxycholate	0.100
Sodium lauryl sulphate	0.050
Sodium chloride	3.700
Dipotassium hydrogen phosphate	3.300
Potassium dihydrogen phosphate	1.000
Agar	15.000

PRINCIPLE

Essential nutrients are provided by tryptone, tryptose, peptone and yeast extract provide essential nutrients especially nitrogenous for the coliforms. Lactose is the fermentable carbohydrate. Growth of gram-positive organisms are inhibited by sodium sulphite, sodium deoxycholate and basic fuchsin. Phosphates functions to buffer the medium. Coliforms are lactose fermenters which lead to the formation of acetaldehyde and reacts with sodium sulphite and basic fuchsin to form red colonies and similar coloration of the medium. Lactose non-fermenters form colorless colonies.

In the first step of enrichment, cotton absorbent pad is impregnated with Lauryl Sulphate Broth. Through membrane filter water sample is passed is aseptically placed on it and it is incubated without inverting for 2 hours at 35°C in a humid atmosphere. It consists of sodium sulphite and basic fuchsin to inhibit the growth of gram-positive bacteria atmosphere, instead of bile salts. After completion of incubation, the membrane filter is aseptically transferred to the M-Endo Agar LES plate and further it is incubated at 35°C for 24 hours. Another way is to place the membrane filter pad inside the lid of Petri plate of M-Endo Agar LES and then impregnated with 2 ml Lauryl Sulphate Broth. Further, it is incubated at 35°C for 1 - 1½ hours. In the second step, the prepared membrane filter is kept directly on the agar surface and incubated as

mention above. Presumptive coliforms are characterized by golden green colonies with metallic sheen within 24 hours of incubation.
Coliform density calculation. The formula for calculating the count is as follows: Total coliform colonies/100 ml =coliform colonies /ml of sample filtered x 100 (: 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).

INSTRUCTION FOR USE

- Dissolve 51.00 grams in 980 ml purified/distilled water.
- Heat to boiling the medium to dissolve completely. Do not autoclave.
- Cool to 45-50°C and add 20 ml of 95% ethanol aseptically.
- Mix and dispense 4 ml amounts into 60 mm Petri plates. In large plates, use sufficient medium to give 1.5 mm depth. Do not expose plates directly to sunlight.

Caution: Basic fuchsin is a potential carcinogenic, avoid inhalation and contamination on skin.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Homogeneous free flowing powder, Light pink to purple
Appearance of prepared medium : Red colored slightly opalescent gel forms in Petri plates
pH (at 25°C) : 7.20 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Color of the colony (on membrane filter)	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	Good-luxuriant	>=50 %	Pink with metallic sheen	35-37°C	20-24 Hours
Klebsiella aerogenes	13048	50-100	Good-luxuriant	>=50 %	Pink to red (may have sheen)	35-37°C	20-24 Hours
Salmonella Typhi	6539	50-100	Luxuriant	>=70%	Colorless to very light pink	35-37°C	20-24 Hours
Staphylococcus aureus subsp. aureus	25923	50-100	Inhibited	0%	-	35-37°C	20-24 Hours
Klebsiella pneumoniae	13883	50-100	Good-luxuriant	>=50 %	Pink to red	35-37°C	20-24 Hours
Salmonella Typhimurium	14028	50-100	Luxuriant	>=70%	Colorless to very light pink	35-37°C	20-24 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water andWastewater,23rd ed., APHA, Washington, D.C.
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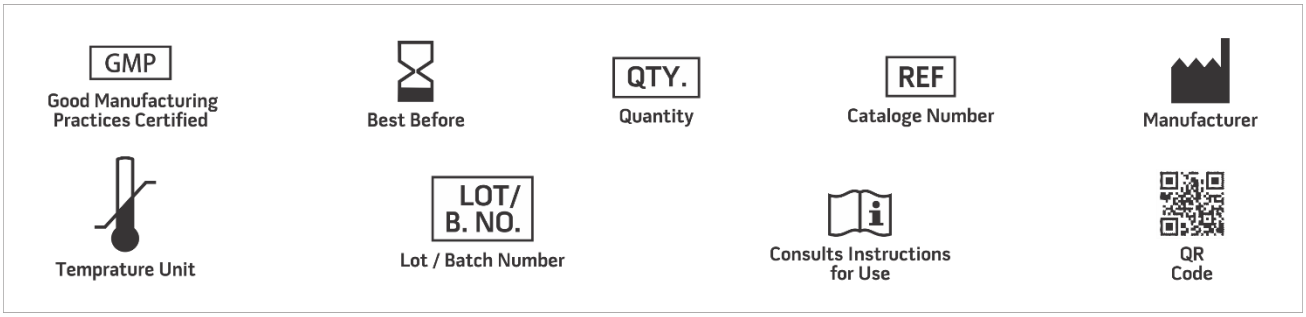
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5thEd., American Public Health Association, Washington, D.C.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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