Ready Prepared Media

Technical Information

M-Endo Agar Plate

Product Code: PM 2106

Application: 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

Principle & 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 (1, 6). 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.

Type of specimen

Water samples

Specimen Collection and Handling

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
After use, contaminated materials must be sterilized by autoclaving before discarding .

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets .

Limitations

1. If the inoculum is too heavy, the sheen may be suppressed.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Avoid exposure of the Agar Plates to light, as it may lead to photo oxidation and decrease productivity of the medium.

Quality Control

Appearance
Sterile M-Endo LES Agar in 90 mm disposable plate.
Quantity of medium
25ml of medium in disposable plate
Colour of medium
Dark pink to purple coloured medium
Reaction
7.00-7.40
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 20 - 24 hours.

Organism	Inoculum(CFU)	Growth	Colour of colony (on membrane basis)
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	pink with metallic sheen
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	good-luxuriant	pink to red (may have sheen)
Salmonella Typhi ATCC6539	50-100	luxuriant	Colourless to very light pink
Staphylococcus aureussubsp. Aureus ATCC 25923 (00034*)	>=10^4	inhibited	
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	Pink to red
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	Colourless to very light pink

Key : (*) Corresponding WDCM numbers (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

- On receipt store between 2-8°C Use before expiry date on the label.
- Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Further Reading

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Cruickshank R., Duguid J. P., Marmion B. P., Swain R. H. A., (Eds.), Medical Microbiology, 1975, 12th Ed. Vol. II, Churchill Livingstone
3. Endo S., 1904, Zentralbl. Bakteriol., Abt. 1, Orig.35:109-110.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.
6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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