



M-Lauryl Sulphate Broth

M1023I

Intended Use:

Recommended for enumeration of *Escherichia coli* and coliforms in water using membrane filtration technique. The composition and performance criteria are in accordance with ISO 9308-1:2014.

Composition**

Ingredients	g/ L
Peptic digest of animal tissue	40.000
Yeast extract	6.000
Lactose	30.000
Sodium lauryl sulphate	1.000
Phenol red	0.200
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 77.2 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired and sterilize by steaming for 30 minutes on three consecutive days or by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Burman (1) substituted bile salts with teepol in Membrane Enriched Teepol Broth, the membrane filtration test medium used to detect coliform organisms in water. Membrane or M-Lauryl Sulphate Broth recommended (2, 3) is similar to M-Enriched Teepol Broth except teepol has been replaced by sodium lauryl sulphate. Sodium lauryl sulphate was found to be an adequate reproducible substitute. Recently ISO Committee (4) has recommended a slight modification (M1023I) of M-Lauryl Sulphate

Broth for enumeration of coliforms in water. *E. coli* and coliform count should be made on separate samples of water. The volumes should be chosen so as the number of colonies on the membrane lies between 10 and 100. The water samples are filtered through sterile membrane filter and then placed face upward on an absorbent pad saturated with M-Lauryl Sulphate Broth. The membranes should be incubated in a container which does not allow evaporation to occur. Water-tight metal containers placed in an accurate water bath are required for incubation of membranes at 44°C. Burman (5) recommended the following incubation temperatures and durations.

Unchlorinated waters:

Coliform organisms: 4 hours at 30°C followed by 14 hours at 35°C

Escherichia coli : 4 hours at 30°C followed by 14 hours at 44°C

Non-chlorinated organisms benefit from 4 hours incubation at 30°C but chlorinated organisms require 6 hours incubation at 25°C. After incubation, yellow colonies are formed which should be confirmed further. No colonies should be assumed on appearance of as nil count. On appearance of small colonies of an intermediate colour, the membrane should be returned for incubation for full period. *E. coli* forms yellow coloured colonies on membranes when incubated at 44°C. Subculture to Lactose Broth (M1003) and Tryptone water (M463I) to confirm gas and indole production respectively after 24 hours incubation at 44°C. Coliform organisms which give yellow colonies on membranes incubated at 35-37°C should be subcultured in Lactose Broth (M1003). This can be incubated at 37°C for 48 hours to check gas production.

Type of specimen

Water samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(6) 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. Due to varying nutritional requirements, some strains may be encountered that grow poorly.
2. If the inoculum is too heavy, the sheen may be suppressed.

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.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Colour and Clarity of prepared medium

Red coloured clear solution without any precipitate

Reaction

Reaction of 7.72% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 18 hours at different temperatures

Organism	Inoculum (CFU)	Growth on membrane(at 35-37°C)	Colour of Colony on Membrane	Growth on membrane (at 43-45°C)	Colour of Colony on Membrane
Cultural Response					
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	yellow	luxuriant	yellow
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	yellow	inhibited	

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

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 (7,8).

Reference

1. Burman N. P., 1967, Proc. Soc. Wat. Treat. Exam., 16:40.
2. Joint Committee of PHLS and the Standing Committee of Analysis, 1980, J. Hyg. Camb., 85:181.
3. Stanfield G. and Irving T. E., 1981, Water Research, 15:469.
4. International Organization for Standardization, 1990, Draft ISO/DIS: 9308-1.
5. Burman N. P., 1967, Rec. Adv. in Bacteriological Examination of waters; C.H. Collins (Ed.), Butterworth, London
6. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

7.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

8.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.

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Disclaimer :

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