m-Endo Broth, 2 mL

Product Number: 6500

Medium Composition	
Lactose	12.5 g
Papaic Digest of Soy Flour	10.0 g
Enzymatic Digest of Animal Tissue	5.0 g
Pancreatic Digest of Casein	5.0 g
Sodium Chloride	5.0 g
Potassium Phosphate, dibasic	4.375 g
Potassium Phosphate, monobasic	1.375 g
Sodium Sulfite	2.1 g
Yeast Extract	1.5 g
Sodium Lauryl Sulfate	0.05 g
Sodium Deoxycholate	0.1 g
Basic Fuchsin	1.05 g
Final pH: 7.2 ± 0.2 at 25°C	

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Physical Characteristics
Appearance of Medium: Hazy to opalescent with or without a precipitate, peach to pink
pH at 25°C: 7.2 ± 0.2

Intended Use

Ampouled m-Endo Broth, 2 mL is used for enumerating coliforms in water by the membrane filtration method.

Product Summary

m-Endo Broth, 2 mL is a ready to use medium for membrane filtration testing. This medium is prepared according to the formula of Fifield and Schaufus,[1] and used for the detection of coliforms in water. It is recommended by the American Public Health Association (APHA) in total coliform membrane filtration procedure for testing water, wastewater, and foods.[2,3] The US Environmental Protection Agency (EPA) specifies using m-Endo broth in the total coliform methods for testing drinking water, surface water, and saline water.[4,5] The total coliform test is the primary indicator of bacteriological quality for potable water, distribution system water, and public water supplies because it is a larger measure of pollution than the fecal coliform test.[4,5]

Principles of the Procedure

Enzymatic digest of casein, enzymatic digest of animal tissue, and papaic digest of soy flour provide nitrogen, carbon, and minerals in m-Endo Broth. Yeast extract is a source of vitamins and trace elements to stimulate bacterial growth. Potassium phosphates are buffering agents. Sodium chloride maintains the osmotic balance. Lactose serves as a carbohydrate source. Sodium lauryl sulfate and sodium deoxycholate are selective agents used to inhibit gram-positive bacteria. Basic fuchsin is a pH indicator. Sodium sulfite is added to decolorize the basic fuchsin solution. Ethanol aids in the homogeneity of the solution and as a selective agent.

Lactose is the fermentable carbohydrate. Lactose positive colonies exhibit a red color caused by the aldehyde reaction with the sodium sulfite and basic fuchsin. The development of a metallic sheen occurs when the organism produces aldehydes with the fermentation of lactose. Lactose non-fermenting bacteria form clear, colorless colonies.

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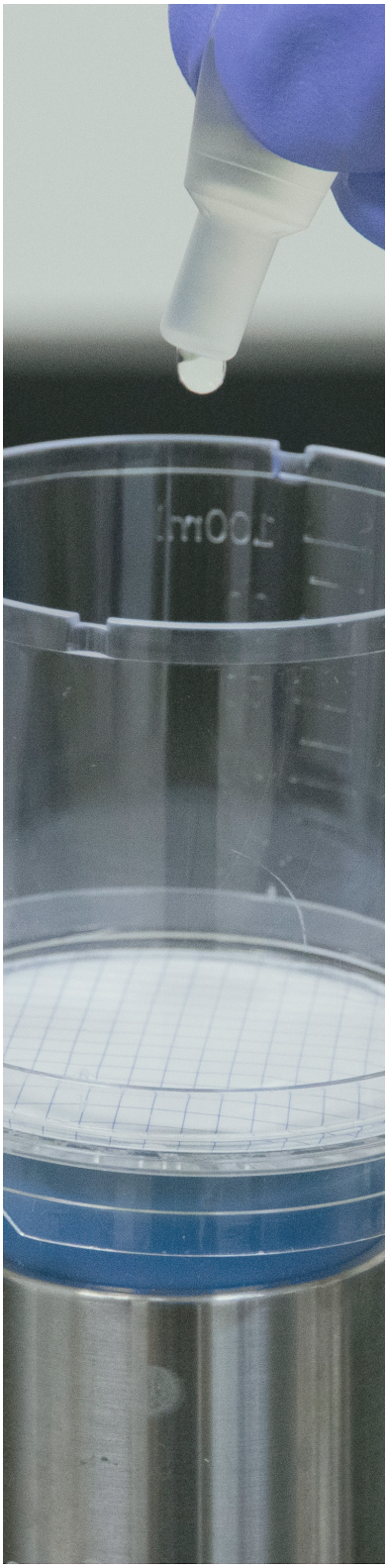

Test Procedure

Preparation

1. Assemble the manifold or filtration flask that will supply the vacuum source, complete with rubber stopper.
2. Using a gentle twisting motion, secure the funnel adapter into the stopper.
3. Using the same gentle twisting motion, secure the NEOGEN Filter onto the funnel adapter.

Filtration Procedure

1. Remove filtration cover and carefully pour the sample onto the filter.
2. Apply vacuum just long enough to pull the sample through the filter. (If using a manifold, open only one valve at a time.)
3. Rinse the inside walls of the filter funnel with approximately 20 mL of sterile buffered solution. Apply vacuum just long enough to pull the solution through the filter, and turn off vacuum. Note: this step is optional if only water is being tested.
4. Briefly remove the filter and its funnel adapter from the stopper to release any remaining vacuum pressure, and then resecure into the stopper.
5. Add m-Endo Broth onto the top of the filter. When doing so, be careful not to touch the filter with the tip of the ampoule.
6. Very briefly apply vacuum so that the media does not pool on top of the filter, and is visible underneath the filter. (Note: the media has been soaked correctly into the filter if there is a small pocket of air around the bottom port. The filter should be moist, but not oversaturated or dry.)
7. Remove and appropriately discard the plastic funnel. Place the filtration system cover over the filter/base assembly converting the unit to a petri dish for sample incubation.
8. Remove the filter from the funnel adapter and place a plug on the open bottom port.
9. Place the filtration plate into the incubator inverted so that the cover is on the bottom, and incubated at 35 ± 2°C. Record results after 18–48 hours.
10. Dispose of test materials in accordance with all applicable local, state, and federal regulations.

Expected Cultural Response

Sterile water was added to sterile filtration units and inoculated with the cultures listed below. The inoculum was filtered followed by the ampoulized m-Endo Broth and the filtration housing removed. Plates were incubated aerobically at 35 ± 2°C and examined for growth at 18–24 hours.

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Microorganisms	Approx . Inoculum (CFU)	Expected Results
Uninoculated Media	N/A	No Growth
Enterobacter aerogenes — ATCC 13048	10–300	≥ 85% Recovery, dark pink to red colonies, may elicit a green metallic sheen
Escherichia coli — ATCC 25922	10–300	≥ 85% Recovery, red colonies with green metallic sheen
Salmonella enteritidis — ATCC 13076	10–300	≥ 85% Recovery, colorless to pink colonies
Salmonella typhimurium — ATCC 14028	10–300	≥ 85% Recovery, colorless to pink colonies
Staphylcoccus aureus — ATCC 25923	300–10,000	Suppressed to Inhibited

Results: Examine filters for the presence of red colonies. All red colonies that have the characteristic metallic sheen are coliforms. The metallic green-gold sheen can cover all or part of the colony. Report the coliform density in terms of total coliforms/100 mL. Lactose non-fermenting bacteria form clear, colorless colonies.

Storage: Store Ampouled m-Endo Broth, 2 mL at 2–8°C.

Expiration: Refer to expiration date printed on the front of the box container.

Limitations of the Procedure

1. Analyze sample as soon as possible after collection.
2. Samples containing colloidal or suspended particulate material can clog the membrane filter, thereby prevent filtration, or cause spreading of bacterial colonies which could interfere with colony identification.
3. If the inoculum is too heavy, the sheen may be suppressed.
4. Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies, including dark red or nucleated colonies without sheen.

NEOGEN Items		
6500	m-Endo Broth, 2 mL	Box of 50
6550	NEOGEN Filter — White	Box of 50

References

1. Fifield, C. W., and C. P. Schaufus. 1958. J. Am. Water Works Assoc. 50:193-196.
2. Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
3. Bordner, R., and J. Winter (eds.). 1978. Microbiological methods for monitoring the environment, water, wastes. EPA-600/8-78-017 Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
4. U. S. Environmental Protection Agency. 2007. R9 Laboratory SOP1101. Membrane filtration coliform analysis.
5. U. S. Environmental Protection Agency. 1992. Manual for the certification of laboratories analyzing drinking water. EPA