m-ENDO AGAR (7724)

Intended Use

m-Endo Agar is used for the enumeration of coliforms in water by the membrane filtration method.

Product Summary and Explanation

The coliform group, especially *Escherichia coli,* are used as indicators of fecal pollution in water, for assessing the effectiveness of water treatment and disinfection, and for monitoring water quality. m-Endo Agar is prepared according to the formula of McCarthy, Delaney, and Grasso,[1] and used in testing water for coliforms by a two-step membrane filtration procedure. Lauryl Tryptose Broth is used as the preliminary enrichment, resulting in higher coliform counts.

The American Public Health Association (APHA) recommends m-Endo Agar in standard total coliform membrane filtration procedure for testing water, wastewater, and foods.[2,3] The US EPA specifies using m-Endo Agar in the total coliform methods for testing water.[4,5] m-Endo Agar is also known as LES (Lawrence Experimental Station) Endo Agar.[1]

Principles of the Procedure

Tryptose, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue, provide nitrogen, carbon, and minerals in m-Endo Agar. Yeast Extract is a source of vitamins and trace elements to stimulate bacterial growth. Potassium Phosphates are the 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 of Gram-positive bacteria. Basic Fuchsin is a pH indicator. Sodium Sulfite is added to decolorize the Basic Fuchsin solution. Ethanol aids is the homogeneity of the solution and as a selective agent. Agar is the solidifying agent.

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.

Formula / Liter

Lactose	9.4 g
Tryptose	7.5 g
Enzymatic Digest of Casein	3.7 g
Enzymatic Digest of Animal Tissue	3.7 g
Sodium Chloride	3.7 g
Potassium Phosphate, dibasic	3.3 g
Sodium Sulfite	1.6 g
Yeast Extract	1.2 g
Potassium Phosphate, monobasic	1.0 g
Basic Fuchsin	0.8 g
Sodium Deoxycholate	0.1 g
Sodium Lauryl Sulfate	0.05 g
Agar	15 g

Final pH: 7.2 ± 0.2 at 25°C

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

Supplement

Non-denatured Ethanol, 20 mL

Precautions

1. For Laboratory Use.
2. TOXIC. Harmful if swallowed, inhaled, or absorbed through skin. May cause allergic reaction and breathing difficulties to sensitive individuals. May cause irritation to skin, eyes, and respiratory tract. Possible carcinogen.

Directions

1. Suspend 51 g of the medium in 1 liter of purified water containing 20 mL of non-denatured Ethanol.
2. Heat with frequent agitation and boil to completely dissolve the medium.
3. Avoid overheating. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light purple.

Prepared Appearance: Prepared medium is red to purple and none to trace hazy.

Expected Cultural Response: Cultural response on m-Endo Agar incubated aerobically in a humidified environment at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
Escherichia coli ATCC® 25922	10 - 100	Good to excellent	Green, metallic sheen
Enterobacter aerogenes ATCC® 13048	10 - 100	Good to excellent	Green, metallic sheen
Salmonella typhimurium ATCC® 14028	10 - 100	Good to excellent	Pink to red
Staphylococcus aureus ATCC® 25923	> 1000	Inhibited	---

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure, Enrichment Method

1. Invert the dish and place an absorbent pad in the lid of a Petri dish.
2. Add 1.8 – 2.2 mL of Lauryl Tryptose Broth to each pad.
3. Place a membrane filter, through which the sample has passed, onto the pad of Lauryl Tryptose Broth.
4. Incubate aerobically for 1.5 to 2 hours at 35°C.
5. Transfer the incubated membrane filter from the Lauryl Tryptose Broth pad to a new pad which 1.8 – 2.0 mL of m-Endo Agar has been added. Proceed following the Single-Step Method, Step 4.

Single Step Method

1. Place a membrane filter absorbent pad inside a sterile 60 mm Petri dish.
2. Add 1.8 – 2.0 mL m-Endo Broth to each pad.
3. Filter the sample through a membrane filter.
4. Place membrane filter top side up on the pad using a rolling motion to avoid entrapping air bubbles.
5. Incubate aerobically in an inverted position for 20 – 24 hours at 35 ± 0.5°C.
6. Observe and count all colonies that are red and have a metallic sheen.

Results

Following incubation, examine membrane filters for presence of colored 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 coliform / 100 mL.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Protect from moisture and light.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if medium has changed from the original color. Expiry applies to medium in its intact container.

Limitations of the Procedure

1. If the inoculum is too heavy, the sheen may be suppressed.
2. Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies, including dark red or nucleated colonies without sheen.

Packaging

m-Endo Agar	**Code No.**	**7724A**	**500 g**
		7724B	**2 kg**
		7724C	**10 kg**

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. **Downes, F. P. and K. Ito** (eds.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
4. **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.
5. **U. S. Environmental Protection Agency.** 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, U. S. Environmental Protection Agency, Cincinnati, OH.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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