m-TEC AGAR (7421)

Intended Use

m-TEC Agar is used with urea for the isolation and enumeration of thermotolerant *Escherichia coli* from water using the membrane filtration technique.

Product Summary and Explanation

Escherichia coli is used as an indicator of fecal pollution in water. Several tests are available for enumerating *E. coli* based on its ability to grow at elevated temperatures and indole production.[1,2] The membrane filter procedure is recognized in Standard Methods as an alternate test procedure.[3] m-TEC is an abbreviation for membrane thermotolerant *E. coli*.

In 1981, Dufour et al. developed a simple and accurate membrane filter technique for rapid enumeration of *E. coli*.[4] In this study, the researchers were able to quantitate *E. coli* on m-TEC Agar within 24 hours without requiring subculture and identification of isolates. Dufour et al. recovered *E. coli* from marine, estuarine, and fresh water samples.[4]

Principles of the Procedure

Enzymatic Digest of Animal Tissue provides nitrogen, carbon, and minerals in m-TEC Agar. Yeast Extract is a source of vitamins and trace elements. Lactose serves as a carbon source. Potassium Phosphate is a buffering agent. Sodium Lauryl Sulfate and Sodium Deoxycholate are selective agents against gram-positive bacteria. Bromcresol Purple and Bromphenol Red are pH indicators. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Animal Tissue	5 g
Yeast Extract	3 g
Lactose	10 g
Sodium Chloride	7.5 g
Potassium Phosphate	4.3 g
Sodium Lauryl Sulfate	0.2 g
Sodium Deoxycholate	0.1 g
Bromcresol Purple	0.08 g
Bromphenol Red	0.08 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

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

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

m-TEC Agar

1. Suspend 45.3 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Dispense 4 – 5 mL amounts into 10 x 50 mm petri dishes, allow to solidify.

Urea Substrate

1. Combine 2 g urea and 10 mg phenol red in 100 mL purified water.
2. Adjust pH to 5.0 ± 0.3
3. Store at 2 - 8°C. Use within one week.

Note: Other methods may recommend an alternative pH. [3,6] Prepare substrate according to recommended guidelines.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light grey-green beige.

Prepared Appearance: Prepared medium at 45 - 50°C is clear to trace hazy and dark purple.

Expected Cultural Response: Cultural response on m-TEC Agar at 44.5°C after 24 – 48 hours incubation.

Microorganism	Response	Reactions w/ Urease Substrate
Enterococcus faecalis ATCC® 29212	inhibited	---
Escherichia coli ATCC® 8739	growth	yellow colonies
Escherichia coli ATCC® 35150	growth	yellow colonies
Escherichia coli ATCC® 35218	growth	yellow colonies
Pseudomonas aeruginosa ATCC® 27853	inhibited	---

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

Test Procedure

1. Follow membrane filter procedure described in Standard Methods.[1]
2. Incubate inoculated plates for 2 hours at 35°C to resuscitate injured cells.
3. Transfer plates to a 44.5 ± 0.5°C waterbath or incubator and incubate for 20 ± 2 hours.
4. Place a 50 mm absorbent pad into petri dish. Add approximately 2 mL of urea substrate to pad (pad should be saturated with urea substrate without any standing liquid in petri dish).
5. Transfer countable filters to pads saturated with urea substrate.
6. After 15 - 20 minutes, count all yellow to yellow-brown colonies with the aid of a stereoscopic microscope.

Results

Yellow to yellow-brown colonies (urease negative) may be presumptively identified as *E. coli*.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

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 intact container when stored as directed.

Limitations of the Procedure

1. Due to varying nutritional requirements, some strains may grow poorly or fail to grow on this medium.
2. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
3. The urease test is required to presumptively identify *E. coli*.
4. Choose a water sample size that will result in 20 - 80 colonies per filter. Higher counts may not provide accurate urease test results.
5. Do not trap air bubbles underneath the filter.

Packaging

m-TEC Agar	**Code No.**	**7421A**	**500 g**
		7421B	**2 kg**
		7421C	**10 kg**

References

1. **Mara, D. D.** 1973. A single medium for the rapid detection of *Escherichia coli* at 44°C. J. Hyg. **71**:783-785.
2. **Pugsley, A. P., L. J. Evision, and A. James.** 1973. A simple technique for the differentiation of *Escherichia coli* in water examination. Water RES. **7**:1431-1437.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Dufour, A. P., E. R. Strickland, and V. J. Cabelli.** 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. **41**:1152-1158.
5. **Dufour, A. P., and V. J. Cabelli.** 1975. Membrane filter procedure for enumerating the component genera of the coliform group in seawater. Appl. Microbiol. **29**:826-833.
6. **1996 Annual Book of ASTM Standards,** Water and Environmental Technology (PCN: 01-11-296-16). ASTM, West Conshohocken, PA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.