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 is trace to slightly hazy and dark purple.

Expected Cultural Response: Cultural response on m-TEC Agar using the membrane filtration technique and incubated aerobically at 44.5°C. Cultures were examined for growth after 20 ± 2 hours. Filters were transferred to a pad saturated with urea substrate and held at room temperature for 15 to 20 minutes. Urease – negative, thermotolerant *E. coli* colonies are yellow to yellow-brown.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Urease Reaction
Enterococcus faecalis ATCC® 29212	10^3	Marked to Complete inhibition	N/A
Escherichia coli ATCC® 8739	10 - 300	Good to excellent	Negative (Yellow to brown colonies)
Escherichia coli ATCC® 35150	10 - 300	Good to excellent	Negative (Yellow to brown colonies)
Escherichia coli ATCC® 35218	10 - 300	Good to excellent	Negative (Yellow to brown colonies)
Proteus vulgaris ATCC® 13315	10 - 300	Markedly suppressed to inhibited	N/A
Pseudomonas aeruginosa ATCC® 27853	10^3	Markedly suppressed to inhibited	Colorless

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.

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. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
2. The urease test is required to presumptively identify *E. coli*.
3. Choose a water sample size that will result in 20 - 80 colonies per filter. Higher counts may not provide accurate urease test results.
4. 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 (517)372-9200 or fax us at (517)372-2006.