m TEC Agar

Intended Use

m TEC Agar is used for isolating, differentiating and rapidly enumerating thermotolerant *Escherichia coli* from water by membrane filtration and an *in situ* urease test.

Summary and Explanation

m TEC is an acronym for "membrane Thermotolerant *E. coli*." *Escherichia coli* is widely used as an indicator of fecal pollution in water, and there are many procedures for enumerating *E. coli* based on its ability to grow at elevated temperatures and produce indole from tryptophan.[1,2] The determination of indole production in conjunction with the most-probable-number procedure often requires the use of another medium and additional incubation time.

In 1981, Dufour et al. developed a simple, accurate, nonlethal membrane filter technique for the rapid enumeration of *E. coli*.[3] This medium, m TEC Agar, quantifies *E. coli* within 24 hours without requiring subculture and identification of isolates. The authors reported that they were able to recover *E. coli* from marine, estuarine and fresh water samples.

m TEC Agar and urea substrate are recommended for use in the detection of *E. coli* when evaluating the microbiological quality of recreational waters.[4,5]

Principles of the Procedure

m TEC Agar contains sufficient nutrients to support the growth of *E. coli*. Peptone is a source of nitrogen, amino acids, carbon and amino acids. Yeast extract provides trace elements, vitamins and amino acids. Monopotassium phosphate and dipotassium phosphate offer buffering capabilities. Lactose is a fermentable carbohydrate and carbon source. Sodium lauryl sulfate and sodium desoxycholate are selective against gram-positive bacteria. Bromcresol purple and bromphenol red are indicator components. Agar is the solidifying agent.

Formula

Difco™ m TEC Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	5.0	g
Yeast Extract	3.0	g
Lactose	10.0	g
Sodium Chloride	7.5	g
Monopotassium Phosphate	1.0	g
Dipotassium Phosphate	3.3	g
Sodium Lauryl Sulfate	0.2	g
Sodium Desoxycholate	0.1	g
Bromcresol Purple	0.08	g
Bromphenol Red	0.08	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 45.3 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. (Cool to 45-50°C and dispense 4-5 mL amounts into 50 × 10 mm Petri dishes and allow to solidify; store in the refrigerator.)
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ m TEC Agar

Dehydrated Appearance:	Green to grayish tan, free-flowing, homogeneous.
Solution:	4.53% solution, soluble in purified water upon boiling. Solution is deep purple with red cast, slightly opalescent.
Prepared Appearance:	Deep purple with red cast, slightly opalescent.
Reaction of 4.53% Solution at 25°C:	pH 7.3 ± 0.2

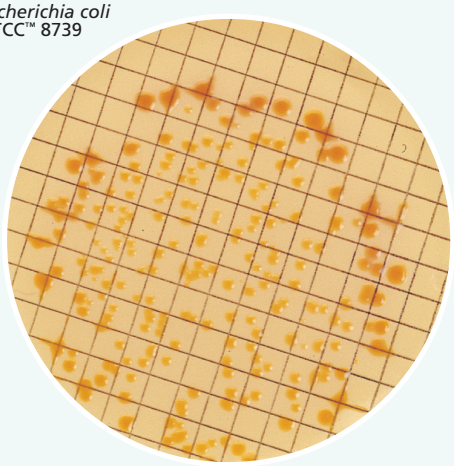
Cultural Response

Difco™ m TEC Agar

Prepare the medium per label directions. Inoculate using the membrane filter technique and incubate the plates at 35 ± 2°C for 2 hours. Transfer plates and incubate at 44.5 ± 0.5°C for 22 ± 2 hours. After incubation, remove filters and place over pads saturated with approximately 2 mL of urease substrate. Count yellow to yellow-brown colonies (urease negative) after 15-20 minutes.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Escherichia coli	8739	20-80	Good	Yellow to yellow-brown

Escherichia coli
ATCC™ 8739



Procedure

1. Follow applicable membrane filter procedures.[4,5]
2. Incubate inoculated plates for 2 hours at 35 ± 2°C to resuscitate injured cells.
3. Transfer the plates to a 44.5 ± 0.5°C waterbath or incubator and incubate for 22 ± 2 hours.
4. Transfer countable filters to pads saturated with urea substrate. Prepare urea substrate by combining 2 g urea and 10 mg phenol red in 100 mL of purified water and adjust the pH to 5.0 ± 0.2. Store at 2-8°C and use within 1 week.

NOTE: Other methods may recommend an alternative pH.[4,5] Prepare substrate according to recommended guidelines.

5. After 15-20 minutes, count all yellow to yellow-brown colonies with the aid of a stereoscopic microscope.

Expected Results

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

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. Plates containing more than 80 colonies are not recommended because high counts may not provide accurate urease test results.
4. Do not trap air bubbles underneath the filter.

References

1. Mara. 1973. J. Hyg. *71*:783.
2. Pugsley, Evision and James. 1973. Water Res. 7:1431.
3. Dufour, Strickland and Cabelli. 1981. Appl. Environ. Microbiol. *41*:1152.
4. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater. 21st ed., online. American Public Health Association, Washington, D.C.
5. American Society for Testing and Materials. 1996. Annual Book of ASTM Standards. Water and Environmental Technology (PCN: 01-110296-16). ASTM, West Conshohocken, Pa.

Availability

Difco™ m TEC Agar

SMWW

Cat. No. 233410 Dehydrated – 100 g