mTEC Agar, Modified

Intended Use

Modified mTEC Agar is a selective culture medium used for the chromogenic detection and enumeration of thermotolerant *Escherichia coli* in water by the membrane filtration technique. It conforms with U.S. Environmental Protection Agency (USEPA) Approved Method 1603: Escherichia coli (E. coli) *in Water by Membrane Filtration Using Modified membrane-Thermotolerant* Escherichia coli *Agar (modified mTEC)*.

Summary and Explanation

mTEC is an acronym for "membrane Thermotolerant *E. coli*." *E. coli* is widely used as an indicator of fecal pollution in water. This organism has a high correlation with gastroenteritis in fresh water environments.[1] In 1986, the USEPA recommended that *E. coli* be used as a bacterial water quality indicator to monitor recreational waters.[2]

Many procedures have been developed for enumerating *E. coli* based on its ability to grow at elevated temperatures and produce indole from tryptophan.[3,4] The determination of indole production in conjunction with the most-probable-number procedure often requires the use of another medium and additional incubation time.

Dufour developed a membrane filtration procedure using mTEC agar for the rapid enumeration of thermotolerant *E. coli*.[5,6] This alternative two-step test procedure quantified *E. coli* within 24 hours without requiring subculture and identification of isolates. However, the membrane filter had to be transferred after initial incubation at an elevated temperature to a urea substrate/phenol red-saturated pad.

Modified mTEC Agar was developed by the USEPA in 1998[7,8] as a single-step procedure that does not require the transfer of the membrane filter to another substrate. The modified medium contains the chromogen, 5-bromo-6-chloro-3-indolyl-β-D-glucuronide. This chromogen is catabolized to glucuronic acid by *E. coli* strains that produce the enzyme β-D-glucuronidase to form red- or magenta-colored colonies, enabling confirmatory identification of *E. coli* in 24 hours. Red or magenta colonies can be verified as *E. coli* in instances where required in evidence gathering or for performing quality control for the initial use of this test.[8]

This medium is recommended for testing the presence of *E. coli* as an indicator organism for fecal contamination in fresh recreational water. This allows for a wide range of sample volumes or dilutions to be analyzed by membrane filtration for the detection and enumeration of *E. coli* levels in water. The USEPA-published false-positive rate is <1% and false-negative rate is 4% from a variety of environmental water samples.[8]

Principles of the Procedure

Modified mTEC 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. Lactose is a fermentable carbohydrate and carbon source. Sodium chloride maintains osmotic equilibrium. Monopotassium and dipotassium phosphates offer buffering capabilities. Sodium lauryl sulfate and sodium desoxycholate are selective against gram-positive bacteria. The chromogen, 5-bromo-6-chloro-3-indolyl-β-D-

User Quality Control

Identity Specifications

Difco™ Modified mTEC Agar

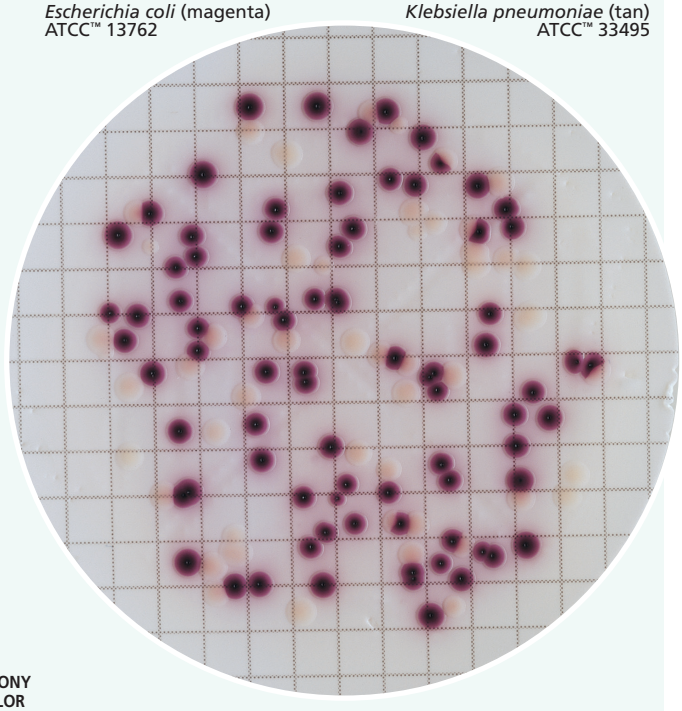
Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.56% solution, soluble in purified water upon boiling. Solution is light to medium tan, very slightly to slightly opalescent, without significant precipitate.
Prepared Appearance:	Light tan, clear to very slightly opalescent, without significant precipitate. Upon removal from 2-8°C storage, plates may exhibit a crystal precipitate that disappears upon warming to room temperature. This is a typical characteristic of the medium and is acceptable.
Reaction of 4.56% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Modified mTEC Agar

Prepare the medium per label directions. Inoculate using the membrane filtration technique and incubate at 35°C for 2 hours. Transfer plates and incubate at 44.5 ± 0.2°C for approximately 22-24 hours. Count all red or magenta colonies.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	19433	20-80	Marked to complete inhibition	–
Escherichia coli	13762	20-80	Good	Red or magenta
Proteus mirabilis	25933	20-80	Good	Tan



glucuronide, is catabolized to form glucuronic acid and a red- or magenta-colored compound by *E. coli* that produce the enzyme β-D-glucuronidase. Agar is the solidifying agent.

Formula

Difco™ Modified mTEC 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
Dipotassium Phosphate	3.3	g
Monopotassium Phosphate	1.0	g
Sodium Lauryl Sulfate	0.2	g
Sodium Desoxycholate	0.1	g
5-Bromo-6-chloro-3-indolyl-β-D-glucuronide	0.5	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 45.6 g of the powder in 1 L 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 in a water bath.
4. Determine pH of medium (remove an aliquot and cool to room temperature) and adjust pH to 7.3 ± 0.2 by aseptically adding sterile 1N NaOH.
5. Dispense 5 mL amounts into 9 × 50 mm or 15 × 60 mm plates and allow to solidify.
6. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Upon removal from 2-8°C storage, plates may exhibit a crystal precipitate that disappears upon warming to room temperature. This is a typical characteristic of the medium and is acceptable.

Procedure

1. Collect and prepare water samples in accordance with recommended guidelines.[9,10]
2. Test required sample volumes following the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.[9] Select sample volumes to produce 20-80 colonies on the membrane filter.
3. After sample has been filtered, aseptically remove membrane filter from filter base and roll it onto Modified mTEC Agar to avoid the formation of bubbles between the membrane and the agar surface.
4. Invert inoculated plates and incubate for 2 hours at 35 ± 0.5°C to resuscitate injured cells.
5. After a 2-hour incubation at 35 ± 0.5°C, transfer the plates to a plastic bag, seal the bag, and place it onto a rack in a 44.5 ± 0.2°C water bath for 22 - 24 hours.
6. After the 22-24 hour incubation, remove the plates from the water bath and count and record the number of red or magenta colonies using an illuminated lens with a 2-5× magnification or a stereoscopic microscope.
7. Calculate and report the number of *E. coli* colonies per 100 mL of sample.

Expected Results

Red to magenta colonies may be presumptively identified as *E. coli*. Refer to the USEPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.[11]

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. 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 test results.
3. Minimize the exposure of Modified mTEC Agar to light before and during incubation, as light may destroy the chromogen.

References

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5. Eaton, Clesceri, and Greenberg (ed.). 1995. Standard methods for the examination of water and wastewater. 19th ed. American Public Health Association, Washington, D.C.
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8. U.S. Environmental Protection Agency. 2002. Method 1603: *Escherichia coli (E. coli)* in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar (modified mTEC). Publication EPA-821-R-02-023. USEPA Office of Water, Office of Science and Technology, Washington, DC.
9. 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.
10. ASTM International. 2002. Annual book of ASTM standards. Water and environmental technology. ASTM International, West Conshohocken, Pa.
11. Bordner, Winter and Scarpino (ed.). 1978. Microbiological methods for monitoring the environment: water and wastes. Publication EPA-600/8-78/017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Availability

Difco™ Modified mTEC Agar

EPA

Cat. No.	214884	Dehydrated – 100 g
	214880	Dehydrated – 500 g

BBL™ Modified mTEC Agar

EPA

Cat. No.	215044	Prepared Plates – Pkg. of 20*
	215046	Prepared Plates – Ctn.of 100*

**Store at 2-8°C.*