mEI Agar

Intended Use

mEI Agar is a selective culture medium used for the chromogenic detection and enumeration of enterococci in water by the single-step membrane filtration technique. It conforms with U.S. Environmental Protection Agency (USEPA) Approved Method 1600: *Enterococci in Water by Membrane Filtration Using membrane*-Enterococcus *Indoxyl*-β-*D-Glucoside Agar (mEI)*.

Summary and Explanation

Enterococci are found in the feces of humans and other warm-blooded animals. Although some strains are ubiquitous and are not related to fecal pollution, the presence of enterococci in water is an indication of fecal pollution and the possible presence of enteric pathogens.[1] In epidemiological studies conducted by the USEPA, it was found that the presence of enterococci had a higher correlation with swimming-associated gastroenteritis in fresh and marine water environments than fecal coliforms.[2] In 1986, the USEPA recommended that both *Escherichia coli* and enterococci be used as bacterial water quality indicators to monitor recreational waters.[3]

A two-step membrane filter (MF) method[4] was developed by Levin et al. to measure enterococci in fresh and marine recreational waters. Using mE agar, the method required a 48-hour incubation and a transfer of the membrane to another substrate medium, Esculin Iron Agar, to differentiate enterococci.

In 1997, the USEPA improved on the mE agar formulation by reducing the triphenyltetrazolium chloride component and adding the chromogen, indoxyl-β-D-glucoside. The new medium, mEI Agar,[1,5] was developed as a single-step procedure that does not require the transfer of the membrane filter to another substrate. Observation of a blue halo around colonies in 24 hours is confirmatory for the presence of enterococci. A wide range of sample volumes or dilutions can

User Quality Control

Identity Specifications

Difco™ mEI Agar

Dehydrated Appearance:	Light to medium beige, free-flowing, homogeneous.
Solution:	7.2% solution, soluble in purified water upon boiling. Solution is medium to dark amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent.
Reaction of 7.2% Solution at 25°C:	pH 7.1 ± 0.2

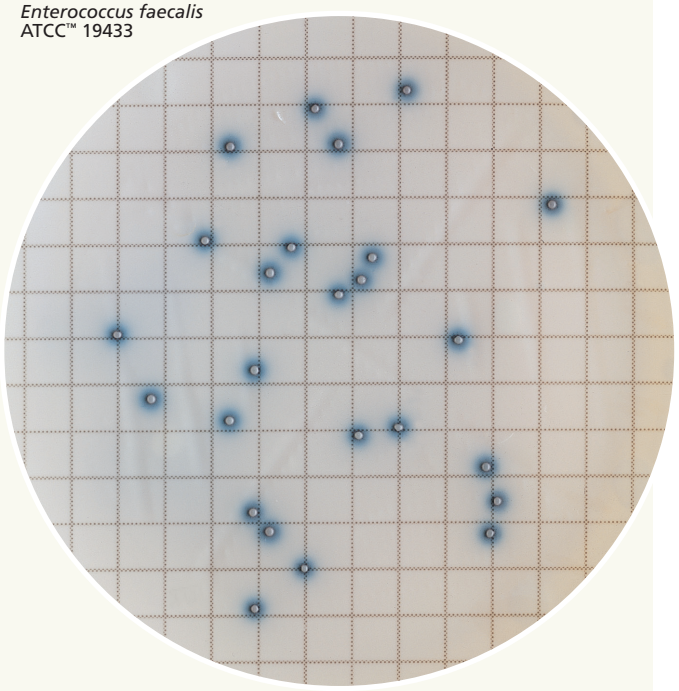
Cultural Response

Difco™ mEI Agar

Prepare the medium per label directions. Inoculate and incubate at 41 ± 0.5°C for 24 ± 2 hours. Count all colonies with blue halos.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
Enterococcus faecalis	19433	20-80	Good	Blue halo
Enterococcus faecium	19434	20-80	Good	Blue halo
Escherichia coli	25922	20-80	Marked to complete inhibition	–

Enterococcus faecalis
ATCC™ 19433



be tested by this single-step MF procedure for the detection and enumeration of enterococci in potable, fresh, estuarine, marine and shellfish-growing waters.

BD mEI Agar conforms to the 1986 revisions to the bacteriological ambient water quality criteria, that included the indicator bacteria *E. coli* and enterococci, which provide better correlation with swimming-associated gastrointestinal illness. In response to this health risk, the USEPA established the Beaches Environmental Assessment Closure and Health (Beach) Program. This method is published for use in the Beach Program.[5]

The USEPA published false-positive rate is 6.0% and false-negative rate is 6.5%.[5] Colonies having a blue halo can be verified as enterococci by appropriate biochemical procedures in instances where required in evidence gathering or for performing quality control for the initial use of the test.[5]

Principles of the Procedure

mEI Agar contains peptone that supplies nitrogen and carbon compounds. Sodium chloride maintains osmotic equilibrium. Esculin is hydrolyzed by enterococci to form esculetin and dextrose. Cycloheximide inhibits fungi. Sodium azide acts as a selective agent to inhibit gram-negative bacteria. Yeast extract provides trace elements, vitamins and amino acids. The addition of the chromogen indoxyl-β-D-glucoside results in the production of an insoluble indigo blue complex by β-D-glucosidase-positive enterococci, which diffuses into the surrounding medium, forming a blue halo around the colony.[6] Agar is incorporated into the medium as the solidifying agent.

Formula

Difco™ mEI Agar

Approximate Formula* Per Liter		
Peptone	10.0	g
Sodium Chloride	15.0	g
Esculin	1.0	g
Cycloheximide	0.05	g
Sodium Azide	0.15	g
Yeast Extract	30.0	g
Indoxyl-β-D-glucoside	0.75	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 72 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 and cool in a 50°C water bath.
4. Prepare a solution of 0.24 g of nalidixic acid in 5 mL of purified water. Add a few drops of 0.1 N NaOH to dissolve. Add this solution to 1 L of mEI medium.
5. Add 0.02 g of triphenyltetrazolium chloride separately to the mEI medium and mix well.
6. Dispense 5 mL amounts into 9 × 50 mm or 15 × 60 mm plates and allow to solidify.
7. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Collect and prepare water samples in accordance to recommended guidelines.[7,8]
2. Test sample volumes following the membrane filtration procedure described in *Standard Methods for the Examination of Water and Wastewater*.[7] Select sample volumes to produce 20-60 colonies on the membrane filter.
3. After sample has been filtered, aseptically remove membrane filter from filter base and roll it onto mEI Agar to avoid the formation of bubbles between the membrane and the agar surface.
4. Invert inoculated plates and incubate for 24 ± 2 hours at 41 ± 0.5°C.
5. After incubation, count and record the number of colonies with a blue halo using an illuminated lens with a 2-5× magnification.
6. Calculate and report the number of enterococci colonies per 100 mL of sample.

Expected Results

Colonies with a blue halo regardless of color may be presumptively identified as enterococci. Refer to the USEPA Microbiology Methods Manual, Part II, Section C, 3.5 for general counting rules.[9]

Limitations of the Procedure

1. Choose a water sample size that will result in 20-60 colonies per filter.
2. Minimize the exposure of mEI Agar to light before and during incubation, as light may destroy the chromogen.
3. Overheating may cause darkening of the medium.[2]

References

1. U.S. Environmental Protection Agency. 1997. Method 1600: Membrane filter test method for enterococci in water. Publication EPA-821-R-97-004a. Office of Water, USEPA, Washington, D.C.
2. U.S. Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: enterococci and *Escherichia coli*. Publication EPA/821/R-97/004. Office of Science and Technology, USEPA, Washington, D.C.
3. U.S. Environmental Protection Agency. 1986. Bacteriological ambient water quality criteria: availability. Fed. Reg. *51*(45):8012.
4. Levin, Fischer and Cabelli. 1975. Appl. Microbiol. *30*:66.
5. U.S. Environmental Protection Agency. 2002. Method 1600: Enterococci in water by membrane filtration using membrane-enterococcus indoxyl-β-D-glucoside agar (mEI). Publication EPA-821-R-02-022. USEPA Office of Water, Office of Science and Technology, USEPA, Washington, DC.
6. Messer and Dufour. 1998. Appl. Environ. Microbiol. *64*:678.
7. 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.
8. ASTM International. 2002. Annual book of ASTM standards. Water and environmental technology. ASTM International, West Conshohocken, Pa.
9. 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™ mEI Agar

EPA SMWW

Cat. No.	214885	Dehydrated – 100 g
	214881	Dehydrated – 500 g

BBL™ mEI Agar

Cat. No.	215045	Prepared Plates – Pkg. of 20*
	215047	Prepared Plates – Ctn. of 100*

**Store at 2-8°C.*