m E Agar • Esculin Iron Agar

Intended Use

m E Agar is used with nalidixic acid and triphenyltetrazolium chloride in isolating and differentiating enterococci from water by membrane filtration and in an *in situ* esculin test on Esculin Iron Agar.

Esculin Iron Agar (EIA substrate) is used for enumerating enterococci from water by membrane filtration based on esculin hydrolysis.

Summary and Explanation

Enterococcus species are a subgroup of fecal streptococci that includes *E. faecalis*, *E. faecium*, *E. gallinarum* and *E. avium*.[1] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10°C and 45°C.[1] The enterococci portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1]

Slanetz and Bartley[2] first reported quantitating enterococci by the membrane filter method in 1957. A wide range of levels of enterococci in water can be enumerated and detected because small or large volumes of water can be analyzed by the membrane filter technique.[3] In 1961, Kenner et al.[4] described the KF method for detecting and quantitating fecal streptococci. In 1966, Isenberg et al.[5] reported a plating procedure with differentiation based on esculin hydrolysis. Levin, Fischer and Cabelli[6] compared the KF method with Isenberg's plating method, and found the latter method resulted in better recovery of fecal streptococci. They developed m E Agar as a primary isolation medium for enterococci, and Esculin Iron Agar as an *in situ* substrate test medium for identifying organisms capable of hydrolyzing esculin.[6]

Two research projects by the U.S. Environmental Protection Agency (USEPA) evaluated the relationships between swimming-associated illness and the ambient densities of indicator bacteria.[7,8] The studies demonstrated that enterococci have a better correlation with swimming-associated illness for both marine and fresh waters than fecal coliforms. *Escherichia coli* has a correlation in fresh water equal to enterococci but does not correlate as well in marine waters.[7,8] This suggests that enterococci may be better indicator organisms for some recreational waters.[7,8]

m E Agar and Esculin Iron Agar are prepared according to the formulas specified in standard methods.[1] These media are used in the membrane filter technique for the isolation of fecal streptococcus and enterococcus groups.[1] This procedure can be used to test marine and fresh water sources.

m E Agar with the addition of 0.075% indoxyl-β-D-glucoside (m EI Agar) is recommended by the USEPA as a one-step procedure for the isolation and identification of enterococci in recreational water.[9] This method is used in the USEPA Beaches Environmental Assessment Closure and Health (BEACH) Program. The use of m EI Agar eliminates the necessity of transferring the incubated membrane to Esculin Iron Agar.

Principles of the Procedure

m E Agar is a highly selective and differential primary isolation medium that supports good growth of enterococci. Peptone and yeast extract provide carbon, nitrogen, minerals, vitamins and other growth factors for organism growth. Sodium chloride maintains the osmotic balance of the medium. Nalidixic acid and sodium azide act as selective agents to inhibit gram-negative bacteria. Cycloheximide inhibits fungi. At the concentration in the formula, 2,3,5-triphenyltetrazolium chloride (TTC) dyes enterococci colonies. TTC slightly inhibits growth of other microorganisms. In addition, the elevated incubation temperature of 41°C inhibits some indigenous microbial flora. Esculin is hydrolyzed by enterococci to form esculetin and dextrose. The esculetin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a black to reddish brown complex that appears in the medium surrounding the colonies. The production of black to reddish brown complex verifies the colonies as enteroccci and facilitates their enumeration. Agar is the solidifying agent.

User Quality Control

Identity Specifications

Difco™ m E Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	7.12% solution, soluble in purified water upon boiling. Solution is light to medium amber with bluish cast, very slightly opalescent.
Prepared Appearance:	Light to medium amber with blue cast, slightly opalescent.
Reaction of 7.12% Solution at 25°C:	pH 7.1 ± 0.2

Difco™ Esculin Iron Agar

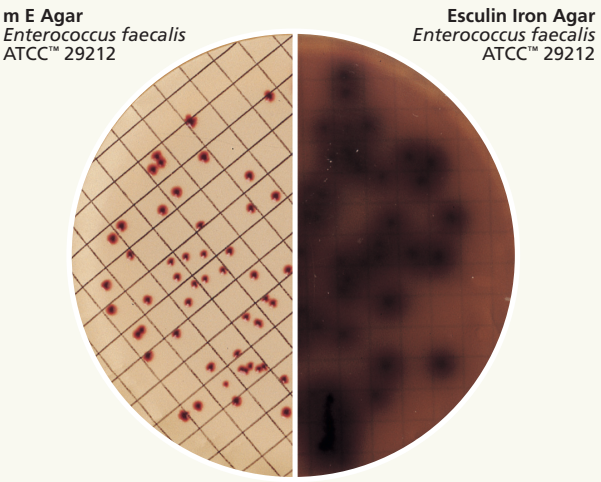
Dehydrated Appearance:	Tan to dark tan, free-flowing, homogeneous.
Solution:	1.65% solution, soluble in purified water upon boiling. Solution is medium amber with bluish cast, very slightly opalescent.
Prepared Appearance:	Medium amber with blue cast, slightly opalescent.
Reaction of 1.65% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Difco™ m E Agar and Difco™ Esculin Iron Agar

Prepare m E Agar per label directions and pour into 9 x 50 mm plates. Dilute the test organisms and filter through membrane filters. Place the filters on m E Agar plates and incubate the plates in an upright position for 48 hours at 41 ± 0.5°C. Remove the filters and place over prepared Esculin Iron Agar plates. After 20 minutes of incubation at 41 ± 0.5°C, count colonies giving positive esculin reaction (formation of black or reddish brown precipitate).

ORGANISM	ATCC™	INOCULUM CFU/10 mL	RECOVERY ON M E AGAR	REACTION ON ESCULIN IRON AGAR
Enterococcus faecalis	29212	20-60	Good/pink to red colonies	Black or reddish brown ppt
Enterococcus faecalis	33186	20-60	Good/pink to red colonies	Black or reddish brown ppt
Escherichia coli	25922	20-60	Marked to complete inhibition	None



Formulae

Difco™ m E Agar

Approximate Formula* Per Liter

Yeast Extract	30.0	g
Peptone	10.0	g
Sodium Chloride	15.0	g
Esculin	1.0	g
Cycloheximide	0.05	g
Sodium Azide	0.15	g
Agar	15.0	g

Difco™ Esculin Iron Agar

Approximate Formula* Per Liter

Esculin	1.0	g
Ferric Ammonium Citrate	0.5	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

Difco™ m E Agar

1. Suspend 7.12 g of the powder in 100 mL 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°C.
4. Add 0.024 g of nalidixic acid and 1.5 mL TTC Solution 1% (0.015 g Triphenyl Tetrazolium Chloride). Adjust to pH 7.1 if necessary.
5. Dispense 4-6 mL into 9 × 50 mm Petri dishes.
6. Test samples of the finished product for performance using stable, typical control cultures.

NOTE: Nalidixic acid is soluble in water with an alkaline pH.

Difco™ Esculin Iron Agar

1. Suspend 1.65 g of the powder in 100 mL of purified water (16.5 g 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°C.
4. Dispense 4-6 mL into 9 × 50 mm Petri dishes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Follow the membrane filter procedure described in *Standard Methods for the Examination of Water and Wastewater*.[1]
2. Choose a sample size so that 20-60 colonies will result.
3. Place the filter on an m E Agar plate and incubate for 48 hours at 41 ± 0.5°C.
4. After incubation, remove the filter from m E Agar and place on an Esculin Iron Agar plate.
5. Incubate Esculin Iron Agar at 41 ± 0.5°C for 20 minutes.

Expected Results[1]

Pink to red enterococci develop a black or reddish-brown precipitate on the underside of the filter. Count colonies using a fluorescent lamp and a magnifying lens. Report results as estimated number or organisms per 100 mL of water.

Limitations of the Procedure

1. m E Agar and Esculin Iron Agar should be used in sequence.
2. Incubation at 41 ± 0.5°C is recommended.
3. Approximately 10% false-positive esculin reactions may be expected. When used as m EI Agar, USEPA reports a 6.0% false positive and 6.5% false negative rate with m E Agar.

References

1. 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.
2. Slanetz and Bartley. 1957. J. Bacteriol. *74*:591.
3. American Society for Testing and Materials. 1996. Annual book of ASTM standards. Section 11, Water and environmental technology. PCN: 01-110296-16. ASTM, West Conshohocken, Pa.
4. Kenner, Clark and Kabler. 1960. Appl. Microbiol. *9*:15.
5. Isenberg, Goldberg and Sampson. 1970. Appl. Microbiol. *20*:433.
6. Levin, Fischer and Cabelli. 1975. Appl. Microbiol. *30*:66.
7. Cabelli. 1981. Health effects criteria for marine recreational waters. U.S. Environmental Protection Agency. EPA-600/1-80-031. Cincinnati, Ohio.
8. Dufour. 1983. Health effects criteria for fresh recreational waters. U.S. Environmental Protection Agency. Cincinnati, Ohio.
9. U.S. Environmental Protection Agency. 1997. EPA method 1600: Membrane filter test method for enterococci in water. USEPA. EPA-821-R-97-004. Washington, D.C.

Availability

Difco™ m E Agar

EPA SMWW

Cat. No. 233310 Dehydrated – 100 g
233320 Dehydrated – 500 g

Difco™ Esculin Iron Agar

EPA SMWW

Cat. No. 248810 Dehydrated – 100 g

Difco™ TTC Solution 1%

EPA SMWW

Cat. No. 231121 Tube – 30 mL*

**Store at 2-8°C.*