m Enterococcus Agar

Intended Use

m Enterococcus Agar, also referred to as m Azide Agar, is used for isolating and enumerating enterococci in water and other materials by membrane filtration or pour plate technique.

Summary and Explanation

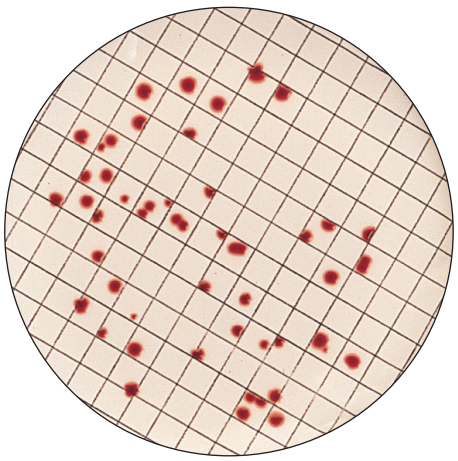
The enterococcus group is a subgroup of the fecal streptococci that includes *E. faecalis, E. faecium, E. gallinarum,* and *E. avium*.[1] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C.[1] The enterococcal portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1] m Enterococcus Agar is used in standard methods for the detection of fecal streptococcus and enterococcus groups using the membrane filtration technique.[1]

m Enterococcus Agar was developed by Slanetz et al.[2] for the enumeration of enterococci by the membrane filtration technique. A modification of m Enterococcus Agar, adding triphenyltetrazolium chloride (TTC), was described by Slanetz and Bartley[3]. This modified medium proved to be a superior membrane filtration medium for the enumeration of enterococci. Increased recovery and larger colonies were obtained by incubating the inoculated membranes on the agar surface instead of on pads saturated with liquid medium. The membrane filtration method has the advantages of being simpler to perform, not requiring confirmation and permitting a direct count of enterococci in 48 hours. Burkwell and Hartman[4] added 0.2% sodium carbonate and 0.05% polysorbate 80 to m Enterococcus Agar to increase the sensitivity for the direct plating method.

Principles of the Procedure

Peptone provides nitrogen, minerals and amino acids. Yeast extract is the vitamin source and dextrose supplies carbon. Dipotassium phosphate acts as a buffer for the medium.

Enterococcus faecalis
ATCC™ 19433



User Quality Control

Identity Specifications

Difco™ m Enterococcus Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 4.2% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ m Enterococcus Agar

Prepare the medium per label directions. Inoculate using the membrane filter technique. Incubate in humid atmosphere at 35 ± 0.5°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	19433	20-60	Good	Light pink to red
Enterococcus faecalis	29212	20-60	Good	Light pink to red
Escherichia coli	25922	10^3	Marked to complete inhibition	–

Sodium azide is the selective agent to suppress the growth of gram-negative organisms. Agar is the solidifying agent. Triphenyl tetrazolium chloride (TTC) is the dye used as an indicator of bacterial growth. TTC is reduced to the insoluble formazan inside the bacterial cell, resulting in the production of red colonies.

Formula

Difco™ m Enterococcus Agar

Approximate Formula* Per Liter

Tryptose	20.0	g
Yeast Extract	5.0	g
Dextrose	2.0	g
Dipotassium Phosphate	4.0	g
Sodium Azide	0.4	g
Agar	10.0	g
2,3,5-Triphenyl Tetrazolium Chloride	0.1	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 42 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. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and dispense into 50 x 9 mm Petri dishes to a depth of 4-5 mm (approximately 4-6 mL).
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Collect water samples as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9060[1] or by laboratory policy.

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9230C.[1]
2. Choose a sample size so that 20-60 colonies will result.
3. Transfer the filter to agar medium in a Petri dish, avoiding air bubbles beneath the membrane.
4. Let plates stand for 30 minutes.
5. Invert plates and incubate at 35 ± 0.5°C for 48 hours.

Direct plating procedure

1. Inoculate medium with a specimen using the streak plate method.
2. Incubate plates at 35 ± 2°C for 24-48 hours.

Expected Results[1]

Count all light and dark red colonies as enterococci. Count colonies using a fluorescent lamp and a magnifying lens.

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, Bent and Bartley. 1955. Public Health Rep. *70*:67.
3. Slanetz and Bartley. 1957. J. Bacteriol. *74*:591.
4. Burkwell and Hartman. 1964. Appl. Microbiol. *12*:18.

Availability

Difco™ m Enterococcus Agar

ISO SMWW

Cat. No.	274610	Dehydrated – 100 g
	274620	Dehydrated – 500 g