Esculin Iron Agar

M1044

Esculin Iron Agar is used for verification of enterococcal colonies on membrane filters through which water samples have been filtered and which have been incubated on M-Enterococcus Agar, Modified (M1048).

Composition**

Ingredients	Gms / Litre
Esculin	1.000
Ferric ammonium citrate	0.500
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 16.5 grams in 1000 ml distilled water. Heat to boiling with frequent stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C and pour into sterile Petri plates to a depth of 4-5 mm.

Principle And Interpretation

Enterococci are indicators of the sanitary quality of recreational waters, since they occur in faeces of humans and warm-blooded animals (1). Detection and quantitation of Enterococci is necessary because gastroenteritis is associated with swimming in recreational water, which is dependant of enterococcal densities (2). Esculin Iron Agar is used in conjunction with M-Enterococcus Agar, Modified, (M1048) for verification of enterococcal colonies in fresh and marine recreational water, as recommended by APHA (3). Esculin in the medium is hydrolyzed by Enterococci to form esculetin and dextrose. Esculetin reacts with the iron salt (ferric ammonium citrate) and produces a dark brown to black complex, which appears around the colonies.

In the membrane filtration technique, two media, namely M-Enterococcus Agar, Modified (M1048) and Esculin Iron Agar (M1044) are used in conjunction, where the former serves as a selective medium while the later confirms the identification of colonies on the basis of its ability to hydrolyze esculin. The membrane filter used to filter the test water sample is aseptically placed on M-Enterococcus Agar, Modified (M1048) and incubated at 40-42°C for 48 hours. After incubation the membrane is aseptically transferred to Esculin Iron Agar (M1044) plate and incubated at 40-42°C for 20 minutes. After incubation count and record the number of pink to red colonies with black or reddish brown precipitate on the underside of the membrane.If required, magnifying glass or fluorescent lamp may be used for counting the visible colonies. Following formula is used for the final calculation (3).

Enterococci / 100 ml = No of enterococcal colonies/ Volume of sample filtered X 100

Quality Control

Appearance
Light yellow to light brown homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Medium amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 1.65% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH
6.90-7.30

Cultural Response

M1044: Cultural characteristics observed after an incubation at 40-42°C for 18-24 hours on M-Enterococcus Agar, Modified (M1048) and after 20 minutes at 40-42°C on Esculin Iron Agar (M1044).

Organism	Growth	Colour of Colony	Esculin Hydrolysis
Escherichia coli ATCC 25922	none-poor		negative reaction
Enterococcus faecalis ATCC 29212	good-luxuriant	pink to red	positive reaction, brown to black precipitate around colonies.

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. U. S. Environmental Protection Agency, 1997, EPA Method 1600: Membrane Filter Test Method for Enterococci in Water, EPA-821-R-97-004, Washington, D.C.
2. Cabelli et al, 1979, Am. J. Public Health, 69:690.
3. Eaton A. D., Clesceri L. S. and Greenberg A. E., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.

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