Esculin Iron Agar

M1044

Intended Use:

Recommended for verifying 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 purified / distilled water. Heat to boiling with frequent stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°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

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(3) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

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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