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-Entercoccus Agar, Modified (M1048).

Composition**

Ingredients	g / L
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).

$$\text{Enterococci/100ml} = \frac{\text{No. of enterococcal colonies}}{\text{Volume of sample filtered (ml)}} \times 100$$

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 (4).

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.

Limitations :

1. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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 25ÁC. pH : 7.1Ñ0.2

pH

6.90-7.30

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Colour of Colony	Esculin Hydrolysis
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor		negative reaction
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	pink to red	positive reaction, brown to black precipitate around colonies.

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

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.

4.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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