M-Enterococcus Agar Base, Modified

M1048

Intended Use:

Recommended for recovery of Enterococci in water samples using membrane filtration technique, along with Esculin Iron Agar for identification.

Composition**

Ingredients	Gms / Litre
Gelatin peptone	10.000
Yeast extract	30.000
Sodium chloride	15.000
Sodium azide	0.150
Esculin	1.000
Cycloheximide	0.050
Nalidixic acid	0.250
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 71.45 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 15 ml of sterile 1% TTC Solution (FD057). Mix well and pour into sterile Petri plates.

Principle And Interpretation

M-Enterococcus Agar Base, Modified was developed for the enumeration and identification of Enterococci in sanitary quality of recreational water according to USEPA (7). Cabelli et al (3) established the correlations between enterococcal densities and gastroentitis associated with swimming in recreational waters. This medium is also useful for the detection and quantitiation of Enterococci from potable, fresh, esturine, marine and shellfish growing waters (1). This medium contains gelatin peptone and yeast extract, which provide the carbonaceous and nitrogenous nutrients, minerals, vitamins and other growth factors. Sodium chloride maintains isotonic conditions of the medium beside the provision of essential ions to variety of organisms.

Sodium azide, Cycloheximide and Nalidixic acid inhibit large number of bacteria and fungi and thus makes the medium selective. Esculin is hydrolyzed by bacterial enzyme to esculetin and dextrose (6). TTC is reduced by Enterococci to insoluble formazan, a red coloured complex inside the bacterial cell resulting in pink to red coloured colonies.

Type of specimen

Water samples

Specimen Collection and Handling:

In this membrane filter procedure, two culture media namely M-Enterococcus Agar Base, Modified and Esculin Iron Agar (M1044) are used for the enumeration and identification of Enterococci where M-Enterococcus Agar, Modified serves as a selective medium while Esculin Iron Agar (M1044) confirms the identification of colonies on the basis of ability of organisms to hydrolyze esculin. Initially the membrane filter that has been used to filter the water is placed on to M-Enterococcus Agar, Modified plate and incubated at 41°C for 48 hours and after incubation transferred to the Esculin Iron Agar plate and further incubated at 41°C for 20 minutes.

After incubation, count and record the colonies on those membrane filters containing 20 - 60 pink to red colonies with black or reddish-brown precipitate on the underside of the membrane. If required, magnification glass and fluorescent lamp may be used for counting the visible colonies. Following formula is used for the final calculation (2).

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

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 7.14% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation at 40-42°C for 48 hours with added sterile 1% TTC solution (FD057) on M-Enterococcus Agar Base, Modified (M1048) and at 40-42°C for 20 minutes on Esculin Iron Agar (M1044).

Organism	Inoculum (CFU)	Growth	Colour of colony (on membrane)	Esculin hydrolysis
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	-	
Enterococcus faecalis ATCC 29212 (00087*)	50-100	good-luxuriant	pink-red (on membrane filter)	positive reaction, black to brown precipitate on the underside of membrane filter under individual colony

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°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 (4,5).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

2. Bordner, Winter and Scarpino (Eds.), 1978, EPA - 600/8-78-017 USEPA, Office of Research and Development, Environmental Monitoring and Support Laboratory Cincinnati, Ohio.

3. Cabelli, Dufour, Levin, et al, 1979, Am. J. Public Health 69:690.

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

5. 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.

6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

7. U. S. Environmental Protection Agency, 1997, EPA Method 1600: Membrane Filter test Methods for Enterococci in water, USEPA, EPA-821-R-97-004, Washington D. C.

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Disclaimer :

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