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 (1). Cabelli et al (2) 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 (3).

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 (5). 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 (4).

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

:DUQLQJ DQG 3UHFDXWLRQV

5HDG WKH ODEHO EHIRUH RSHQLQJ WKH FRQWDLQHU :HDU SURWHFWLYH JORYHV SURWHFWLYH FORWKLQJ H\H SURWHFWLRQ IDFH SURWHFWLRQ)ROORZ JRRG PLFURELRORJLFDO ODE SUDFWLFHV ZKLOH KDQGOLQJ VSHFLPHQV DQG FXOWXUH 6WDQGDUG SUHFDXWLRQV DV SHU HVWDEOLVKHG JXLGHOLQHV VKRXOG EH IROORZHG ZKLOH KDQGOLQJ FOLQLFDO VSHFLPHQV 6DIHW\ JXLGHOLQHV PD\ EH UHIHUUHG LQ LQGLYLGXDO VDIHW\ GDWD VKHHWV

Limitations :

1. Well isolated colonies must be used to avoid erroreous results.

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 40-42oC for 20 minutes on Esculin Iron Agar (M1044).

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

Reference

1.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.
2.Cabelli, Dufour, Levin, et al, 1979, Am. J. Public Health 69:690.
3.Greenberg A. W., Eaton A. D. and Clesceri L. S. (Eds.), 1998, Standard Methods for the Examination of Water and Waste Water, 20th ed., APHA, Washington DC.
4.Bordner, Winter and Scarpino (Eds.), 1978, EPA - 600/8-78-017 USEPA, Office of Research and Development, Environmental Monitoring and Support Laboratory Cincinnati, Ohio.
5.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

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