Technical Information

M-Enterococcus Agar Base, Modified

Product Code: DM 2048

Application: - M-Enterococcus Agar, Modified is used for the recovery of Enterococci in water samples using membrane filter technique alongwith Esculin Iron Agar for the identification.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	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

Principle & Interpretation

M-Enterococcus Agar Base, Modified was devised 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 the growth of 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.

In this membrane filter procedure, two culture media namely M-Enterococcus Agar Base, Modified and Esculin Iron Agar (DM2044) are used for the enumeration and identification of Enterococci where M-Enterococcus Agar, Modified serves as a selective medium while Esculin Iron Agar (DM2044) 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].

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

Methodology

Suspend 71.45 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C and aseptically add 15 ml of sterile 1% TTC Solution (MS2057). Mix well and pour into sterile Petri plates.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Cycloheximide is very toxic. Avoid skin contact or aerosol formation and inhalation.

Quality Control

Physical 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 Range 6.90-7.30

Cultural Response/ characteristics

DM 2048: Cultural characteristics observed after an incubation at 40-42°C for 48 hours with added sterile 1% TTC solution (MS2057) on M-Enterococcus Agar Base, Modified (DM2048) and at 40-42°C for 20 minutes on Esculin Iron Agar (DM2044).

Organism	Inoculum (CFU)	Growth	Colour of colony (on membrane)	Esculin hydrolysis
Escherichia coli ATCC 25922	>=10^3	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

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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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