M-Enterococcus Agar Base

M1108

M-Enterococcus Agar Base is a selective medium used in membrane filtration procedures as well as a direct plating medium, for isolation and enumeration of Enterococci in water, sewage, food or other materials.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Yeast extract	5.000
Dextrose	2.000
Dipotassium phosphate	4.000
Sodium azide	0.400
2,3,5-Triphenyl tetrazolium chloride	0.100
Agar	10.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 41.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT OVERHEAT OR AUTOCLAVE. Add 0.5 ml polysorbate 80 and 2 ml of 10% aqueous solution of sodium carbonate, if desired. Dispense into 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.

Principle And Interpretation

This medium was devised by Slanetz, Bent and Bartley (1) for the enumeration of Enterococci by the membrane filter technique. Slanetz and Bartley (2) modified it by the addition of Triphenyl Tetrazolium Chloride (TTC) and found that larger colonies and higher counts were obtained by placing membrane filters directly on the agar surface than on pads saturated with liquid medium. This medium is highly selective. Burkwell and Hartman used polysorbate 80 (0.5 ml/l) and sodium carbonate (2 ml of a 10% aqueous solution per litre) to increase sensitivity for direct plating of foods and increasing colony size (3). As per standard methods, M-Enterococcus Agar is used for the detection of faecal *Streptococcus* and *Enterococcus* groups using the membrane filtration technique (4).

Casein enzymic hydrolysate and papaic digest of soyabean meal, yeast extract, dextrose act as source of carbon, nitrogen and other essential growth nutrients. Sodium azide inhibits gram-negative organisms. TTC serves as a rapid indicator of bacterial growth. TTC is reduced to insoluble formazan inside the bacterial cells, which gives red colouration to colonies.,,

For filtration, choose a sample size so that 20-60 colonies will result. Transfer the filter aseptically to agar medium, avoiding air bubbles beneath the membrane. The medium can also be directly inoculated by streaking the specimen and incubating the plates at 35-37°C for 24-48 hours. Incubate the plates at 35°C for 48 hours. After incubation, count all light and dark red colonies as Enterococci.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium
Light pink coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24 - 48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Colour of colony (on membrane)
Escherichia coli ATCC 25922	>=10^3	inhibited	
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant	pink - dark red (maroon)

Storage and Shelf Life
Store below 30°C in tightly closed container and use freshly prepared medium. Use before expiry date on the label.

Reference
1. Slanetz, Bent and Bartley, 1955, Publ. Health. Rep., 70:67.
2. Slanetz and Bartley, 1957, J. Bact., 74:591.
3. Burkwell and Hartman, 1964, Appl. Microbiol., 12:18.
4. Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA, Washington, D.C.

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