Enterococcus Confirmatory Agar

M392

Enterococcus Confirmatory Agar is recommended for confirming the presence of Enterococci in water supplies and other sources.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	5.000
Dextrose	5.000
Sodium azide	0.400
Methylene blue	0.010
Agar	15.000
Final pH (at 25°C)	8.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.41 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in 100 ml quantities in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the agar tubes to cool in a slanted position.

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

Enterococcus Confirmatory Agar formulated by Sandholzer and Winter (2) is used for the detection of Enterococci in water supplies, swimming pools, sewage etc. Enterococci are found as normal flora in the gastrointestinal tracts of humans and animals. They are becoming increasingly important agents of human diseases largely because of their resistance to antimicrobial agents to which other Streptococci are generally susceptible (3). The *Enterococcus* is a subgroup of the fecal Streptococci group that includes *Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum* , and *Enterococcus avium* (1). Enterococci are differentiated from other Streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C (1).

The ability of organisms to grow in the presence of variable amounts of sodium chloride is a test that has been used to characterize several bacteria, including the viridans Streptococci. It is useful for presumptive identification of the Entercoccal group D organisms which have the specific ability to grow in the presence of 6.5% NaCl incorporated into the medium. A positive test is the presence of bacterial growth in the medium. If the organism is bile esculin positive and grows in 6.5% NaCl broth, the organism is an *Enterococcus* species and if the organism is bile esculin positive and fails to grow in the 6.5% NaCl broth, the organism belongs to a group D Streptococci. The enterococcal portion of the faecal *Streptococcus* group is a valuable bacterial indicator for determining the extent of faecal contamination of recreational surface waters (1).

Casein enzymic hydrolysate, yeast extract, dextrose provide essential growth nutrients for Enterococci. Sodium azide inhibits contaminating flora. The positive presumptive tests are confirmed by inoculating from Enterococcus Presumptive Broth (M419) to Enterococcus Confirmatory slant-broth combination prepared with an Azide Agar medium (Enterococcus Confirmatory Agar, M392) overlaid with a Salt Azide Penicillin Broth (Enterococcus Confirmatory Broth, M394). A negative catalase test is considered confirmed positive evidence of the presence of Enterococci. Single strength medium can be used for small inoculum. Production of acid and turbidity in an azide presumptive broth when incubated at 45°C is considered positive presumptive evidence for the presence of Enterococci, which is confirmed by inoculating on Confirmatory Agar (M392).

Quality Control

Appearance

Light yellow to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light blue coloured clear to slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 3.04% w/v aqueous solution at 25°C. pH : 8.0±0.2

pH
7.80-8.20

Cultural Response
M392: Cultural characteristics observed after an incubation at 45°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922	>=10^3	inhibited
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant

Storage and Shelf Life
Store below 30°C in tightly clsed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. 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.
2. Sandholzer and Winter, 1946, Commercial Fisheries Leaflet T1a
3. Edwards M. S., Baker C. J., 1990, Principles and Practice of Infectious Diseases, 3rd Ed., pp 1554-1563, New York

Revision : 2 / 2015