

# **Technical Information**

### **Enterococcus Confirmatory Agar**

Product Code: DM 1392

Application: - 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 perfor	mance parameters	

### **Principle & Interpretation**

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 <sup>(3)</sup>. The Enterococcus is a subgroup of the fecal Streptococci group that includes Enterococcus faecalis, Enterococcus faecium, Enterococcus aallinarum, 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  $10^{\circ}$ C and  $45^{\circ}$ C  $^{(1)}$ . The enterococcal portion of the faecal *Streptococcus* group is a valuable bacterial indicator for determining the extent of faecal contamination of recreational surface waters <sup>(1)</sup>. Sandholzer and Winter <sup>(2)</sup> devised Enterococcus Confirmatory Agar for the detection of Enterococci in water supplies, swimming pools, sewage etc.

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 (DM1419) to Enterococcus Confirmatory slant-broth combination prepared with an Azide Agar medium (Enterococcus Confirmatory Agar, DM1392) overlaid with a Salt Azide Penicillin Broth (Enterococcus Confirmatory Broth, DM1394). 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 (DM1392).

## Methodology

Suspend 30.41 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense in 100 ml quantities in tubes and sterilize by autoclaving at 15 lbs pressure (12 1°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.

## Quality Control

#### **Physical Appearance**

ight 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 range 7.80-8.20

Cultural Response/ characteristices

DM 1392: Cultural characteristics observed after an incubation at 45°C for 18-24 hours.

Organism Inoculum (CFU) Growth

Escherichia coli ATCC 25922 >=10³ inhibited

Enterococcus faecalis ATCC 29212 50-100 good-luxuriant

### 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. 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

### Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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