Technical Information

Enterococcus Confirmatory Broth

Product Code: DM 1394

Application: - Enterococcus Confirmatory broth 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
Sodium chloride	65.000
Methylene blue	0.010
Final pH (at 25°C)	8.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Enterococcus Confirmatory Broth is formulated by Sandholzer and Winter [1] for the detection of Enterococci in water supplies, swimming pools, sewage etc. Enterococcus Confirmatory Broth has the same formula as Enterococcus Confirmatory Agar (DM1392) except agar, sodium chloride and Penicillin, which is used to detect Enterococci from crabmeat and oysters etc. 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 [2].

Casein enzymic hydrolysate, yeast extract, dextrose provide essential growth nutrients for Enterococci. Sodium azide inhibits gram-negative organisms. Penicillin has inhibitory effect on Staphylococci. 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 in / on Confirmatory Broth (DM1394).

Methodology

Suspend 80.41 grams of powder media in 1000 ml distilled water. Shake well & heat if necessary 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. Cool to room temperature and add 65 units of Penicillin to each 100 ml of broth prior to use.

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

Quality Control

Physical Appearance
Cream to yellow may have slight green tinge homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow coloured, clear solution which aquires greenish tinge at the surface on standing

Reaction
Reaction of 8.04% w/v aqueous solution at 25°C. pH : 8.0±0.2

pH range 7.80-8.20

Cultural Response/ characteristics

DM 1394: 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

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

Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

1. Sandholzer and Winter, 1946, Commercial Fisheries Leaflet T1a .
2. 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.

Disclaimer :

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