



Enterococcus Confirmatory Broth

Intended Use:

Recommended for confirming the presence of Enterococci in water supplies and other sources.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	5.000
Dextrose (Glucose)	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

Directions

Suspend 80.41 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense in 100 ml quantities in tubes and sterilize by autoclaving at 15 lbs pressure $(121^{\circ}C)$ for 15 minutes. Cool to room temperature and add 65 units of Penicillin to each 100 ml of broth prior to use.

Principle And Interpretation

Enterococcus Confirmatory Broth is formulated by Sandholzer and Winter (4) for the detection of Enterococci in water supplies, swimming pools, sewage etc. Enterococcus Confirmatory Broth has the same formula as Enterococcus Confirmatory Agar (M392) 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 (1).

Tryptone, yeast extract, dextrose provide nitrogeneous and carbonaceous compounds, long chain amino acids and other 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 (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 in / on Confirmatory Broth (M394).

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. (1) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

M394

Quality Control

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

pН

7.80-8.20

Cultural Response

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

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	>=10 ⁴	inhibited
Enterococcus faecalis ATC 29212 (00087*)	CC 50-100	good-luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. Sandholzer and Winter, 1946, Commercial Fisheries Leaflet T1a

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Disclaimer :

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