MUD SF Broth Base

M1343

MUD SF Broth Base is recommended for the detection and enumeration of intestinal Enterococci in surface and waste water by miniaturized method (MPN). The composition and performance criteria of this medium are as per the specifications laid down in ISO 7899-1:1998(E).

Composition**

ISO specification - MUD/SF medium	
Ingredients	**g / L**
Tryptose	40.000
Monopotassium phosphate	10.000
D-Galactose	2.000
Polyoxyethylenesorbitan monooleate (Tween 80)	1.500
MUD (4-Methylumbelliferyl-β-D-glucoside)	0.150
Final pH (at 25°C)	7.5±0.2

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Ingredients	**g / L**
Tryptose	40.000
Potassium dihydrogen phosphate	10.000
D-Galactose	2.000
Tween 80 (Polysorbate 80)	1.500
4-Methylumbelliferyl-β-D-glucoside (MUD)	0.150
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 5.36 grams in 100 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 - 50°C and aseptically add rehydrated contents of one vial of NAT Selective Supplement (FD148) and 1 ml of sterile 1% TTC Solution (FD057). Mix well and dispense as desired.

Note: As per ISO 7899-1:1998(E), the selective agents- Nalidixic acid, Thallium acetate (NAT Selective supplement, FD148) and 2,3,5-Triphenyl tetrazolium chloride (TTC Solution, FD057) and Flourescence substrate- MUD (4-Methylumbelliferyl-β-D-glucoside) are added in Base and sterilized by filtration through a membrane of average pore size 0.2µm

Principle And Interpretation

The normal habitat of faecal Streptococci is the gastrointestinal tract of warm-blooded animals. The *Enterococcus* group is a subgroup of the faecal Streptococci that includes *E. faecalis*, *E. faecium*, *S. gallinarum* and *S. avium*. The Enterococci are differentiated from other Streptococci by their ability to grow in high sodium chloride concentration i.e. 6.5% at pH 9.6 and at 10°C to 45°C. The Enterococci portion of the faecal *Streptococcus* group is a valuable bacterial indicator for determining the extent of faecal contamination of recreational surface waters. The multiple tube techniques are applicable primarily to raw and chlorinated waste-water and sediments and can be used for fresh and marine waters.

MUD SF Broth is prepared as per the formula accepted by ISO committee under the specification ISO 7899-1:1998 for detection and enumeration of Enterococci in surface and waste water by miniaturized method (MPN) (1).

Tryptose provides carbonaceous, nitrogenous and other essential growth nutrients. Galactose serves as energy source and Potassium dihydrogen phophatebuffers the medium well. Tween 80, (Polyoxyethylene sorbitan monooleate) provides fatty acids. MUD (4-Methylumbelliferyl-β -D-glucoside) is added as fluorogenic substance. Intestinal Enterococci are capable of anaerobic growthat 44°C and of hydrolyzing 4-methylumbelliferyl-β-D-glucoside (MUD) in the presence of thallium acetate, nalidixic acid (as FD) and 2, 3, 5-Triphenyltetrazolium chloride resulting in blue fluorescence.

The diluted sample is inoculated in a row of microtitre plate wells containing dehydrated culture medium. Once the microtitre plate is inoculated, cover with disposable sterile adhesive tape and incubate the plate at 44°C for minimum of 36 hours and maximum 72 hours. Observe under UV light at 366 nm in the dark after an incubation period of 36 to72 hours. The presence of Enterococci is indicated by fluorescence resulting from the hydrolysis of MUD. The results are reported as Most Probable Number per 100 ml.

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 (1,2). 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 complete 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.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Colour and Clarity of prepared medium
Light amber coloured clear solution
Reaction
Reaction of 5.3% w/v aqueous solution at 25°C. pH : 7.5±0.2
pH
7.30-7.70
Cultural Response
Productivity : Cultural response was observed with added TTC Solution 1% (FD057) and NAT Selective Supplement (FD148) after an incubation at 44 ± 0.5°C for 44±4 hours.
Selectivity : Cultural response was observed with added TTC Solution 1% (FD057) and NAT Selective Supplement (FD148) after an incubation at 44 ± 0.5°C for 44±4 hours.

Organism	Inoculum (CFU)	Growth	Fluorescence at 366 nm
Productivity			
Enterococcus faecalis WDCM 00176	50-100	luxuriant	positive, blue
*Enterococcus hirae ATCC 8043 (00089)**	50-100	luxuriant	positive, blue
Enterococcus faecium WDCM 00178	50-100	luxuriant	positive, blue
Selectivity			
*Aerococcus viridans ATCC 11563 (00061)**	>=10^4	inhibited	
*Lactococcus lactis ATCC 19435 (00016)**	>=10^4	inhibited	
*Staphylococcus epidermidis ATCC 14990 (00132)**	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°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 (3,4).

References

1. International Organization for Standardization (ISO), ISO 7899-1:1998.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.

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