Mannitol Salt Broth

M383

Mannitol Salt Broth is used for the selective isolation of presumptive pathogenic Staphylococci.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Beef extract	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 96.02 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Note : This product contains 7.5% sodium chloride as one of its ingredients. On repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, therefore we strongly recommend storage in tightly closed containers in dry place away from bright light.

Principle And Interpretation

Mannitol Salt Broth is prepared as suggested by Chapman (1) and is used for the selective isolation of pathogenic Staphylococci. This medium is recommended for the detection and enumeration of coagulase-positive Staphylococci in milk (2) food (3) and other specimens. Mannitol Salt Broth is used for the isolation of presumptive pathogenic staphylococci. Pathogenic staphylococci ferment mannitol and produce a yellow coloured medium.

The medium contains beef extract and proteose peptone which makes it very nutritious as they provide essential growth factors and trace nutrients. Many other bacteria except Staphylococci are inhibited by 7.5% sodium chloride. Mannitol is the fermentable carbohydrate source. The differential action of the medium is attributed to D-Mannitol. *Staphylococcus aureus* ferments mannitol to produce yellow coloured medium. Most coagulase-negative species of Staphylococci and Micrococci do not ferment mannitol and therefore the medium remains red in colour. The colour of the medium is due to the reactivity of phenol red to the pH of the medium; phenol red is red at pH 8.4 and yellow at 6.8. Presumptive *Staphylococcus* showing yellow coloured medium should be further tested for production of coagulase.

A possible *S. aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (4). Few strains of *S. aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (4).

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Colour and Clarity of prepared medium
Red coloured clear solution in tubes

Reaction
Reaction of 9.6% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response

M383: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Colour of medium
Escherichia coli ATCC 25922	>=10^3	inhibited	
Staphylococcus aureus ATCC 25923	50-100	good-luxuriant	yellow
Staphylococcus epidermidis ATCC 12228	50-100	fair-good	red

Storage and Shelf Life

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

Reference

1. Chapman G.H., 1945, J. Bact., 50:201.
2. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
3. Bacteriological Analytical Manual, 1995, Food and Drug Administration, 8th ed., AOAC, International, U.S.A.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore

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