Mannitol Salt Agar

MH118

Mannitol Salt Agar is used for selective isolation of pathogenic Staphylococci from pharmaceutical products in accordance with Microbial Limit Test by harmonized method of USP/EP/BP/JP/IP (Medium 13).

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Pancreatic digest of casein	5.000
Beef extract	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
pH after sterilization (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 111.02 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

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

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e *Staphylococcus aureus* is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (1). Staphylococci have the unique ability of growing on a high salt containing media (2). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5% NaCl was studied by Chapman (3). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (1, 4-7). The additional property of lipase activity of *Staphylococcus aureus* can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (8).

It is also used in the performance of microbial limit tests for the selective isolation of *Staphylococcus.* The formulation is in accordance with the harmonization of USP/EP/BP/JP/IP (9,10,11,13,14)

The medium contains beef extract, pancreatic digest of casein and peptic digest of animal tissue 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 fermentation of which leads to acid production, detected by phenol red indicator.

S.aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of *S.aureus* are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of *S.aureus* should be confirmed by performing the coagulase test [tube or slide (1)]. Lipase activity of *S.aureus* can be detected by supplementing the medium with egg yolk emulsion

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) (12). 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 (12).

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Red coloured clear to slightly opalescent gel forms in Petri plates

pH
7.20-7.60

Growth Promotion Test
Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BPJP/IP, after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Growth promoting properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for <=18 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100cfu (at 30-35°C for 18-72 hours).

Inhibitory properties
No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100cfu (at 30-35°C for >= 72 hours).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Growth Promoting + Indicative						
Staphylococcus aureus ATCC 6538	50 -100	luxuriant	25 -100	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
Inhibitory						
Escherichia coli ATCC 8739	>=10^3	inhibited	0	0 %		>=72 hrs
Additional Microbiological testing						
Staphylococcus aureus ATCC 25923	50 -100	luxuriant	25 -100	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
Staphylococcus epidermidis ATCC 12228	50 -100	fair - good	15 -40	30 -40 %	red	18 -72 hrs
Staphylococcus epidermidis ATCC 14990	50 -100	fair - good	15 -40	30 -40 %	red	18 -72 hrs
Proteus mirabilis ATCC 12453	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922	>=10^3	inhibited	0	0%		>=72 hrs
Escherichia coli NCTC 9002	>=10^3	inhibited	0	0%		>=72 hrs

Enterobacter aerogenes ATCC 13048	>=10^3	inhibited	0	0%	>=72 hrs

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.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C. ,,
2.Koch P. K., 1942, Zentralbl. Bakteriol. Parasitenkd. Abt. I Orig.149:122.
3.Chapman G. H., 1945, J. Bacteriol., 50:201.
4.Hitchins A. D., Tran T. and McCarron J. E., 1995, FDA Bacteriological Analytical Manual, 8th Ed., AOAC International, Gaithersburg, Md.
5.Davis J. G., 1959, Milk testing, 2nd Ed., Dairy Industries Ltd, London.
6.American Public Health Association, 1966, Recommended Methods for the Microbiological Examination of Foods, 2nd Ed, APHA, New York.
7.Silverton R. E. and Anderson M. J., 1961, Handbook of Medical Laboratory Formulae, Butterworths, London.
8.Gunn B. A., Dunkelberg W. E. and Creitz J. R., 1972, Am. J. Clin. Pathol., 57:236.
9.British Pharmacopoeia, 2011,The Stationery office British Pharmacopoeia
10.European Pharmacopoeia, 2011, EDQM.
11.The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention, Rockville, MD.
12.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
13.Japanese Pharmacopoeia, 2008
14.Indian Pharmacopoeia, 2010, Govt. of India, Ministry of Health and Family Welfare, New Delhi

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