Technical Information

Mannitol Salt Agar

Product Code: DM 1118

Application: - MacConkey Broth Purple w/BCP is used for presumptive identification of coliforms from variety of specimens such as water, milk and food etc.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Beef extract	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
Final pH (25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Staphylococci although widespread in nature, 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 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 also recommended for the isolation of coagulasepositive 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 (MS2045) in which the lipase activity can be visualized as yellow opaque zones around the colonies [(8)].

Beef extract and proteose peptone supply essential growth factors and trace nutrients to the growing bacteria. Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. Mannitol is the fermentable carbohydrate, 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) (DM1002) [(9)]. 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 [(9)].

Methodology

Suspend 111.02 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, add 5% v/v Egg Yolk Emulsion (MS2045). Mix well and pour into sterile Petri plates.

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.

Quality Control

Physical 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
Reaction
Reaction of 1.1% w/v aqueous solution at 25°C.pH:-7.4±0.2
pH range 7.20-7.60
Cultural Response/ characteristics
DM 1118: Cultural characteristics observed after an incubation at 35-37°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation period
Staphylococcus aureus ATCC 6538	50-100	luxuriant	25-100	>=50 %	yellow/white colonies surrounded by yellow zone	18-72 hours
Escherichia coli ATCC 8739	>=10^3	inhibited	0	0 %		>=72 houre
Staphylococcus aureus ATCC 25923	50-100	luxuriant	25-100	>=50 %	yellow/white colonies surrounded by yellow zone	18-72 hours
Staphylococcus epidermidis ATCC 12228	50-100	fair - good	15-40	30-40%	red	18-72 hours
Staphylococcus epidermidis ATCC 14990	50-100	fair - good	15-40	30-40%	red	18-72 hours
Proteus mirabilis ATCC 12453	50-100	none-poor	0-10	0-10%	yellow	18-72 hours
Escherichia coli ATCC 25922	>=10^3	inhibited	0	0%		>=72 houre
Escherichia coli NCTC 9002	>=10^3	inhibited	0	0%		>=72 houre
Enterobacter aerogenes ATCC 13048	>=10^3	inhibited	0	0%		>=72 houre

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

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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. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore

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