Coagulase Mannitol Agar Base

M272

Coagulase Mannitol Agar Base is recommended for the primary isolation and differentiation of pathogenic Staphylococci from clinical specimens or for classifying pure cultures.

Composition**

Ingredients	Gms / Litre
Brain heart infusion	5.000
Casein enzymic hydrolysate	10.500
Papaic digest of soyabean meal	3.500
Sodium chloride	3.500
Mannitol	10.000
Bromo cresol purple	0.020
Agar	14.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 47.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118 - 121°C (12-15 lbs pressure respectively)for 15 minutes. Cool to 45 - 50°C. Add 7 - 15% v/v sterile, pre-tested, rabbit plasma to basal medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The genus *Staphylococcus* comprises 28 accepted or proposed species, 14 of which may be encountered in human clinical specimens. Staphylococci are generally found on the skin and mucous membranes of humans and other animals. Some of the pathogenic staphylococci in both humans and animals produce an enzyme called coagulase and detection of this enzyme is used in the laboratory to identify these organisms (6).

These media are used for the isolation of *Staphylococcus aureus* from clinical specimens and for differentiation of *S.aureus* from other species on the basis of coagulase production and mannitol fermentation. Chapman for the first time introduced a medium for selective isolation and differentiation of Staphylococci (1). Tellurite-glycine media were designed by Zebovitz et al (2) and Marwin (3) for selectively isolating coagulase-positive Staphylococcal species. Present medium is based on Esber and Faulconer formulation (5). Mutant or old cultures of *S.aureus* may be weak coagulase producers. They should be freshly sub cultured and rechecked. *Escherichia coli* ferments mannitol and may be weakly coagulase positive. Coagulase production is dependent on the presence of a fermentable sugar like mannitol in this case. It is also dependent on the presence of a protein factor in the brain heart infusion and blood plasma (4). When mannitol is fermented, the pH of the medium surrounding the coagulase positive colonies drops. This drop in pH is indicated by the change in colour of the bromocresol purple indicator, which turns yellow and exhibits yellow zones around the colonies.

An opaque area of coagulated plasma forms around the colonies of coagulase positive organisms. *Staphylococcus epidermidis* is coagulase negative and mannitol non-fermenting species, which does not change the colour of the medium. Coagulase negative species may ferment mannitol and produce a yellow zone around the colonies but an opaque zone will not be formed.

Quality Control

Appearance

Light yellow to light grey homogeneous free flowing powder

Gelling

Firm, comparable with 1.45% Agar gel

Colour and Clarity of prepared medium

Purple coloured, slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.7% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M272: Cultural characteristics observed with added 7-15% v/v sterile pretested, rabbit plasma at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Mannitol fermentation	Coagulase production
Cultural Response					
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%	positive reaction, yellow colour	positive reaction, colonies surrounded by opaque zone
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	>=70%	negative reaction, purple colour	negative reaction, no opaque zone formation

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, 1944, J. Bacteriol., 48:113
2. Zebovitz, Evans and Nivens, 1955, J. Bacteriol., 70:686.
3. Marwin, 1958, Am. J. Clin. Pathol., 30:470.
4. Schaub and Merrit, 1960, Bull. Johns Hopkins Hosp., 106:25.
5. Esber and Faulconer, 1959, Am. J. Clin. Pathol., 32:192.
6. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company

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