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Mannitol Salt Agar for the isolation of *Staphylococcus aureus*

Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus* from clinical and non-clinical specimens. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. It is a selective medium prepared according to the recommendations of Chapman for the isolation of presumptive pathogenic staphylococci.

Composition of Mannitol Salt Agar

Ingredients	Gms / Litre
Pancreatic Digest of Casein	5.0 gm
Peptic Digest of Animal Tissue	5.0 gm
Beef Extract	1.0 gm
Sodium Chloride	75.0 gm
D-Mannitol	10.0 gm
Phenol Red	0.025 gm
Agar	15.0 gm
Total	**111. 025 gm**

Distilled Water = 1000 ml

Final pH 7.4 ± 0.2 at 25°C.

Principle of Mannitol Salt Agar

Mannitol Salt Agar contains **peptones** and **beef extract,** which supply nitrogen, vitamins, minerals and amino acids essential for growth. The 7.5% concentration of **sodium chloride** results in the partial or complete inhibition of bacterial organisms other than staphylococci. **Sodium chloride** also supplies essential electrolytes for transport and osmotic balance. **Mannitol** is the fermentable carbohydrate, fermentation of which leads to acid production, detected by **phenol red** indicator, aids in the differentiation of staphylococcal species. Coagulase positive staphylococci (e.g., *Staphylococcus aureus*) produce yellow colonies and a surrounding yellow medium while coagulase negative staphylococci produce red colonies and no color change of the phenol red indicator. **Agar** is the solidifying agent.

Addition of 5% v/v **Egg Yolk Emulsion** enables the detection of lipase activity of staphylococci along with mannitol fermentation. The salt clears the egg yolk emulsion and lipase production is detected as yellow opaque zone around the colonies.

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Uses of Mannitol Salt Agar

1. It is used for the selective isolation and differentiation of *Staphylococcus aureus* from clinical samples.
2. It is also used for the enumeration of staphylococci in food and dairy products.
3. This medium is also included in the Bacteriological Analytical Manual for cosmetics testing.
4. It is also used in the bacteriological examination of swimming pool water, spas and drinking water using membrane filtration

Preparation of Mannitol Salt Agar

1. Suspend 111.025 gm of MRS media in 1000 ml of distilled water.
2. Boil to dissolve the media completely.
3. Autoclave at 121°C for 15-20 minutes.
   (**Optional**: Add 5% v/v Egg Yolk Emulsion)
4. Cool to 45-50°C and pour into petri dishes.

Result Interpretation on Mannitol Salt Agar

Yellow colonies of *Staphylococcus aureus* | ***Staphylococcus aureus* and *Serratia marcescens* on MSA**

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Organisms	Results
Staphylococcus aureus	Yellow colonies with yellow zones.
Staphylococci other than *S. aureus (*e.g. *Staphylococcus epidermidis)*	Colorless or Red colonies with red zones.
Streptococci	No growth to trace growth.
Micrococci	Large white to orange.
Gram-negative bacteria	No growth to trace growth.

Quality Control on Mannitol Salt Agar

Positive Control: *Staphylococcus aureus* ATCC 6538, Medium-sized yellow colonies

Negative Control: *Escherichia coli* ATCC 25922, Partial to Complete Inhibition.

Limitations of Mannitol Salt Agar

1. Several *Staphylococcus* species other than *S. aureus* are mannitol positive and produce yellow colonies surrounded by yellow zones on this medium (e.g. *S. capitis, S. xylosus, S. cohnii, S. sciuri, S. simulans,* and other species). Therefore, further biochemical tests are necessary for the identification of S. aureus or other species.
2. Most organisms other than staphylococci are inhibited by the high salt concentration found in Mannitol Salt Agar except for some halophillic marine organisms.
3. A few strains of *Staphylococcus aureus* may exhibit a delayed fermentation of mannitol. Negative plates should be re-incubated overnight before discarding.
4. Presumptive *Staphylococcus aureus* must be confirmed with a coagulase test.

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