MANNITOL SALT AGAR

PRODUCT:

Plated Media:

Mannitol Salt Agar P1900, P8061 (contact plate)

Tubed Media:

Mannitol Salt Agar T6892 (slant)

PURPOSE:

Mannitol Salt Agar is a highly selective medium designed for the recovery and isolation of pathogenic staphylococci. This medium meets the U.S. Pharmacopeia (USP) standards in performing microbial examination of nonsterile products.

PRINCIPLE:

In 1942 Koch[4] described the tolerance of *Staphylococcus aureus* to high concentrations of sodium chloride. Chapman[2] formulated a medium which incorporated 7.5% sodium chloride into an agar containing mannitol and a phenol red indicator for the recovery of pathogenic staphylococci. Most strains of coagulase-positive staphylococci grow on the medium, producing colonies with yellow zones as a result of the fermentation of mannitol. Coagulase-negative strains may be inhibited or produce small colonies with no color change in the surrounding medium. Other bacteria are generally inhibited, so that a heavy inoculum of a culture containing mixed flora will not result in an overgrowth.

FORMULA:

Approximate, per liter deionized filtered water.

Beef Extract	1.0 g
Peptic Digest of Animal Tissue	5.0
Pancreatic Digest of Casein	5.0
Sodium Chloride	75.0
D-Mannitol	10.0
Agar	15.0
Phenol Red	25.0 mg

Final pH 7.4 ± 0.2 at 25°C

PRECAUTIONS:*

For *in vitro* diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Limitations: While this media is selective for pathogenic staphylococci, a coagulase test should always be done to complete the identification. Further biochemical procedures for the differentiation of staphylococci may be found in appropriate references.[1,3,5]

Rarely, strains of streptococci, particularly enterococci, may grow on this media, and some strains, e.g., *E. faecalis, E. faecium*, and *S. bovis*, will ferment mannitol.

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference materials.[3,5] In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory without delay.

Method of Use: Prior to inoculation, the media should be brought to room temperature. Inoculate clinical specimens directly onto the media surface using generally accepted procedures suitable to the nature of the specimen. The procedure should be one that enhances the development of isolated colonies. Incubate inoculated plates aerobically at 35°C for 24 hours. Most strains of

S. aureus capable of fermenting mannitol will do so within 24 hours. However, delayed fermentation of mannitol may occur with a few strains of *S. aureus*, so negative plates should be incubated for an additional 24 hours before being discarded.

Interpretation:

Positive: Growth of smooth, raised colonies; yellow color change in the medium. (Possible growth of *Staphylococcus aureus*; further identification required.)[3,5]

Negative: Growth of smooth, raised colonies; no color change in the medium; or inhibition of growth.

Material Required but Not Provided: Standard microbiological supplies and equipment are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):	**Expected Results:**
Staphylococcus aureus (25923)	Growth; yellow colonies with yellow zones
Staphylococcus epidermidis (12228)	Growth; pink colonies with pink zones by 48 hours
Proteus mirabilis (12453)	Inhibition

Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration. Mannitol Salt Agar should appear translucent and light pink in color.

BIBLIOGRAPHY:

1. Branson, D., *Methods in Clinical Microbiology — A Manual of Tests and Procedures*, Charles C. Thomas, Springfield, Ill., 1972.
2. Chapman, G. H., 1945. *J. Bacteriol.*, *50*:201.
3. Forbes, B. A., et al., Bailey and Scott’s Diagnostic Microbiology, 12th ed. C. V. Mosby, St. Louis, 2007.
4. Koch, F. E., *Zentralbl. Bakteriol. Parasetenkd.*, Abt. 1942. 1 Orig., *149*:122.
5. Murray, P.R., et al., Manual of Clinical Microbiology, 9th ed., American Society for Microbiology, Washington, D. C., 2007.
6. United States Pharmacopeia 30 - NF 25, Chapter 62, Microbial examination of nonsterile products: Tests for specified microorganisms, 2007.

*For more detailed information, consult appropriate references.

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