MANNITOL SALT AGAR

- For in vitro use only - Catalogue No. PM30

Our Mannitol Salt Agar is a selective medium used for the differential isolation of staphylococci.

Koch first reported the tolerance of *Staphylococcus aureus* to high concentrations of sodium chloride in 1942. Chapman devised the original medium by adding salt to phenol red mannitol agar; he found that pathogenic, coagulase-positive staphylococci grew well on the medium while other staphylococci grew poorly. Mannitol Salt Agar is not completely selective for staphylococci. Some group D enterococci may exhibit growth with mannitol fermentation; however, catalase test and gram morphology should distinguish between enterococci and staphylococci. Prolonged incubation (≥ 48 hours) may also allow growth of *Micrococcus*, *Bacillus*, and some species of *Serratia*.

Pancreatic digest of casein, peptic digest of animal tissue, and beef extract are the nutritional sources that provide the bacterial cells with the essential elements required for growth. Mannitol can be utilized and fermented by pathogenic staphylococci to produce acid. Acid produced during fermentation is detected by phenol red, a pH indicator that changes from red to yellow when a sufficient amount of acid is produced. The high sodium chloride level suppresses the growth of most bacteria, other than staphylococci, and allows for direct inoculation from heavily contaminated sources without the concern of overgrowth by other bacteria.

Mannitol Salt Agar is recommended for isolating pathogenic staphylococci form a variety of clinical samples; it has also been indicated in the past for the testing and enumeration of coagulase-positive staphylococci from foods.

Formula per Litre of Medium

Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
D-Mannitol	10.0 g
Sodium Chloride	75.0 g
Beef Extract	1.0 g
Phenol Red	0.025 g
Agar	15.0 g

pH 7.4 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using an inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. If the specimen is contained on a swab, roll the swab several times over a small area near the edge of the plate and streak the plate for isolation with a sterile loop starting where the swab was inoculated.
3. Incubate aerobically at 35°C.
4. Examine plates after 24 hours. Re-incubate plates an additional 24 hours and examine before discarding.

Interpretation of Results

Typical colonial morphology on MSA:

Staphylococcus aureus – Large, bright yellow, opaque colonies with yellow halos

Other staphylococci – Small to large, non-pigmented colonies with reddish-purple halos

Streptococci – Inhibited

Micrococci – Large, white to orange colonies

Gram-negative bacteria – Inhibited

Potential *Staphylococcus aureus* isolates must be confirmed as coagulase positive and sub-

cultured onto a non-selective medium for further testing. The ability to clot plasma continues to be the most widely used criterion for the identification of pathogenic staphylococci.

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Staphylococcus aureus ATCC 25923	Growth, colonies with yellow halos
Staphylococcus epidermidis ATCC 12228	Growth, colonies with red halos
Proteus mirabilis ATCC 12453	Inhibition (partial)

Storage and Shelf Life

Our Mannitol Salt Agar should be stored away from direct light at 4 to 8°C with the medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

References

1. Koch FE. Elektivnährboden für staphylokokken. Zentralbl Bakteriol Parasitenkd Infektionskr Hyg Abt I 1942; 149:122.
2. Chapman GH. The significance of sodium chloride in studies of staphylococci. J Bacteriol 1945; 50:201.
3. Porter A, Nicholson J. A contact slide method for the isolation of staphylococci. J Clin Pathol 1961; 14:557.
4. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
5. Isenberg HD, Ed. Clinical microbiology procedures handbook. Washington, DC: ASM, 1992.
6. United States Pharmacopeial Convention. The United States pharmacopeia. 23rd ed. Rockville, MD: The United States Pharmacopeial Convention, 1995.
7. NCCLS. Quality assurance for commercially prepared microbiological culture media. 2nd ed. NCCLS document M22-A2. Wayne, PA: NCCLS, 1996.
8. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis: Mosby, 1998.

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