Mannitol Salt Agar (NCM0078)

Intended Use
Mannitol Salt Agar is used for the isolation of staphylococci in a laboratory setting. Mannitol Salt Agar is not intended for use in the diagnosis of disease or other conditions in humans. Conforms to Harmonized USP/EP/JP Requirements.

Description
Chapman formulated Mannitol Salt Agar to isolate staphylococci by inhibiting growth of most other bacteria with a high salt concentration. Chapman added 7.5% Sodium Chloride to Phenol Red Mannitol Agar and noted pathogenic strains of staphylococci (coagulase-positive staphylococci) grew luxuriantly and produced yellow colonies with yellow zones. Non-pathogenic staphylococci produced small red colonies with no color change to the surrounding medium.

Mannitol Salt Agar is highly selective and samples from heavily contaminated sources may be streaked onto this medium without danger of overgrowth. Mannitol Salt Agar is recommended for isolating pathogenic staphylococci from samples, cosmetics, and microbial limit tests.

Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products, turning the phenol red pH indicator from red to yellow. Typical pathogenic staphylococci ferment mannitol and form yellow colonies with yellow zones. Typical non-pathogenic staphylococci do not ferment mannitol and form red colonies.

Enzymatic digest of casein, enzymatic digest of animal tissue, and beef extract provide the nitrogen, vitamins, and carbon in Mannitol Salt Agar. D-Mannitol is the carbohydrate source. In high concentrations, sodium chloride inhibits most bacteria other than staphylococci. Phenol red is the pH indicator. Agar is the solidifying agent.

Typical Formulation

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

Final pH: 7.4 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions
Refer to SDS

Preparation
1. Suspend 111 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.

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Test Procedure

Refer to appropriate references using Mannitol Salt Agar for the isolation and identification of staphylococci.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light red-orange to light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and peach to pink.

Expected Cultural Response: Cultural response on Mannitol Salt Agar at 33-37°C after 18-24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Recovery	Reactions
Escherichia coli ATCC® 8739	> 1000	Complete Inhibition	---
Escherichia coli ATCC® 25922	> 1000	Complete Inhibition	---
Staphylococcus aureus ATCC® 6538	10 - 100	10-100	Off-white to pale to light yellow colonies
Staphylococcus aureus ATCC® 25923	10 - 100	10-100	Off-white to pale to light yellow colonies
Staphylococcus aureus NCTC 11940	10 - 100	10-100	Off-white to pale to light yellow colonies
Staphylococcus epidermidis ATCC® 12228	10 - 100	10-100	Colorless to pink colonies

The organisms listed are the minimum that should be used for quality control testing.

Results

Staphylococci will grow on this medium, while the growth of most other bacteria will be inhibited. Coagulase-positive staphylococci will produce luxuriant growth of yellow colonies and may have a yellow halo around the colony. Coagulase-negative staphylococci will produce small colorless to pink colonies with no color change to the medium.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

References

1. European Pharmacopoeia 10th Edition (2020)
2. United States Pharmacopeia National Formulary 2018: USP 41 NF 36
3. Japanese Pharmacopeia 17th Edition (2017)

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5. Kloos, W. E., and T. L. Bannerman. 1995. *Staphylococcus* and *Micrococcus*. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.

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