

Mannitol Salt Agar

SMH118

Intended Use:

Recommended for the selection and subculture of *Staphylococcus aureus* in accordance with the harmonized method of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Mannitol Salt Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized, hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium in tubes as butts /slants or in plates as desired and allow to solidify. If on plate, either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically.

Note : This product contains 7.5% Sodium chloride as one of its ingredients. On repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, therefore we strongly recommend storage in tightly closed containers in dry place away from bright light.

Principle And Interpretation

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e *Staphylococcus aureus* is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (10). Staphylococci have the unique ability of growing on a high salt containing media (8). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5%NaCl was studied by Chapman (2). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (10, 5,3,12,11). The additional property of lipase activity of *Staphylococcus aureus* can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (4).

HM peptone B and proteose peptone supply essential growth factors and trace nutrients to the growing bacteria.

Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator.

S.aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of *S.aureus* are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones.

Presumptive coagulase-positive yellow colonies of *S. aureus* should be confirmed by performing the coagulase test [tube or slide] (10). Lipase activity of *S.aureus* can be detected by supplementing the medium with egg yolk emulsion.

A possible *S.aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (9). Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (9).

Type of specimen

Please refer disclaimer Overleaf.

Clinical samples: pus; Food and dairy samples, water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (12,1).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. A possible *S.aureus* must be confirmed by the coagulase test.
2. The organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (9).
3. Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (9).

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Sterile Mannitol Salt Agar in glass bottle.

Colour of medium

Red coloured coloured medium

Quantity of medium

100ml of medium in glass bottle.

Reaction

7.20-7.60

Sterility Test

Passes release criteria

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Incubation temperature
Cultural Response <i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
<i>Escherichia coli</i> ATCC 8739 >=10 ³ (00012*)		inhibited	0 %		>=72 hrs
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs

<i>Staphylococcus epidermidis</i> ATCC 14990 (00132*)	50 -100			red	18 -72 hrs
<i>Proteus mirabilis</i> ATCC 12453	50 -100	none-poor	0 -10 %	yellow	18 -72 hrs
<i>Escherichia coli</i> ATCC 25922 (00013*)	$\geq 10^3$	inhibited	0%		≥ 72 hrs
<i>Escherichia coli</i> NCTC 9002	$\geq 10^3$	inhibited	0%		≥ 72 hrs
<i>Enterobacter aerogenes</i> ATCC 13048 (00175*)	$\geq 10^3$	inhibited	0%		≥ 72 hrs

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 15-25°C Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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4. Gunn B. A., Dunkelberg W. E. and Creitz J. R., 1972, Am. J. Clin. Pathol., 57:236.
5. Hitchins A. D., Tran T. and McCarron J. E., 1995, FDA Bacteriological Analytical Manual, 8th Ed., AOAC International, Gaithersburg, Md.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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10. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
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12. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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