IFU

Instructions for Use

MANNITOL SALT AGAR (MSA)

Cat. no. J330	Tryptic Soy Agar (TSA) / Cetrimide (CET) / Mannitol Salt Agar (MSA), 15x100mm Triplate, 7ml/section	10 plates/bag
Cat. no. J79	All Purpose Quadplate (Eosin Methylene Blue (EMB) / Mannitol Salt Agar (MSA) / Mycobiotic Agar / Blood Agar 5%, 15x100mm Quadplate, 5ml/section	10 plates/bag

INTENDED USE

Hardy Diagnostics Mannitol Salt Agar (MSA) is recommended for use as a selective and differential medium for the isolation of pathogenic staphylococci.

SUMMARY

Koch reported the use of a medium containing 7.5% sodium chloride as a selective agent for the isolation of staphylococci in 1942.[5]The results were confirmed and improved by Chapman in 1945 by the addition of this salt concentration to Phenol Red Mannitol Agar, as *Staphylococcus aureus* usually ferments mannitol.[3] Non-pathogenic staphylococci usually show less luxuriant growth on this medium after the incubation period.

A sodium chloride, concentration of 7.5%, is nearly ten times the usual concentration seen in most media. It serves to inhibit most organisms except staphylococci in mixed flora specimens. The beef extract and peptones supply the essential elements carbon, nitrogen, and sulfur. Mannitol is added to show the fermentation capabilities of the organisms. Acid production as the result of fermentation of this sugar results in the formation of colonies with a yellow zone. Those staphylococci that do not ferment mannitol show a purple or red zone around the colonies.

Mannitol Salt Agar (MSA) is recommended by the American Public Health Association for the enumeration of staphylococci in food and dairy products.[9,10]

FORMULA

Ingredients per liter of deionized water:*

Sodium Chloride	75.0gm
Proteose Peptone	10.0gm
Mannitol	10.0gm
Beef Extract	1.0gm
Phenol Red	0.025gm
Agar	15.0gm

Final pH 7.4 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1,2,4,7] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

Method of Use: Allow the plates to warm to room temperature and the agar surface to dry before inoculating. Heavily inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35-37ºC for 24-48 hours. Examine colonial morphology.

INTERPRETATION OF RESULTS

Mannitol fermentors such as *S. aureus* appear as yellow colonies with yellow zones in the media. Non-mannitol fermentors such as *S. epidermidis*, if present, will have clear pink to red colonies with no yellow color change in the medium. Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1,2,4,7]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Most organisms other than staphylococci are inhibited by the high salt concentration found in Mannitol Salt Agar except for some halophillic marine organisms.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Mannitol Salt Agar (Cat. no. J330):					
Staphylococcus aureus ATCC® 6538	J	18hr	35°C	Aerobic	Growth; yellow colonies and media at 18-24 hours
Escherichia coli ** ATCC® 8739	B	72hrs	35°C	Aerobic	Partial to complete inhibition
Mannitol Salt Agar (Cat. no. J79):					
Staphylococcus aureus ATCC® 25923	A	24-48hr	35°C	Aerobic	Growth; yellow colonies and media
Staphylococcus epidermidis ATCC® 12228	A	24-48hr	35°C	Aerobic	Variable growth; red colonies and media if present
Proteus mirabilis ATCC® 12453	B	24hr	35°C	Aerobic	Partial to complete inhibition
Escherichia coli ATCC® 8739	A	72hr	30-35°C	Aerobic	Partial to complete inhibition

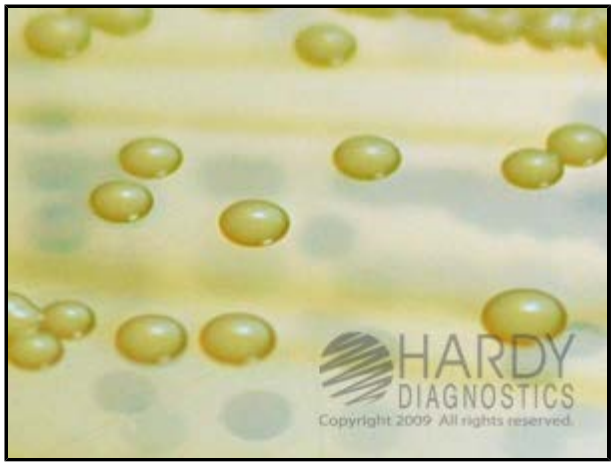
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

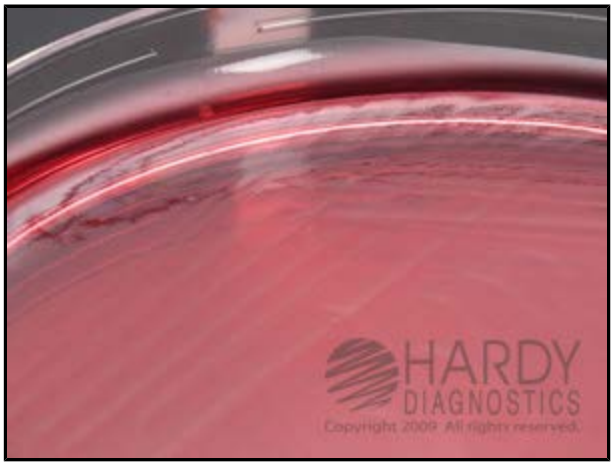
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Mannitol Salt Agar (MSA) should appear clear, slightly opalescent, and pinkish-red in color.



Staphylococcus aureus (ATCC® 25923) colonies growing on Mannitol Salt Agar. Incubated aerobically for 48 hours at 35ºC.



Proteus mirabilis (ATCC® 12453) growth inhibited on Mannitol Salt Agar. Incubated aerobically for 48 hours at 35ºC.

REFERENCES

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3. Chapman, G.H. 1945. *J. Bacteriol.*; 50:201.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

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6. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

7. Jorgensen, J.H., et al. 2015. *Manual of Clinical Microbiology*, 11th ed. American Society for Microbiology, Washington, D.C.

8. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

9. American Public Health Association. 1993. *Standard Methods for the Examination of Dairy Products*, 16th ed. APHA, Washington, D.C.

10. APHA Technical Committee on Microbiological Methods for Foods. 2001. *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed. APHA, Washington, D.C.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]