



# Instructions for Use

# MANNITOL SALT AGAR (MSA), USP

<u>Cat. no. G40</u>	Mannitol Salt Agar (MSA), USP, 15x100mm Plate, 18ml	10 plates/bag
<u>Cat. no. P98</u>	Mannitol Salt Agar (MSA), USP, Contact Plate, 12ml	10 plates/bag

# **INTENDED USE**

Hardy Diagnostics Mannitol Salt Agar (MSA), USP is recommended for use as a selective and differential medium for the isolation of pathogenic staphylococci. The medium complies with the harmonized European, U.S., and Japanese Pharmacopoeias for determining the microbial quality of non-sterile products.

These products are not intended to be used for the diagnosis of human disease.

# SUMMARY

Koch reported the use of a medium containing 7.5% sodium chloride as a selective agent for the isolation of staphylococci in 1942. The results were confirmed and improved by Chapman in 1945 by the addition of salt to Phenol Red Mannitol Agar, as *Staphylococcus aureus* usually ferments mannitol. 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 fermentation capability of the organism. 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.<sup>(2,3)</sup> The medium is prepared according to the U.S. Pharmacopoiea Standard formula for Mannitol Salt Agar.<sup>(5)</sup>

MSA Contact Plates (Cat. no. P98) are to be used to test for contamination of surfaces by pathogenic staphylococci.

## **FORMULA**

Ingredients per liter of deionized water:\*

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

Final pH 7.4 +/- 0.2 at 25°C.

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

## **STORAGE AND SHELF LIFE**

Storage: Upon receipt store at 2-8°C. 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 dating 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 quality control incubation times.

Refer to the document "Storage" on the Hardy Diagnostics Technical Document website for more information.

## PRECAUTIONS

#### Catalog No. G40

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at <u>www.cdc.gov/ncidod/dhqp/gl\_isolation.html</u>.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.* 

Sterilize all biohazard waste before disposal.

Refer to the document "<u>Precautions When Using Media</u>" on the Hardy Diagnostics <u>Technical Document</u> website for more information.

Refer to the document <u>SDS Search</u> instructions on the Hardy Diagnostics website for more information.

#### Catalog No. P98

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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at <a href="http://www.cdc.gov/ncidod/dhqp/gl\_isolation.html">www.cdc.gov/ncidod/dhqp/gl\_isolation.html</a>.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.* 

Sterilize all biohazard waste before disposal.

Refer to the document "<u>Precautions When Using Media</u>" on the Hardy Diagnostics <u>Technical Document</u> website for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics website for more information.

#### PROCEDURE

Prepare a sample using a 1:10 dilution of not less than 1.0gm of product to be examined as described in <61> and use 10mL or quantity corresponding to 1.0gm or 1.0mL to inoculate a suitable amount (main suitability of test method) of TSB, USP and homogenize. Incubate enrichment per <61>. Subculture TSB, USP enrichemnt to MSA, USP, and incubate at 30-35°C for 18-72 hours.

Method of Use, Contact Plates: Aseptically remove lid of contact plate. Gently place on area to be tested. Using a rolling motion, keep the plate in place, making sure all of the agar surface touches the testing area. Do not wipe or rub the contact plate on the surface being tested. Cover with the lid, and incubate and examine as described above.

## **INTERPRETATION OF RESULTS**

The presence of *S. aureus* is indicated by the growth of yellow or white colonies surrounded by a yellow zone. Confirm colonies by identification tests.<sup>(2-5)</sup>

## LIMITATIONS

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

Most organisms other than staphylococci are inhibited by the high salt concentration found in Mannitol Salt Agar except for some halo-tolerant organisms such as *Enterococcus faecalis*.

Refer to the document "<u>Limitations of Procedures and Warranty</u>" on the Hardy Diagnostics <u>Technical Document</u> website for more information.

# MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Deculto
		Time	Temperature	Atmosphere	Results
Staphylococcus aureus** ATCC <sup>®</sup> 6538	J	18-72hr	30-35°C	Aerobic	Growth; yellow colonies and media at 18-24 hours
Staphylococcus aureus** ATCC <sup>®</sup> 25923	А	24-48hrs	35°C	Aerobic	Growth; yellow colonies and media at 24-48 hours
Proteus mirabilis** ATCC <sup>®</sup> 12453	В	24hrs	35°C	Aerobic	Partial to complete inhibition
<i>Escherichia coli**</i> ATCC <sup>®</sup> 8739	А	18-72hrs	30-35°C	Aerobic	Partial to complete inhibition

\* Refer to the document "Inoculation Procedures for Media QC" on the Hardy Diagnostics Technical Document website for more information.

\*\* Tested in accordance with USP <62>.<sup>(4,5)</sup>

#### **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 certificates of analysis (CofA) available from Hardy Diagnostics <u>Certificates of Analysis</u> website. In addition, refer to the following documents on the Hardy Diagnostics <u>Technical Document</u> website for more information on QC: "<u>Introduction to</u> <u>Quality Control</u>" and "<u>Finished Product Quality Control Procedures</u>," or see reference(s) for more specific information.

#### **PHYSICAL APPEARANCE**

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

#### REFERENCES

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

2. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.

3. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

4. The Official Compendia of Standards. USP General Chapter <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests. *USP-NF*. United States Pharmacopeial Convention Inc., Rockville, MD.

5. The Official Compendia of Standards. USP General Chapter <62> Microbiological Examination of Nonsterile Products: Testsfor Specified Microorganisms. *USP-NF*. United States Pharmacopeial Convention Inc., Rockville, MD.

ATCC is a registered trademark of the American Type Culture Collection.

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