

## Mannitol Salt Agar

### Intended Use

Mannitol Salt Agar is a medium used for selective isolation of pathogenic Staphylococci.

### Summary

Koch reported that only Staphylococci grow on agar media containing 7.5% sodium chloride. Chapman studied this phenomenon in detail and concluded that the addition of 7.5% salt to phenol red mannitol agar results in an improved medium for the isolation of plasma coagulating Staphylococci.

Mannitol Salt Agar is recommended by the USP and IP for use in Microbial Limit Tests. It is used for the detection and enumeration of coagulase positive Staphylococci in milk, food and other specimens. This medium is also included in the Bacteriological Analytical Manual for cosmetics testing.

### Principle

Proteose peptone and cara beef extract supplies essential growth factors such as nitrogen, carbon, sulphur and trace nutrients. The 7.5% salt concentration results in partial or complete inhibition of bacteria other than Staphylococci. Mannitol fermentation, results in change in the phenol red indicator, (from red to yellow) which helps in the differentiation of Staphylococcal species. Coagulase negative species of Staphylococci and micrococci do not ferment mannitol and grow as small red colonies surrounded by red or purple zones. Yellow coloured colonies should be tested for production of coagulase. Addition of 5% v/v Egg Yolk Emulsion enables the detection of lipase activity of Staphylococci along with mannitol fermentation. The salt clears the egg yolk emulsion and lipase production is detected as yellow opaque zone around the colonies. Coagulase positive Staphylococci produce colonies surrounded by bright yellow zones while non-pathogenic Staphylococci produce colonies with reddish purple zones.

### Formula\*

Ingredients	g/L
Cara Beef Extract#	1.0
Proteose Peptone	10.0
Sodium Chloride	75.0
D-Mannitol	10.0
Phenol Red	0.025
Agar	15.0
Final pH (at 25°C)	7.4 ± 0.2

\*Adjusted to suit performance parameters.

#Equivalent to Beef Extract

### Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

### Type of Specimen

Clinical samples - Pus, Urine; Food and Dairy samples; Water samples

### Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

## Directions

1. Suspend 111.02 g of the powder in 1000 mL purified / distilled water and mix well.
2. Boil with frequent agitation to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. OPTIONAL: Add 5% v/v Egg Yolk Emulsion (204050370100).
5. Mix well and dispense as desired.

**Note:** As this product contains 7.5 % sodium chloride, on repeated exposure to air and moisture absorption, it has a tendency to form lumps. Hence it is recommended to store this product in a tightly closed container in dry place away from light.

## Quality Control

**Dehydrated Appearance:** Light pink coloured, homogenous, free flowing powder.

**Prepared Appearance:** Red to rose pink coloured, slightly opalescent gel forms in petridishes.

**Growth Promotion Test:** Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 18 to 72 hours.

**Growth Promoting Properties:** The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.

**Indicative Properties:** The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.

**Inhibitory Properties:** No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating >100 cfu of the appropriate microorganism at 30°C-35°C for 72 hours.

### Growth Promoting

#### Organism (ATCC)

*Staphylococcus aureus* subsp.  
*aureus* (6538)

#### Growth

Good

#### Colour of Colony

Yellow colonies surrounded by yellow zone

### Inhibitory

*Escherichia coli* (8739)

Inhibited

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### Additional Microbiological Testing

*Staphylococcus epidermidis* strain  
PCI 1200 (12228)

Good

Red

**Note:** For inhibition no growth of test microorganism should occur.

## Interpretation of Results

Typical colony morphology on Mannitol Salt Agar is as follows:

<i>Staphylococcus aureus</i>	Small to large with yellow zones.
<i>Staphylococci</i> other than <i>S. aureus</i>	Small to large with red zones.
Streptococci	No growth to trace growth.
Micrococci	Large white to orange.
Gram-negative bacteria	No growth to trace growth.

## Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

## Precautions / Limitations

1. Negative plates should be re-incubated overnight before discarding.
2. Presumptive *Staphylococcus aureus* should be confirmed with a coagulase test.
3. A few strains of *S. aureus* may exhibit delayed fermentation of mannitol.

## Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## Reference

1. Chapman, 1945. J. Bact; 50:201.
2. US Pharmacopeial Convention, Inc. 2001. The United States Pharmacopoeia 25/NF 20-2002. The US Pharmacopeial Convention, Inc; Rockville, Md.
3. IP, 1996, Ministry of Health and Family Welfare, Govt. of India, Vol.
4. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8<sup>th</sup> Ed; Rev. A, AOAC, International, Gaithersburg, Md.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

## Product Presentation:

Cat No.	Product description	Pack Size
201130380100	Dehydrated Culture Media	100 g
201130380500	Dehydrated Culture Media	500 g
201130382500	Dehydrated Culture Media	2.5 k
203130820100	Bottle Media	100 mL
203130820250	Bottle Media	6 x 250 mL

 Temperature Limit	 Manufacturer	 <b>LOT</b>	Batch Code	 Date of Manufacture	 This way up	 <b>RO</b> Received on
<b>REF</b> Catalogue Number	 Consult Instructions for use	 Use-by Date	 <b>HC</b>	Hygroscopic keep container tightly closed	<b>OO</b> Opened on	

Revision: 0825/VER-03

## Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.