

## MANNITOL SALT AGAR (MSA) (CHAPMAN MEDIUM) EUROPEAN PHARMACOPOEIA

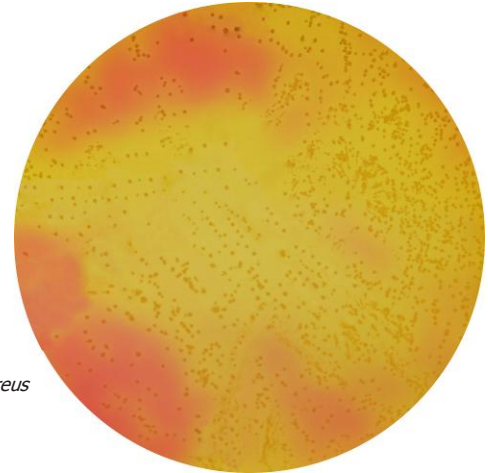
**CAT N°: 1062**

For the isolation and enumeration of pathogenic staphylococci from clinical samples and other materials

### FORMULA IN g/l

Sodium Chloride	75.00	Beef Extract	1.00
D-Mannitol	10.00	Phenol Red	0.025
Pancreatic Digest of Casein	5.00	Bacteriological Agar	15.00
Peptic Digest of Animal Tissue	5.00		

**Final pH 7.4 ± 0.2 at 25°C**



*Staphylococcus aureus*  
ATCC 25923

### PREPARATION

Suspend 111 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 45-50°C, mix well and dispense into plates. The prepared medium should be stored at 8-15°C. The color is red.

The dehydrated medium should be homogeneous, free-flowing and beige-pink in color. If there are any physical changes, discard the medium.

### USES

MANNITOL SALT AGAR (MSA) is a selective medium prepared according to the recommendations of Chapman for the isolation of presumptive pathogenic staphylococci. Most of the other bacteria are inhibited by the high concentration of Sodium chloride.

The Peptone mixture and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Mannitol is the carbohydrate energy source and Phenol red is the pH indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The degradation of mannitol by bacteria produces acidic products that change the color of the medium from pink to yellow. Due to its high content of sodium chloride, a heavy inoculum of the material in study can be used.

The European Pharmacopeia recommends in the Paragraph 2.6.13 "Microbiological examination of non – sterile products: Test for specified micro-organisms". After incubation in Casein Soya Bean Digest Broth at 30-35°C for 18-24 hours, subculture on a plate of Mannitol Salt Agar (MSA), the incubation of the plates at 30-35°C for 18-72 hours for growing promotion test and also to inoculate and incubate *Escherichia coli* ATCC 8739 as negative control. The mannitol fermenting pathogenic staphylococci are large and are surrounded by a yellow zone, colonies of non-pathogenic staphylococci appear as small colonies surrounded by a red or purple zone.

The addition of 5% Egg Yolk Emulsion (Cat. 5152) allows to detect the lipase activity of staphylococci, as well as mannitol fermentation. The high concentration of salt in the medium clears the egg yolk emulsion, and lipase production is detected as a yellow opaque zone around the colonies of staphylococci producing this enzyme. This phenomenon, together with a positive coagulase test, confirms the organism as a pathogenic *Staphylococcus*.

Inoculate and incubate at  $35\pm 2^{\circ}\text{C}$  and observe after 18-24 hours and after 48 hours.

Interpretation: The possible presence of *S. aureus* is indicated by the growth of yellow /white colonies surrounded by a yellow zone. This is confirmed by identification test.

The product complies with the test if colonies of the types described are not present or if the confirmatory identification tests are negative.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of  $35\pm 2^{\circ}\text{C}$  and observed after 18-24 hours and after 48 hours.

Microorganisms	Growth	Colony Color
<i>Escherichia coli</i> ATCC 25922	Inhibited	
* <i>Escherichia coli</i> ATCC 8739	Inhibited	
<i>Enterobacter aerogenes</i> ATCC 13048	Inhibited	
<i>Staphylococcus aureus</i> ATCC 25923	Good	Yellow
* <i>Staphylococcus aureus</i> ATCC 6538	Good	Yellow
<i>Staphylococcus epidermidis</i> ATCC 12228	Acceptable	Red
<i>Staphylococcus epidermidis</i> ATCC 14990	Good	Red

\* According to European Pharmacopoeia 7.0 incubate at  $30-35^{\circ}\text{C}$  during 18-72 hours

## BIBLIOGRAPHY

McColloch Am. J. Vet. Research, 8:173. 1947. Vellilla, Faber, and Pelczar Am. J. Vet. Research, 8:275. 1947.  
Chapman, G.H. 1945 J. Bact. 50:201-203  
European Pharmacopoeia. 7.0



## STORAGE

Once opened keep powdered medium closed to avoid hydration.

