

## Mannitol Salt Agar

MH118

### Intended Use

Recommended for selective isolation of Staphylococci from pharmaceutical products in accordance with Microbial Limit Test by harmonized method of USP/EP/BP/JP/IP.

### Composition\*\*

Ingredients	Gms / Litre
Peptone #	5.000
Tryptone ##	5.000
HM Peptone B ###	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
pH after sterilization ( at 25°C)	7.4±0.2

# Peptic digest of animal tissue

## Pancreatic digest of casein

### Equivalent to Beef extract

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 111.02 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C. Mix well and pour into sterile Petru plates.

*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 (1). Staphylococci have the unique ability of growing on a high salt containing media (2). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5%NaCl was studied by Chapman (3). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (1,4,5,6,7). 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 (8). It is also used in the performance of microbial limit tests for the selective isolation of *Staphylococcus*. The formulation is in accordance with the harmonization of USP/EP/BP/JP/IP (9,10,11,12,13).

The medium contains HM peptone B, tryptone and peptone which makes it very nutritious as they provide carbon, nitrogen compounds, long chain amino acids, vitamins and other essential growth factors and trace nutrients. Many other bacteria except Staphylococci are inhibited by 7.5% sodium chloride. 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 (1)]. 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) (14). 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 (14).

### Type of specimen

Pharmaceutical samples

### Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (9,10,11,12,13).

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

### Warning and Precautions:

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 specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations

1. This medium is a selective medium, some strains of *Staphylococcus aureus* may exhibit a delayed fermentation of mannitol.

### 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

Light yellow to pink homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

#### pH

7.20-7.60

#### Growth Promotion Test

Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BPJP/IP, after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

#### Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating  $\leq 100$  cfu(at 30-35°C for  $\leq 18$  hours).

#### Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating  $\leq 100$ cfu (at 30-35°C for 18-72 hours).

#### Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating  $\geq 100$ cfu (at 30-35°C for  $\geq 72$  hours).

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
<b>Growth Promoting + Indicative</b>						
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	50 -100	luxuriant	25 -100	≥50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
<b>Inhibitory</b>						
<i>Escherichia coli</i> ATCC 8739 (00012*)	≥10 <sup>3</sup>	inhibited	0	0 %		≥72 hrs
<b>Additional Microbiological testing</b>						
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50 -100	luxuriant	25 -100	≥50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
<i>Staphylococcus epidermidis</i> ATCC 12228 (00036*)	50 -100	fair - good	15 -40	30 -40 %	red	18 -72 hrs
<i>Staphylococcus epidermidis</i> ATCC 14990 (00132*)	50 -100	fair - good	15 -40	30 -40 %	red	18 -72 hrs
<i>Proteus mirabilis</i> ATCC 12453	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -72 hrs
<i>Escherichia coli</i> ATCC 25922 (00013*)	≥10 <sup>3</sup>	inhibited	0	0%		≥72 hrs
<i>Escherichia coli</i> NCTC 9002	≥10 <sup>3</sup>	inhibited	0	0%		≥72 hrs
# <i>Klebsiella aerogenes</i> ATCC 13048	≥10 <sup>3</sup>	inhibited	0	0%		≥72 hrs

Key: (#) Formerly known as *Enterobacter aerogenes*, (\*) Corresponding WDCM numbers

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (15,1).

## Reference

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