

Mannitol salt agar (Ph.Eur., USP, JP)

Code 84622.0500

Also know as

Mannitol salt phenol red agar, MSA.

Intended use

For the selective isolation, cultivation and enumeration of staphylococci from clinical and non-clinical specimens.

Formula * - Composition in g/L

Pancreatic digest of casein.....	5.000
Pancreatic digest of animal tissue	5.000
Beef extract.....	1.000
Sodium chloride.....	75.000
D-Mannitol.....	10.000
Phenol red	0.025
Agar.....	15.000

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

Final pH 7.4 ± 0.2 at 25 °C.

Instructions for preparation

Dissolve 111 g in 1 litre of purified water by bringing to the boil with frequent shaking. Sterilise in the autoclave at 121 °C for 15 minutes..

Principle of the method and general information

Mannitol salt agar is a selective and differential medium for the isolation and presumptive identification of pathogenic staphylococci from clinical specimens, foodstuffs, pharmaceutical products and other samples. The medium, prepared according to the formula described by Chapman, contains a high concentration of salt (7.5 %), which inhibits the growth of most bacteria except *Staphylococcus* spp. The production of acids through the fermentation of mannitol, changes the pH of the medium causing a shift of the indicator (phenol red) from red to yellow.

Presumptive pathogenic staphylococci grow very well on the medium producing large colonies surrounded by yellow halos. Non-pathogenic staphylococci grow less luxuriously, forming small colonies surrounded by purple halos.

Instruction for use

For laboratory use only.

Prepare the product to be examined according to the suitable Standard or Norm.

For the detection of *Staphylococcus aureus* in non-sterile pharmaceutical products, European Pharmacopoeia recommends to prepare a 1:10 dilution with not less than 1 g of the product to be examined in Buffered sodium chloride peptone broth (Art. N° 84605.0500) and use 10 ml or a quantity corresponding to 1 g or 1 ml to inoculate a suitable amount of Tryptic soy broth (Art. N° 84675.0500); mix and incubate at 30-35°C for 18-24 hours.

Subculture on plates of Mannitol salt agar and incubate at 30-35°C for 18-72 hours.

The possible presence of *Staphylococcus aureus* is indicated by the growth of yellow/white colonies surrounded by a yellow zone. This is confirmed by identification tests

Limitations

- It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.
- Enterococci may exhibit growth and slight mannitol fermentation on Mannitol salt agar: however, catalase test and Gram morphology should separate the two genera.

- Few strains of *S.aureus* may exhibit a delayed mannitol fermentation; negative plates should be re-incubated and additional 24 hours (total 72 h) before being discarded.
- A possible *S.aureus* must be confirmed by coagulase test; it is recommended that microorganism be sub-cultured to a less inhibitory medium not containing excess salt (e.g. Nutrient agar) to avoid possible interference of salt with coagulase testing or other diagnostic tests.

Quality Control

Physical characteristics:

Appearance of powder	Pink, fine, homogeneous hygroscopic powder
Appearance of prepared medium	Red-orange, limpid
pH (25°C)	7.4 ± 0.2

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity Ratio
Growth promoting test according to Ph.Eur.				
<i>S.aureus</i> ATCC 25923	30-35°C / 18 h / AE	QT / ≥ 100 CFU	Good growth with typical yellow colonies and yellow halo	PR ≥ 0.5
Test for inhibitory properties, according to Ph.Eur.				
<i>E. coli</i> ATCC 8739	30-35°C / 72 h / AE	EC / ≥ 100 CFU	No growth	
Other quality control tests				
<i>S. aureus</i> ATCC 6538	37°C / 24 h / AE	EC	Good growth with yellow colonies and yellow halo	
<i>S. aureus</i> CBS 100	37°C / 24 h / AE	EC	Good growth with yellow colonies and yellow halo	
<i>S. epidermidis</i> ATCC 12228	37°C / 24 h / AE	EC	Good growth with white colonies and violet halo	
<i>P.mirabilis</i> ATCC 12453	37°C / 24 h / AE	MM	Growth partially inhibited	
<i>E. faecalis</i> ATCC 29212	37°C / 24 h / AE	MM	No growth	

Notes

PR (Productivity Ratio): CFU obtained on the culture medium under test / CFU obtained on Tryptic soy agar plate

Incubation atmosphere AE :aerobic incubation

Inoculation method QT : quantitative surface plating method; EC: semi-quantitative, ecometric technique; MM: modified Miles-Misra surface drop method

Microbiological characteristics tested in accordance to Ph.Eur.

ATCC is a registered trade mark of American Type Culture Collection; CBS: strain obtained from Laboratory culture collection

References

- Chapman, G.H. (1945) - J. Bact. 50, 201-203.
- European Pharmacopoeia: 2.6.13 Microbiological examination of non-sterile products: tests for specified micro-organisms

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+10 °C to 30 °C and <60% RH).

Ordering information

84622.0500 Mannitol salt agar (EP/JP/USP) Bottle of 500 g