## Formula * in g/L

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

Final pH 7.4 ±0.2 at 25 °C
* Adjusted and /or supplemented as required to meet performance criteria

## Directions

Suspend 111 g of powder in 1 L of distilled water and bring to the boil. Dispense in tubes or flasks and sterilize in the autoclave at 121ºC for 15 minutes.

## Description

Mannitol Salt Agar is a classical medium for the detection and enumeration of staphylococci. It was described by Chapman and has been adopted by many official organisations. Several modifications of it have been developed, all formulations resulting in media with similar efficiency.
This medium takes advantage of the high saline tolerance of staphylococci, and uses sodium chloride as a selective agent. Only staphylococci and halophilic enterobacteria are able to grow freely at the concentration of salt employed in this medium, while other bacteria are inhibited. It also exploits the correlation between the pathogenicity of staphylococci and their ability ferment mannitol.
Mannitol fermentation results in an accumulation of acid products, indicated by the phenol red indicator turning yellow. A yellow halo surrounds the presumptive pathogenic colonies, while the rest of the medium remains red/orange in colour.

## Technique

Inoculate the plates and incubate at 37 °C for 36 hours or at 30-35 °C for 3 days.

The typical appearance of the colonies after the correct incubation is as follows:
- Presumptive pathogenic staphylococci (coagulase +) are mannitol positive and produce large colonies with a yellow halo.
- Non-pathogenic staphylococci (coagulase -) are usually mannitol negative and produce small colonies without a halo or change in colour.

Coagulase presence must be tested by the classical technique in order to establish its true pathogenic potential.

Note: According to the methodology chosen by the laboratory (Pharmacopeia or other international standards), may be slight variations in incubation times and temperatures, as well as inhibition of *E. coli*, which can be variable depending on the inoculated bacterial population . This medium can normally reduce the bacterial load by up to 3 decimal logarithms.

## Quality control

**Incubation temperature:** 30-35 ºC **Incubation time:** 24 - 48 - 72 h

**Inoculum:** Practical range 10-100 CFU. (Productivity) according to Eur. Pharm. harm.

| Microorganism | Growth | Remarks |
|---|---|---|
| *Escherichia coli* ATCC® 8739 | Inhibited | Selectivity |
| *Staphylococcus epidermidis* ATCC® 12228 | Poor to good (Specificity) | White-pink colonies; Red medium Man(-) |
| *Staphylococcus aureus* ATCC® 25923 | Productivity > 0.50 | White colonies; Yellow medium Man (+) |
| *Staphylococcus aureus* ATCC® 6538 | Productivity > 0.50 | White colonies; Yellow medium Man (+) |

## References

· ATLAS, R.M. & L.C.PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
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· DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
· EUROPEAN PHARMACOPOEIA 10.0 (2020) 10th ed. § 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. EDQM. Council of Europe. Strasbourg.
· FDA (Food and Drug Adminstrations) (1995) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC Internacional Inc. Gaithersburg. MD. USA.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· ISO 22718 Standard (2015) . Cosmetics - Microbiology - Detection of Staphylococcus aureus.
· USP 33 - NF 28 (2011) <62> Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.

## Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).