

## Specification

Selective medium for the isolation of pathogenic staphylococci according to the Pharmacopoeial Harmonized Methodology and the ISO Standard 22718:2006.

## Presentation

10 Prepared bottle  
Bottles 125 ml  
with: 100 ± 3 ml

### Packaging Details

1 box with 10 bottles 125 ml. Non injectable cap.

### Shelf Life

12 months

### Storage

8-25°C

## Composition

Composition (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

## Description /Technique

### 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:

To use, the contents of the bottle should be poured into plates. The melting of the culture medium should be carried out according to the manufacturer's instructions, either in a water bath (100°C) or microwave oven. Never apply direct heat to melt a medium. The melting temperatures and times depend on the shape of the container, the volume of medium and the heat source. Before melting any medium loosen the screwcap of the container to avoid breaking the container. The medium should be melted only once and used. Media with agar should not be melted repeatedly as their characteristics change with each remelting. Overheating should be avoided as much as prolonged heating, especially with regard to media with an acidic or alkaline pH. Once melted pour the plates using aseptic techniques. To inoculate, follow standard laboratory methods or the applicable norms. Spiral plate method, streak plating, econometric methods, dilution banks, spread plating etc...

Inoculate the plates and incubate at 37°C for 36 hours or at 32°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 produces 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: Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the methodology.

**Quality control****Physical/Chemical control**

Color : Reddish

pH: 7.4 ± 0.2 at 25°C

**Microbiological control**

Melt Medium - Pour plates - inoculation Practical range 100±20 CFU; Min. 50 CFU (Productivity)/ 10<sup>4</sup>-10<sup>6</sup> (Selectivity).  
Aerobiosis. Incubation at 30-35°C. Reading at 18-72h

**Microorganism***Escherichia coli* ATCC® 8739, WDCM 00012*Stph. aureus* ATCC® 25923, WDCM 00034*Staphylococcus aureus* ATCC® 6538, WDCM 00032*Stph. epidermidis* ATCC® 12228, WDCM 00036**Growth**

Inhibited

Good. White colonies. Yellow medium.

Good. White colonies. Yellow medium.

Poor to good- White colonies -Red medium

**Sterility Control**

Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH

Check at 7 days after incubation in same conditions

**Bibliography**

- ATLAS, R.M. & L.C.PARKS (1993) Handbook of Microbiological Media. CRC Press. Boca Raton. Fla. USA.
- CHAPMAN (1945) The significance of sodium chloride in studies of staphylococci. J. Bact 50:201.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
- EUROPEAN PHARMACOPOEIA 8.0 (2014) 8th 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 Administrations) (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.