TECHNICAL DATA SHEET

MANNITOL SALT AGAR

DETECTION OF PATHOGENIC STAPHYLOCOCCI

1 INTENDED USE

Mannitol Salt agar is used for the detection of *Staphylococcus aureus* in pharmaceutical products. The typical composition responds to the formulation defined in the European, American and Japanese Pharmacopoeia (EP, USP and JP).

It is also used for the selective isolation, detection and enumeration of pathogenic staphylococci in cosmetic products and filterable water as in swimming pools, potable water or spas.

2 HISTORY

The experiments of Koch showed that staphylococci could tolerate hypersalted media at 7.5%. Chapman confirmed these initial results and observed that staphylococci which coagulated rabbit plasma formed yellow colonies on this medium, while most other bacteria were inhibited.

3 PRINCIPLES

The high sodium chloride concentration inhibits the growth of most bacteria other than staphylococci.

Mannitol fermentation, shown by the color change of the pH indicator (phenol red) to yellow orients the diagnosis.

The demonstration of pathogenic staphylococci is generally confirmed by a coagulase test.

4 TYPICAL COMPOSITION

The composition can be adjusted to obtain optimal performance.

For 1 liter of media:

- Tryptone ..... 5.0 g
- Peptic digest of meat ..... 5.0 g
- Meat extract ..... 1.0 g
- Mannitol ..... 10.0 g
- Sodium chloride ..... 75.0 g
- Phenol red ..... 25.0 mg
- Bacteriological agar ..... 15.0 g

pH of the ready-to-use media at 25 °C: 7.4 ± 0.2.

5 PREPARATION

- Suspend 111.0 g of dehydrated medium (BK030) in 1 liter of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes or flasks.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47°C.
- Pour into sterile Petri dishes (Ø 55 mm or 90 mm according to the application) and let solidify on a cold surface.

✓ **Reconstitution:**
111.0 g/L

✓ **Sterilization:**
15 min at 121 °C

6 INSTRUCTIONS FOR USE

Detection of *Staphylococcus aureus* (Pharmacopoeias)

- Dry in an incubator the 90 mm plates with the covers partially removed.
- Transfer 0.1 mL of the product to analyze to the surface of the plates.
- Spread the inoculum on the surface of the medium with a sterile triangle.
- Incubate at 30-35 °C for 18 to 72 hours.

✓ **Inoculation:**
0.1 mL on surface

✓ **Incubation:**
18-72 h at 30-35 °C

Water testing by membrane filtration

- Aseptically filter the appropriate volume of water through a nitrocellulose membrane.
- Place the membrane, filtered side up, on the surface of the agar prepared as described, insuring a complete contact.
- Incubate at 36 ± 2 °C for 44 ± 4 hours.

✓ **Inoculation:**
Membrane filtration

✓ **Incubation:**
44 h at 36 °C

7 RESULTS

Count the characteristic colonies.
Pathogenic staphylococci form luxuriant white to yellow colonies, surrounded by a yellow ring due to the fermentation of mannitol.
Non-pathogenic staphylococci generally give rise to small red colonies which do not change the color of the medium.
Several strains of *Staphylococcus epidermidis* can ferment mannitol.

See ANNEX 1: PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media: cream to pinkish colored powder, free-flowing and homogeneous.
Prepared media: red agar.

Typical culture response at 30-35 °C (harmonized Pharmacopoeia: EP, USP & JP)

Microorganisms		Growth Productivity Ratio P_R	Characteristics
Staphylococcus aureus [1]	WDCM 00032	$P_R \geq 50$ %	White to yellow colonies surrounded by a yellow zone
Escherichia coli [2]	WDCM 00012	Inhibited, score 0	-

(1) After maximum of 18 hours of incubation, inoculum ≤ 10^2 microorganisms
(2) After minimum of 72 hours of incubation, inoculum > 10^2 microorganismes

9 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C.
The expiration dates are indicated on the labels.

Prepared media in vials (*): 180 days at 2-8 °C.
Prepared media in plates (*): 30 days at 2-8 °C.
(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated media:
500 g bottle..... BK030HA

11 BIBLIOGRAPHY

Chapman, G.H. 1945. The significance of sodium chloride in studies of staphylococci. J. Bacteriol., 50: 201.

Chapman, G.H. 1948. An improved Stone medium for the isolation and testing of food poisonning *staphylococci*. Food Research, 13: 100-105.

European Pharmacopeia. Chapiter 2.6.13. Microbiological examination of non-sterile products: Test for specified microorganisms. Harmonized method.

The United States. Chapter <62> Microbiological examination of non-sterile products: Test for specified products.

The Japanese Pharmacopoeia. Chapter 4.05 Microbial Limit Test II. Microbiological examination of non-sterile products: Test for specified products.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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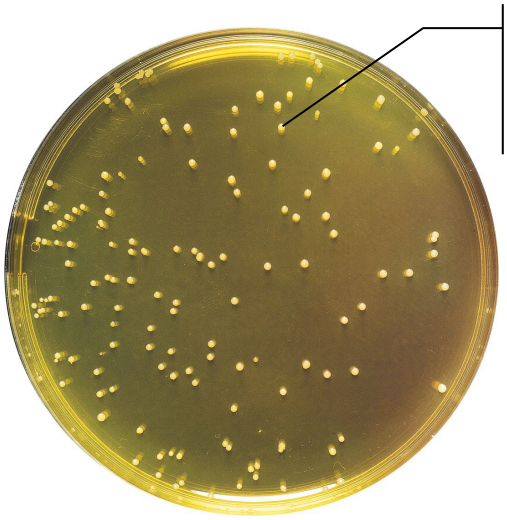
Mannitol Salt Agar

Detection and enumeration of pathogenic staphylococci

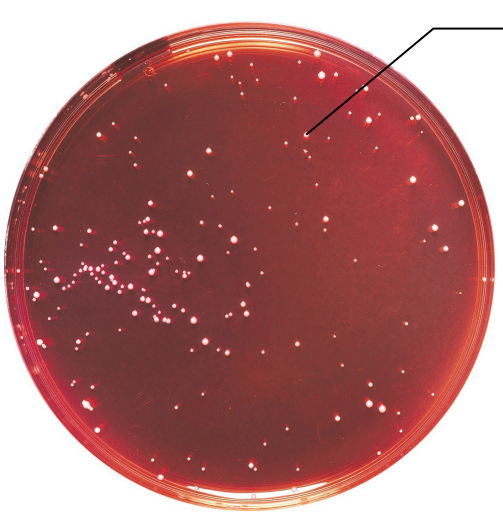
Results:

Growth obtained after 24 hours of incubation at 37 °C (surface inoculation).



Staphylococcus aureus

Characteristic colony:
yellow colony on yellow media, or
surrounded by a yellow halo.



Staphylococcus epidermidis

Non characteristic colony:
white colony on red agar.