Instructions For Use
ENGLISH

Mannitol Salt Agar

Selective medium for isolation and enumeration of staphylococci from clinical and nonclinical specimens.

INTENDED PURPOSE

Selective medium used for isolating pathogenic staphylococci from clinical specimens, food and other materials of sanitary importance. This medium is intended as an aid in the diagnosis, requiring further identification tests to complete the diagnostic results.

DESCRIPTION

Mannitol Salt Agar (MSA) is a medium for the selective isolation of staphylococci while allowing differentiation of mannitol fermenting from non fermenting staphylococci.

This medium is prepared according to recommendations of the harmonized USP/EP/JP method for the detection of *S. aureus* in non sterile pharmaceutical products.

TYPICAL FORMULA*	(g/litre)
Pancreatic Digest of Casein	5.0
Peptic Digest of Animal Tissue	5.0
Beef Extract	1.0
D-Mannitol	10.0
Sodium Chloride	75.0
Phenol Red	0.025
Agar	15.0
Final pH 7.4 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Pancreatic digest of casein, peptic digest of animal tissue and beef extract provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Mannitol is the fermentable carbohydrate. The high salt content of 7.5% inhibits most bacteria other than staphylococci. Phenol red is the pH indicator. Agar is the solidifying agent.

PREPARATION

Dehydrated medium

Suspend 111 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil for 1 minute shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

Medium in bottles

Melt the content of the bottle in a water bath at 100°C (loosing the cap partially removed) until completely dissolved. Then screw the cap and check the homogeneity of the dissolved medium, if it is the case turning the bottle upside down. Cool at 45-50°C, mix well avoiding foam formation and aseptically distribute into Petri dishes.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Autoclave, water bath, sterile Petri plates, test tubes, inoculating loops, swabs, incubator, quality control organisms.

SPECIMENS

Clinical specimens such as faeces, materials from respiratory tract, purulent exudates, wounds, abscesses etc., should be sampled at the acute stage, before antimicrobial therapy (where possible) and examined as soon as possible after collection. Good laboratory practices for collection, transport and storage of the clinical specimens should be applied. Refer to specific guidelines for more information about specimen collection and preparation.

TEST PROCEDURE

Ensure there is no visible moisture on the plates before use.

Inoculate the plates by directly streaking the specimen on the agar surface or spread the sample from an enrichment culture to obtain well-isolated colonies.

Incubate aerobically at 35 ± 2°C for 24-48 h.

Following the harmonized USP/EP/JP method for microbiological examination of non sterile products, inoculate the sample in Tryptic Soy Broth, then subculture on a MSA plate and incubate at 30-35°C for 18-72 hours.

For more details, consult appropriate guidances.

INTERPRETING RESULTS

S. aureus cultivates with yellow or white colonies surrounded by a yellow zone. Confirm by identification tests(*).

Coagulase-negative Staphylococci form small colourless to red colonies with no color change to the medium.

* Suspect colonies can be subcultured to a moderately selective medium such as Baird Parker RPF Agar for the determination of coagulase activity (ISO 6888-2).

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store bottles and prepared plates at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 4 years.
Medium in bottles: 2 years.
Ready-to-use plates: 6 months.

QUALITY CONTROL

Appearance of Dehydrated Medium: Free-flowing, homogeneous, beige-pink.

Appearance of Prepared Medium: Slightly opalescent, pinkish-red.

Expected Cultural Response:

Control strain		Inoculum	Incubation	Criteria	Specification
Staphylococcus aureus	WDCM 00034 (ATCC® 25923; NCTC 12981)	50-100 CFU	24-48 h / 35 ± 2°C	Good growth ($P_R \geq 0.5$)	Yellow colonies with yellow zone
Staphylococcus epidermidis	WDCM 00036 (ATCC® 12228; NCTC 13360)				red colonies
Staphylococcus aureus	WDCM 00193 (ATCC® 6538; NCTC 10788)		18-72 h / 30-35°C		Yellow colonies with yellow zone
Escherichia coli	WDCM 00013 (ATCC® 25922; NCTC 12241)	10^4-10^6 CFU	24-48 h / 35 ± 2°C	Inhibition	—-
Escherichia coli	WDCM 00012 (ATCC® 8739; NCTC 12923)		18-72 h / 30-35°C	Inhibition	—-

A productivity ratio (P_R) of 0.5 is equivalent to a recovery rate of 50%.

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of MSA was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Some strains of *S. capitis*, *S. xylosus*, *S. cohnii*, *S. sciuri*, *S. simulans* are mannitol positive and produce yellow colonies surrounded by yellow zones on this medium.

MSA is intended as an aid in the diagnosis of infectious diseases, requiring further tests to complete the diagnostic results. All identification tests should ideally be performed from non-selective agar.

WARNING AND PRECAUTIONS

1) **For *in vitro* diagnostic use (IVD).**
2) **For laboratory professional use only.**
3) Operators must be trained and have certain experience. Please read the instructions carefully before using the product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.
4) Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.
5) Do not use if the product or packaging appears to be damaged.
6) Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
7) Handle all specimens as if infectious using safe laboratory procedures. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
8) Avoid cross-contamination of samples by using disposable tips and changing them after each sample.
9) Do not mix reagents of different batches. Please use the product within the validity period.
10) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled
11) Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.
12) Ensure laboratory equipment is calibrated and maintained in accordance with the laboratory's procedure.
13) When test results are transmitted from the laboratory to an informatics centre, attention has to be done to avoid erroneous data transfer.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

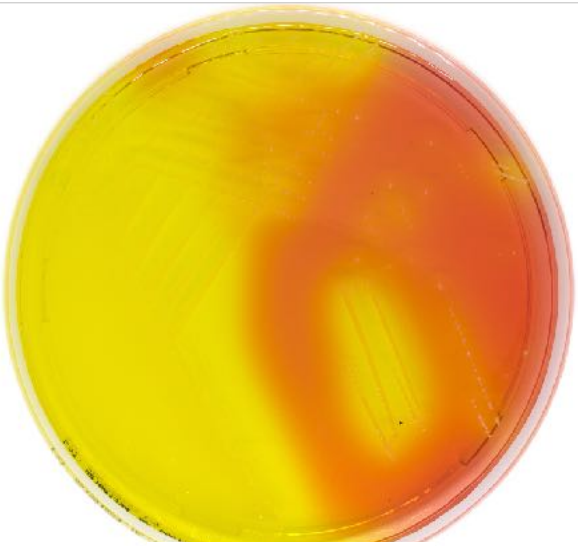
The product is available in the various configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit **liofilchem.com**

Product	Format	Packaging	Ref.
Mannitol Salt Agar (MSA)	Plate 90 mm	20 plates	10030
		100 plates	10030*
	Bottle	6 x 100 ml	402290
		6 x 200 ml	412290
		6 x 500 ml	470080
	Dehydrated media	100 g	620029
		500 g	610029
		5 kg	6100295

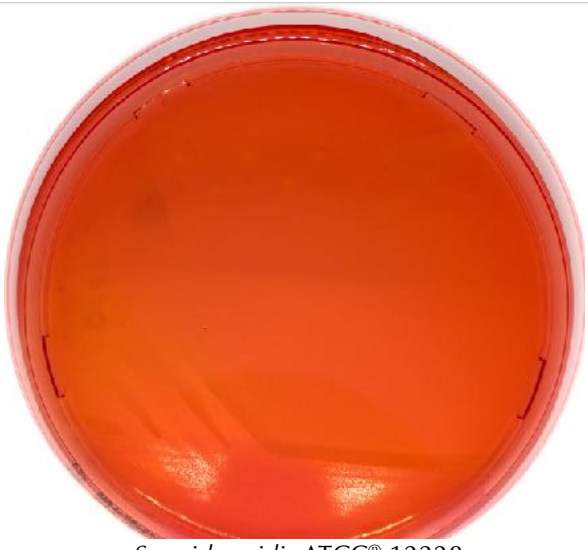
Revision History

Revision	Release Date	Change Summary
1	2024-01-17	Updated: Layout and content in compliance with IVDR 2017/746 Added: Example photographs

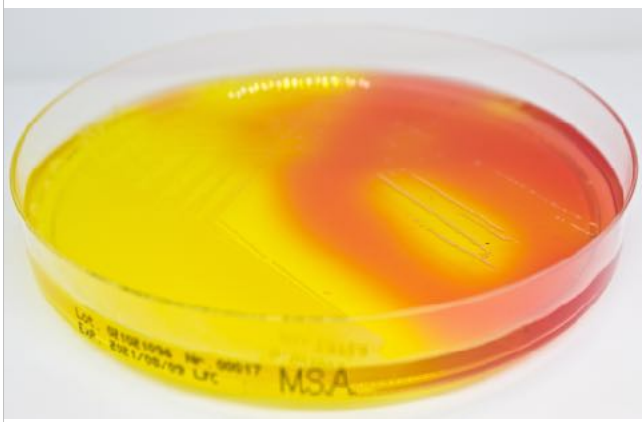
This IFU document and the SDS are available from the online Support Center:
liofilchem.com/ifu-sds

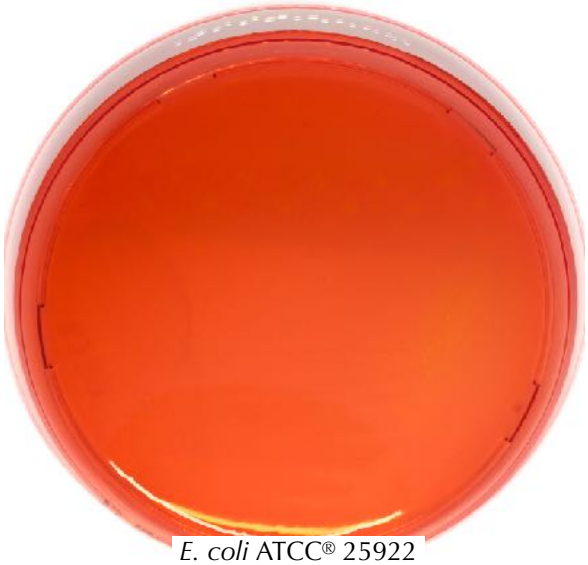


S. aureus ATCC® 25923



S. epidermidis ATCC® 12228





E. coli ATCC® 25922