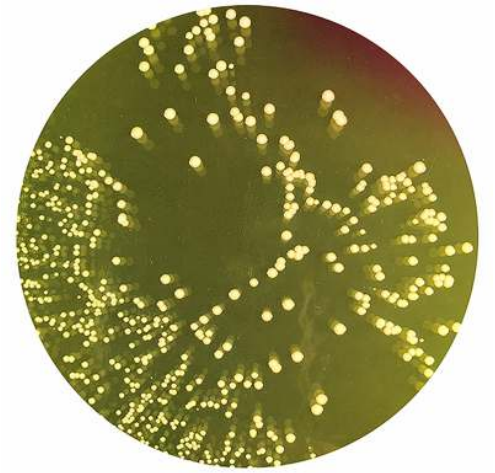
INSTRUCTIONS FOR USE

MANNITOL SALT AGAR

Ready-to-use plates



Mannitol Salt Agar: *S.aureus* colonies

1 - INTENDED USE

In vitro diagnostic device. Selective medium for the isolation and differentiation of staphylococci from clinical and non clinical specimens.

2 - COMPOSITION - TYPICAL FORMULA *

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

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Staphylococcus species are Gram positive, non-motile, non-sporeforming cocci of varying size occurring singly, in pairs and in irregular clusters. The optimum growth temperature is 30°C-37°C. They are facultative anaerobes and have a fermentative metabolism. *S.aureus* is a ubiquitous commensal bacterium on human skins and anterior naris, but frequently causes severe infections in humans.

Mannitol Salt Agar is based on early work by Gordon[1] indicating that the fermentation of mannitol could be used as a mean for differentiating pathogenic from non-pathogenic staphylococci, and by Koch[2] discovering that the presence of 7.5% sodium chloride in media inhibited the growth of most organisms except staphylococci. Chapman[3] utilized this information to formulate phenol-red mannitol agar with 7,5% of sodium chloride.

Mannitol Salt Agar is a selective and differential medium intended for the isolation of staphylococci from clinical specimens[4,5] and for the differentiation of mannitol fermenting from non fermenting staphylococci. In clinical samples, mannitol positive isolates are suggestive of *S. aureus* and should be tested further.[6]

Mannitol Salt Agar meets harmonised EP, USP, JP requirements [7] for the detection of *S.aureus* in non sterile pharmaceutical products and is recommended for the detection of *S.aureus* in cosmetics[8,9].

Peptones provide carbon, nitrogen and trace elements for bacterial growth, sodium chloride at the concentration of 75 g/L creates a high osmotic pressure: staphylococci can withstand the pressure, while this concentration will inhibit the growth of most other gram-positive and gram-negative bacteria[2]. Additionally, the medium contains mannitol as fermentable carbohydrate and phenol red as a pH indicator. When mannitol is fermented acid is produced, which lowers the pH and results in the formation of a yellow area surrounding an isolated colony. Mannitol non-fermenters that withstands the high salt concentration, would display a red to pink area due to peptone breakdown[6].

4 - PHYSICAL CHARACTERISTICS

Medium appearance	red-violet, limpid
Final pH at 20-25 °C	7.4 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mannitol Salt Agar	Ready-to-use plates	541665	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Mannitol Salt Agar is intended for the bacteriological examination of human clinical specimens from contaminated sources, such as faeces, materials from respiratory tract, purulent exudates, wounds, abscesses.[4,5,10] and non-clinical specimens, such as non sterile pharmaceutical products and cosmetics[8,9] . Good laboratory practices for collection, transport and storage of the clinical specimens should be applied.[11] Consult appropriate standard methods for details on sample collection and preparation of non sterile pharmaceutical products and cosmetics [7,8,9]

8- TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Inoculate and streak the specimen with a loop over the four quadrants of the plate to obtain well isolated colonies, ensuring that sections 1 and 4 do not overlap. Alternatively, if the material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate in aerobic conditions at 35-37°C and record the results after 24 and 48 hours.

For the detection of *S.aureus* in non sterile pharmaceutical products follow the test method described by EP and summarized below.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

Use 10 mL or the quantity corresponding to 1 g or 1 mL of sample to inoculate a suitable amount of Tryptic Soy Broth. Mix and incubate at 30–35 °C for 18–24 h. Subculture by streaking on a plate of Mannitol Salt Agar, and incubate at 30–35°C for 18–72 h.

9 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies.
Suspected *S.aureus* colonies are yellow/white surrounded by a yellow zone.
Mannitol non fermenting staphylococci produce small colonies with no colour change of the medium or with red or purple zone.
It is mandatory to confirm putative *S.aureus* isolates recovered on Mannitol Salt Agar.[10]

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[12]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S.aureus ATCC 6538 or 25923	35°C / 18-24 H / A	growth with yellow colonies
S.epidermidis ATCC12228	35°C / 18-24 H /A	growth with small colourless colonies with violet halo
P.mirabilis ATCC 12453	35°C / 18-24 H / A	partially inhibited
E.coli ATCC 8739	35°C / 68-72 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to use plates of Mannitol Salt Agar and of the raw material used for the production of prepared plates (dehydrated Mannitol Agar REF 401665) are tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by a quantitative test with the target strain *S.aureus* ATCC 6538: Mannitol Salt Agar plates are inoculated with decimal dilutions in saline of a colony suspension and incubated at 35°C for 18-24 hours. The colonies are enumerated on both batches and the productivity ratio (*Pr*) is calculated. If *Pr* is ≥ 0,7 and if the colonies' morphology and colour are typical (yellow or white colonies with yellow halo) the results are considered acceptable and conform to the specifications. Furthermore the productivity characteristics are tested by semi-quantitative ecometric technique with the following target-strains: *S.aureus* ATCC 25923, clinical isolate *S.aureus* and *S.epidermidis* ATCC 12228. Colonies of mannitol fermenters are white/yellow in colour with a yellow zone; colonies of mannitol non fermenter (*S.epidermidis*) are white with a violet halo. The amount of growth on the plates after incubation is evaluated and shall be comparable in both batches.
The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with decimal dilutions in saline from 10^{-1} to 10^{-4} of a 0.5 McFarland suspension of the non-target strains *E.faecalis* ATCC 29212, *E.coli* ATCC 8739 and *P.mirabilis* ATCC 12453. After incubation for 24-72 hours, the growth of non-target strains *E.faecalis* and *E.coli* is inhibited at the dilution 10^{-1} and the growth of *P.mirabilis* is partially inhibited.

12 - LIMITATIONS OF THE METHOD

- Enterococci may exhibit growth and slight mannitol fermentation, however catalase test and Gram morphology should separate the two genera.[13]
- Few strains of *S.aureus* may exhibit a delayed mannitol fermentation; negative plates should be re-incubated for additional 24 hours before being discarded.[13]
- A putative *S.aureus* strain must be confirmed by the coagulase test. Subculture the colonies on a non-inhibitory agar for performing the test.
- Mannitol Salt Agar is a selective medium for pathogenic *S.aureus* isolation when distinct colonies are observed after 24 hours of incubation, however, if incubated 48 hours even *Micrococcus* and *Bacillus* and certain *Serratia* strains may grow.[13]
- Media colour change demonstrates mannitol fermentation, not colony colour. This is particularly important as many micrococci are pigmented.[6]
- There are reports that some coagulase negative staphylococci can produce yellow colonies with yellow zone on Mannitol Salt Agar.[14]
- Some target organisms (potentially pathogenic *Staphylococcus* strains) may be inhibited on this medium. The sensitivity of the described procedure varies depending of the clinical specimens, the amount of competitive non-target organisms, the number of target organisms. If it is necessary to detect all potential pathogens, it is advisable to use also a non selective and non differential medium together with Mannitol Salt Agar.
- For the detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains more accurate detection methods, such as molecular techniques, should be applied.
- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates from pure culture for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious disease; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to use plates be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana S.r.l. for the risk reduction linked to infectious animal diseases.

- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

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TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 2	Updated layout and content in compliance with IVDR 2017/746	2020/05
Revision 3	Removal of obsolete classification	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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