MANNITOL SALT BROTH

Dehydrated culture medium

1 - INTENDED USE

Selective liquid medium for the detection and differentiation of staphylococci.

2- COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Peptocomplex	10.000 g
Beef extract	1.000 g
Sodium chloride	75.000 g
Mannitol	10.000 g
Phenol red	0.025 g

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Mannitol Salt Broth is based on early work by Gordon[1] indicating that the fermentation of mannitol could be used as a mean of differentiating pathogenic from non-pathogenic staphylococci, and by Koch[2] discovery 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 media with 7.5% of sodium chloride.

Mannitol Salt Broth is a selective and differential medium intended for enrichment of staphylococci and for their enumeration by MPN or MF techniques.

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 colour. Mannitol non-fermenters that withstand the high salt concentration, would display a red to pink colour due to peptone breakdown[4].

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 96 g in 1000 mL of cold purified water. Mix thoroughly and heat if necessary to completely dissolve the powder. Distribute and sterilize by autoclaving at 121°C for 15 minutes.

5- PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	orange, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	red-orange, limpid
Final pH at 20-25 °C	7.4 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Mannitol Salt Broth	Dehydrated medium	4016662	500 g (5.2 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipette, incubator and laboratory equipment as required, Petri dishes, screwcap tubes, Erlenmeyer flasks, absorbent pads, membrane filters, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Foodstuffs and other materials of sanitary importance. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9- TEST PROCEDURE

Place 1 mL of the sample in a 9 mL tube, or 10 mL in a 90 mL bottle. Alternatively pour 2 mL onto absorbent pad placed in a 55 mm Ø Petri dish and then place the membrane used for the filtration of the sample.

Incubate at 37 °C for 24-48 hours.

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth: yellow tubes presumably contain *S. aureus*, turbid, non-yellow tubes presumably contain other staphylococci.

With membrane filtration, yellow colonies are presumed to be *S. aureus* and red colonies are presumed to be other staphylococci.

Confirm the isolates by coagulase test or latex agglutination test.

11 - 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.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S. aureus ATCC 6538 or 25923	37°C / 18-24 H / A	growth the medium turns yellow
S. epidermidis ATCC12228	37°C / 18-24 H /A	growth, no colour change of the medium
E. coli ATCC 8739	37°C / 24-48 H / A	partially inhibited

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

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12- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Mannitol Salt Broth is tested for productivity, specificity and selectivity by dilution to extinction method, by comparing the results with a previously approved Reference Batch.
Productivity and specificity are tested, by inoculating 1 mL of appropriate decimal dilutions of coagulase positive and coagulase negative staphylococci in test tubes, incubating at 37°C for 36 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). *S. aureus* ATCC 25923 exhibits good growth and the tubes turn yellow; *S. epidermidis* ATCC 12228 exhibits good growth with no colour change of the medium. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Selectivity is tested with the following non-target strains: *E. coli* ATCC 25922 and *P. vulgaris* ATCC 9484. After incubation at 37°C for 36 hours, the growth of non-target strains is partially inhibited.

13 - LIMITATIONS OF THE METHOD

- Enterococci may exhibit growth and slight mannitol fermentation; however, catalase test and Gram morphology should separate the two genera.[5]
- Few strain of *S.aureus* may exhibit a delayed mannitol fermentation; negative tubes should be re-incubated for additional 24 hours before being discarded.[5]
- Mannitol Salt Broth is a selective medium however, if incubated 48 hours, *Micrococcus* and *Bacillus* and certain *Serratia* strains may grow.[5]
- There are reports that some coagulase negative staphylococci can acidify the medium.[6]
- Some target organisms (potentially pathogen *Staphylococcus* strains) may be inhibited on this medium. The sensitivity of the described procedure varies depending of the specimens, the amount of competitive non-target organisms and the number of target organisms.

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- 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 this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium 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 for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- 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.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps). The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type, and the storage method applied (temperature and packaging).

16 - REFERENCES

1. Gordon, M. H. 1903-04. Reports of some characters by which various streptococci and staphylococci may be differentiated and identified. Local British Government Board, Rept. Med. Officer. 33:388–430.
2. Koch, F. E. 1942. Electivnährboden für Staphylokokken. Zentr. Bakt. Parasitenk. I Orig.149:122–124.
3. Chapman, G. H. 1945. The significance of sodium chloride in studies of staphylococci. J.Bacteriol. 50:201–203.
4. Shields P, Tsang AY. Mannitol salt agar plates protocol. American Society for Microbiology (ASM), October 9, 2006.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
6. Thakur P, Nayyar C, Tak V, Karnika Saigal K. Mannitol-fermenting and tube coagulase-negative staphylococcal isolates: unraveling the diagnostic dilemma. J Lab Physicians 2017; 9(1):65–66.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

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REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/09

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

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