BBL™ Mannitol Salt Agar

L007389 • Rev. 08 • November 2015

QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Mannitol Salt Agar is a selective and differential medium for the detection and enumeration of staphylococci from clinical and nonclinical specimens.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Streak the plates for isolation using 18- to 24-h broth cultures diluted 10^3–10^4 CFU/plate for *Staphylococcus* spp. and 10^4–10^5 CFU/plate for *Proteus mirabilis*.
   b. Incubate plates at 35 ± 2 °C in an aerobic atmosphere.
   c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 and 48 h for amount of growth, colony size, pigmentation and selectivity.
3. Expected Results

CLSI Organisms	ATCC®	Recovery	Reaction
**Staphylococcus aureus*	25923	Growth	Colonies have yellow zones at 48 h
**Staphylococcus epidermidis*	12228	Growth	Colonies have red or purple zones at 48 h
**Proteus mirabilis*	12453	Inhibition (partial) at 24 h. Inhibition of swarming at 48 h as compared to TSA II control.	
Additional Organism			
Staphylococcus aureus	13150	Growth	Colonies have yellow zones at 48 h

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Mannitol Salt Agar is used for the selective isolation and enumeration of staphylococci from clinical and nonclinical materials.

V SUMMARY AND EXPLANATION

Koch, in 1942, reported that only staphylococci grow on agar media containing 7.5% sodium chloride.[1] Chapman further studied this phenomenon in greater detail and concluded that the addition of 7.5% sodium chloride to phenol red mannitol agar results in an improved medium for the isolation of plasma-coagulating staphylococci.[2] This is the formulation supplied as **BBL** Mannitol Salt Agar.

VI PRINCIPLES OF THE PROCEDURE

Mannitol Salt Agar is a nutritive medium due to its content of peptones and beef extract, which supply essential growth factors, such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the differentiation of staphylococcal species.

VII REAGENTS

Mannitol Salt Agar

Approximate Formula* Per Liter Purified Water

Beef Extract	1.0 g	D-Mannitol	10.0 g
Pancreatic Digest of Casein	5.0 g	Phenol Red	0.025 g
Peptic Digest of Animal Tissue	5.0 g	Agar	15.0 g
Sodium Chloride	75.0 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions”[3-6] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[7,8] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Mannitol Salt Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge and streak from this inoculated area.

Incubate plates 24–48 h at 35 ± 2 °C in an aerobic atmosphere.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a “dilution” technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Further, growth of each organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas. Better isolation is obtained due to the inhibitory action of the medium.

Typical colonial morphology on Mannitol Salt Agar is as follows:

Staphylococcus aureus	Small to large with yellow zones
Staphylococci other than *S. aureus*	Small to large with red zones
Streptococci	No growth to trace growth
Micrococci	Large, white to orange
Gram-negative bacteria	No growth to trace growth

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[7-12]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
221173	**BD BBL™** Mannitol Salt Agar, Pkg. of 20 plates
221271	**BD BBL™** Mannitol Salt Agar, Ctn. of 100 plates

XIII REFERENCES

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7. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R. H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
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