Mannitol Salt Agar

Intended Use

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

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[1-3] performance specifications, where applicable.

Summary and Explanation

Koch, in 1942, reported that only staphylococci grow on agar media containing 7.5% sodium chloride.[4] 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 plasmacoagulating staphylococci.[5] Mannitol Salt Agar is listed User

Quality Control

Identity Specifications

BBL™ Mannitol Salt Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material and may contain many light to dark red flecks.
Solution:	11.1% solution, soluble in purified water upon boiling. Solution is medium to dark, red to rose; clear to slightly hazy.
Prepared Appearance:	Light to medium rose red, trace orange; clear to hazy.
Reaction of 11.1% Solution at 25°C:	pH 7.4 ± 0.2

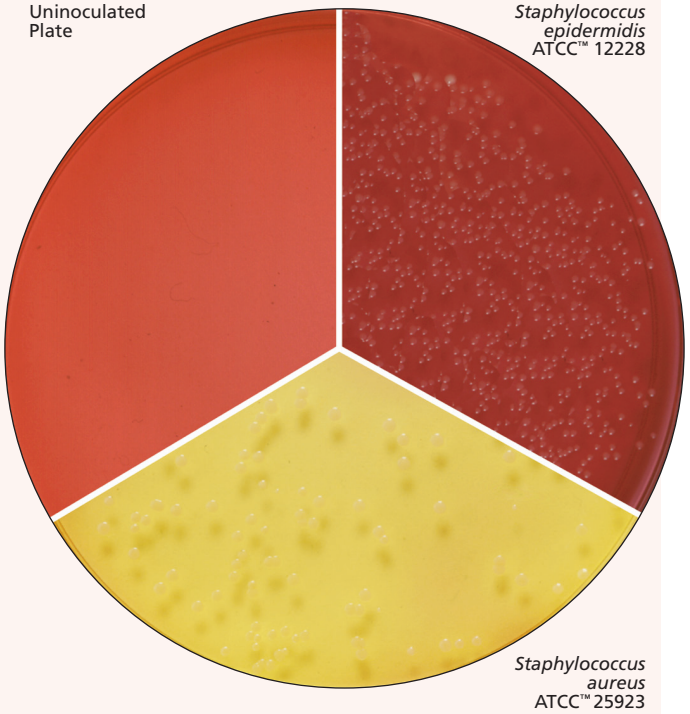
BBL™ Mannitol Salt Agar (prepared)

Appearance:	Light to medium rose red, trace orange; clear to hazy.
Reaction at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Mannitol Salt Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours. Incubate plates with *Staphylococcus aureus* ATCC 6538 and *E. coli* ATCC 8739 at 30-35°C for 18-72 hours.



ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF MEDIUM AROUND COLONY
Proteus mirabilis	12453	$10^4 – 10^5$	Partial to complete inhibition	–
Staphylococcus aureus	25923	$10^3 – 10^4$	Good	Yellow
Staphylococcus epidermidis	12228	$10^3 – 10^4$	Good	Red
Staphylococcus aureus	6538	<100	Growth	N/A
Escherichia coli	8739	>100	No growth	N/A

BBL™ Mannitol Salt Agar (prepared)

Inoculate and incubate at 35 ± 2°C for 48 hours. Incubate plates with *Staphylococcus aureus* ATCC 6538 and *E. coli* ATCC 8739 at 30-35°C for 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF MEDIUM AROUND COLONY
Proteus mirabilis	12453	$10^4 – 10^5$	Partial inhibition	–
Staphylococcus aureus	13150	$10^3 – 10^4$	Good	Yellow
Staphylococcus aureus	25923	$10^3 – 10^4$	Good	Yellow
Staphylococcus epidermidis	12228	$10^3 – 10^4$	Good	Red
Staphylococcus aureus	6538	<100	Growth	N/A
Escherichia coli	8739	>100	No growth	N/A

as one of several media recommended for the enumeration of gram-positive bacteria in cosmetics,[6] clinical specimens,[7-11] and pharmaceutical products.[1] The *USP* General Chapter <62> recommends Mannitol Salt Agar as a test medium for isolating *Staphylococcus aureus* in the Microbiological Examination of Nonsterile Products.[1]

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. Agar is a solidifying agent.

Formula

BBL™ Mannitol Salt Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein	5.0	g
Peptic Digest of Animal Tissue	5.0	g
Beef Extract	1.0	g
Sodium Chloride	75.0	g
D-Mannitol	10.0	g
Phenol Red	25.0	mg
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 111 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[7-11]

For cosmetic and pharmaceutical samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[1,13-15]

Procedure

Refer to appropriate standard references for details on test methods to obtain isolated colonies from specimens or samples using Mannitol Salt Agar.[1,6,7,11] Incubate plates at 35 ± 2°C in an aerobic atmosphere for 24-48 hours, or as instructed in the standard reference.[1,6,7,11]

Expected Results

After the recommended incubation period, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation. Coagulase-positive staphylococci produce growth of yellow colonies with yellow zones. Coagulase negative staphylococci produce small red colonies with no color change to the medium. *Micrococcus* produce large, white to orange colonies, with no color change to the medium. Most other bacteria will be inhibited.

References

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2. European Directorate for the Quality of Medicines and Healthcare. 2008.The European pharmacopoeia, 6th ed., Supp. 1, 4-1-08, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Koch. 1942. Zentralbl. Bakteriol. Parasitenkd. Abt. I Orig. *149*:122.
5. Chapman. 1945. J. Bacteriol. *50*:201.
6. U.S. Food and Drug Administration. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
7. Murray, Baron, Jorgensen, Landry and Pfaller (eds). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
8. Forbes, Sahm and Weissfeld. 2007. Bailey and Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
9. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
10. Winn, Koneman, Allen, Janda, Procop, Schreckenberger and Woods (eds.). 2005. Koneman's Color atlas and textbook of diagnostic microbiology, 6th ed. Lippincott Williams & Wilkins, Baltimore, Md.
11. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Mannitol Salt Agar

BAM BS12 CMPH2 EP JP MCM9 USP

Cat. No.	211407	Dehydrated – 500 g†
	211410	Dehydrated – 5 lb (2.3 kg)†
	293689	Dehydrated – 25 lb (11.3 kg)†

United States and Canada

Cat. No.	221173	Prepared Plates – Pkg. of 20*†
	221271	Prepared Plates – Ctn. of 100*†

Europe

Cat. No.	254027	Prepared Plates – Pkg. of 20*†
	254079	Prepared Plates – Ctn. of 120*†

Japan

Cat. No.	251173	Prepared Plates – Pkg. of 20*

**Store at 2-8°C.*
†QC testing performed according to USP/EP/JP performance specifications.