

PRODUCT SPECIFICATION SHEET

Mannitol Salt Agar Base (DM160)

Intended Use

Mannitol Salt Agar Base (DM160) is used for selective isolation of pathogenic Staphylococci.

Product Summary and Explanation

A selective medium prepared according to the recommendations of Chapman⁽¹⁾ for the isolation of presumptive pathogenic Staphylococci. Most other bacteria are inhibited by the high salt concentration with the exception of some halophilic marine organisms. Chapman added 7.5% sodium chloride to Phenol Red Mannitol Agar and noted that pathogenic strains of Staphylococci (coagulase-positive staphylococci) grew luxuriantly and produce yellow colonies with yellow zones in the surrounding medium. Non-pathogenic Staphylococci produced small red colonies with no colour change to the surrounding medium. Mannitol salt Agar is recommended for the detection and enumeration of coagulase-positive staphylococci in milk⁽²⁾, in food⁽³⁾ and other specimens.⁽⁴⁾ Mannitol salt Agar is selective hence specimens from heavily contaminated sources may be streaked onto this medium without danger of overgrowth.⁽⁵⁾ Mannitol Salt Agar is recommended for isolating pathogenic staphylococci from clinical specimens, cosmetics and for microbial limit tests.⁽⁷⁾ The addition of 5% v/v Egg yolk Emulsion (MS038) to Mannitol Salt Agar enables the lipase activity of Staphylococci to be detected along with mannitol fermentation.⁽⁶⁾ The high concentration of salt in the medium clears the egg yolk emulsion and lipase production is detected as a yellow opaque zone around colonies of Staphylococci which produce this enzyme.

Principles of the Procedure

Beef extract and Proteose peptone supply essential growth factors and trace nutrients to the growing bacteria. Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator. Agar is the solidifying agent. S.aureus ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of S.aureus are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of S.aureus should be confirmed by performing the coagulase test [tube or slide]. Lipase activity of S.aureus can be detected by supplementing the medium with egg yolk emulsion. Coagulase test is used to confirm S.aureus. Few strains of S.aureus may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded.

Formula / Liter

Ingredients	Gms / Litre
Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	75.00
D-Mannitol	10.00
Phenol red	0.025
Agar	15.00
Final pH (at 25°C)	7.4±0.2
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 111.02grams in 1000 ml distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes/validated cycle.
4. If desired, add 5% v/v Egg Yolk Emulsion (MS038).
5. Mix well and pour into sterile Petri plates.

PRODUCT SPECIFICATION SHEET

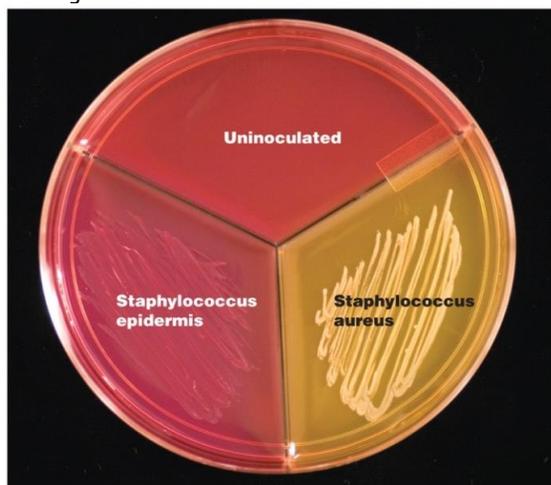
Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing, powder
Prepared Medium	Red coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 11.1% Solution	pH 7.4 + 0.2 at 25°C
Gel Strength	Firm as compared to 1.5% Agar gel

Expected Cultural Response: Cultural response on Mannitol Salt Agar observed after incubation at 35-37°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation period
1.	<i>Staphylococcus aureus</i> ATCC 6538	50 -100	Luxuriant	25 -100	≥50 %	Yellow/white colonies surrounded by yellow zone	18 -72 hrs
2.	<i>Escherichia coli</i> ATCC 8739	≥10 ³	Inhibited	0	0 %	-	≥72 hrs
3.	<i>Staphylococcus aureus</i> ATCC 25923	50 -100	Luxuriant	25-100	≥50 %	Yellow/white colonies surrounded by yellow zone	18 -72 hrs
4.	<i>Staphylococcus epidermidis</i> ATCC 12228	50 -100	Fair - good	15-40	30 -40 %	Red	18 -72 hrs
5.	<i>Staphylococcus epidermidis</i> ATCC 14990	50 -100	Fair - good	15-40	30 -40 %	Red	18 -72 hrs
6.	<i>Proteus mirabilis</i> ATCC 12453	50 -100	None-poor	0-10	0 -10 %	Yellow	18 -72 hrs
7.	<i>Escherichia coli</i> ATCC 25922	≥10 ³	Inhibited	0	0%	-	≥72 hrs
8.	<i>Escherichia coli</i> NCTC 9002	≥10 ³	Inhibited	0	0%	-	≥72 hrs
9.	<i>Enterobacter aerogenes</i> ATCC 13048	≥10 ³	Inhibited	0	0%	-	≥72 hrs

The organisms listed are the minimum that should be used for quality control testing.



Test Procedure

PRODUCT SPECIFICATION SHEET

Inoculate specimens on the medium for a primary isolation or inoculate isolated colonies onto medium for differentiation.

Results

1. Staphylococci will grow on this medium, while the growth of most other bacteria will be inhibited.
2. Coagulase-positive staphylococci will produce luxuriant growth of yellow colonies with yellow zones.
3. Coagulase-negative staphylococci will produce small pink to red colonies with no colour change to medium.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Product Name : Mannitol salt Agar

Product Code : DM160

Available Pack sizes : 100gm / 500gm

References

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5. Kloos, W.E., and T.L. Bannerman. 1995. Staphylococcus and Micrococcus. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.). Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
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7. United States Pharmacopeial Convention. 1995. The United States pharmacopoeia 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
8. Gunn B. A. Dunkelberg W.E. and Creitz J.R. (1972) Am.J. Clin. Path. 57. 236-238.

Further Information

For further information please contact your local MICROMASTER Representative.



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