PRODUCT SPECIFICATION SHEET

Mannitol Salt Agar Plate (RP010H)

Intended Use
Medium for selective isolation and subculture of Staphylococcus aureus in compliance with the harmonized method of USP/EP/BP/JP.

Product Summary and Explanation

Principles of the Procedure
Mannitol Salt Agar is prepared as suggested by Chapman (1) and is used for the selective isolation of pathogenic Staphylococci . This medium is recommended for the detection and enumeration of coagulase-positive Staphylococci in milk (2) food (3) and other specimens. The medium contains beef extract and protease peptone which makes it very nutritious as they provide essential growth factors and trace nutrients. Many other bacteria except Staphylococci are inhibited by 7.5% sodium chloride. Mannitol is the fermentable carbohydrate source. The differential action of the medium is attributed to D-Mannitol. Staphylococcus aureus ferments mannitol to produce yellow colonies with yellow cones. Most coagulase-negative species of Staphylococci and Micrococci do not ferment mannitol and therefore the medium remains red in colour. The colour of the medium is due to the reactivity of phenol red to the pH of the medium; phenol red is red at pH 8.4 and yellow at 6.8. Presumptive Staphylococcus showing yellow coloured medium should be further tested for production of coagulase.

Formula / Liter

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.00
Pancreatic digest of casein	5.00
Beef extract	1.00
Sodium chloride	75.00
D-Mannitol	10.00
Phenol red	0.025
Agar	15.00
Final pH : 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. Prepared plated media are For *in vitro* Diagnostic Use or For Laboratory Use as labelled.
2. Directions for use should be read and followed carefully.
3. 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.
4. Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, since it must be assumed that all specimens/samples collected might contain infectious microorganisms.

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

Quality Control Specifications

Appearance	Sterile Mannitol Salt Agar in 90mm plates
Colour	Red coloured medium
Reaction	pH : 7.20 ± 7.60 at 25°C

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Quantity of medium	25ml of medium in 90mm plates

Sterility Check: Passes release criteria.

Cultural response	Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Expected Cultural Response:

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation period
Growth Promoting + Indicative							
1.	*Staphylococcus aureus ATCC 6538*	50 -100	luxuriant	25 -100	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
Inhibitory							
2.	*Escherichia coli ATCC 8739*	>=10^3	inhibited	0	0 %	--	>=72 hrs
Additional Microbiological Testing							
3.	*Staphylococcus aureus ATCC 25923*	50 -100	luxuriant	25-100	>=50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
4.	*Staphylococcus epidermidis ATCC 12228*	50 -100	fair - good	15-40	30 -40 %	red	18 -72 hrs
5.	*Staphylococcus epidermidis ATCC 14990*	50 -100	fair - good	15-40	30 -40 %	red	18 -72 hrs
6.	*Proteus mirabilis ATCC 12453*	50 -100	none-poor	0-10	0 -10 %	yellow	18 -72 hrs
7.	*Escherichia coli ATCC 25922*	>=10^3	inhibited	0	0%	-	>=72 hrs
8.	*Escherichia coli NCTC 9002*	>=10^3	inhibited	0	0%	-	>=72 hrs
9.	*Enterobacter aerogenes 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

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

Results

1. Coagulase-positive *S. aureus* ferments mannitol and produce yellow coloured colonies surrounded by yellow zones.
2. Coagulase-negative strains of *S. aureus* are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones.
3. Presumptive coagulase-positive yellow colonies of *S. aureus* should be confirmed by performing the coagulase test [tube or slide].[1]

Storage
On receipt, store plates at 15-25°C.

Expiration
Refer to the expiration date stamped on the pack. Prepared plates stored in their original sleeve wrapping at 20-25°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times.

Product Disposal
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

Limitations of the Procedure
1. The type of peptone used in the base may affect pigment production.
2. No single medium can be depended upon to exhibit all pigment-producing *P. aeruginosa* strains.
3. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescin production since this yellowing does not fluoresce.
4. Some nonfermenters and some aerobic sporeformers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.
5. Studies of Lowbury and Collins showed *P. aeruginosa* may lose its fluorescence under UV light if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.
6. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Mannitol Salt Agar Plate
Product Code : RP010H
Available Pack sizes : Pack of 10 plates

References
1. Chapman G.H., 1945, J. Bact., 50:201.
2. Marshall R. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
3. Bacteriological Analytical Manual, 1995, Food and Drug Administration, 8th ed., AOAC, International, U.S.A.

Further Information

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