

Mannitol Salt HiCynth™ Agar Base

MCD118

Mannitol Salt HiCynth™ Agar Base is used as a selective media for the isolation of pathogenic Staphylococci.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.1*	10.000
HiCynth™ Peptone No.5*	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

*Chemically defined peptones

Directions

Suspend 111.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, add 5% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

Note : This product contains 7.5% Sodium chloride as one of its ingredients. On repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, therefore we strongly recommend storage in tightly closed containers in dry place away from bright light .

Principle And Interpretation

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e *Staphylococcus aureus* is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (1). Staphylococci have the unique ability of growing on a high salt containing media (2). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5% NaCl was studied by Chapman (3). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive staphylococci from cosmetics, milk, food and other specimens (1, 4-7). The additional property of lipase activity of *Staphylococcus aureus* can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (8). Mannitol Salt HiCynth™ Agar Base is prepared by using chemically defined peptones free from animal and vegetable peptones to avoid BSE/TSE risks associated with chemical peptones.

HiCynth™ Peptone No.1 and HiCynth™ Peptone No.5 supply essential nitrogen and carbon source, long chain amino acids, 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.

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](1). Lipase activity of *S.aureus* can be detected by supplementing the medium with egg yolk emulsion.

A possible *S.aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (9). 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 (9).

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 11.1% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of colony	Incubation temperature
Cultural Response <i>Staphylococcus aureus</i> ATCC 6538	50 -100	luxuriant	25 -100	≥50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
<i>Escherichia coli</i> ATCC 8739	≥10 ³	inhibited	0	0 %		≥72 hrs
<i>Staphylococcus aureus</i> ATCC 25923	50 -100	luxuriant	25 -100	≥50 %	yellow/white colonies surrounded by yellow zone	18 -72 hrs
<i>Staphylococcus epidermidis</i> ATCC 12228	50 -100	fair - good	15 -40	30 -40 %	red	18 -72 hrs
<i>Staphylococcus epidermidis</i> ATCC 14990	50 -100	fair - good	15 -40	30 -40 %	red	18 -72 hrs
<i>Proteus mirabilis</i> ATCC 12453	50 -100	none-poor	0 -10	0 -10 %	yellow	18 -72 hrs
<i>Escherichia coli</i> ATCC 25922	≥10 ³	inhibited	0	0%		≥72 hrs
<i>Escherichia coli</i> NCTC 9002	≥10 ³	inhibited	0	0%		≥72 hrs
<i>Enterobacter aerogenes</i> ATCC 13048	≥10 ³	inhibited	0	0%		≥72 hrs

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

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