

**Lipovitellin Salt Mannitol Agar Base****M627**

Lipovitellin Salt Mannitol Agar Base is used for selective isolation and identification of pathogenic *Staphylococcus aureus* by detecting lipase production and mannitol fermentation.

**Composition\*\*\***

Ingredients	Gms / Litre
Beef extract	1.000
Peptone, special	10.000
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

**Directions**

Suspend 11.1 grams in 90 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 around 50-55°C and aseptically add 10 ml of sterile 2.0% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

The coagulase-positive species of *Staphylococcus* i.e. *Staphylococcus aureus* is well-documented as a human opportunistic pathogen (1). *S. aureus* is also isolated from recreational water like swimming pools, and are thus indicators of health risk (2-5). *S. aureus* is relatively resistant to the effect of disinfectant like chlorine and sodium chloride. Lipovitellin Salt Mannitol Agar Base, recommended by APHA (6) is used for the selective isolation and identification of pathogenic *S. aureus* by detecting lipase production and mannitol fermentation.

Beef extract and peptone special serve as sources of essential nutrients required for bacterial growth. Sodium chloride in higher concentration makes the medium selective for *Staphylococcus* by inhibiting accompanying flora. D-Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by the pH indicator dye, namely phenol red. Lipovitellin is a lipophosphoprotein, which is combined with lecithin in the yolk of eggs. It is also known as vitellin or ovovitellin and is inhibitory to majority of bacteria except *Staphylococcus*. Egg yolk emulsion serves as a source of lipids for lipase activity.

Inoculate tubes of M-*Staphylococcus* Broth (M1120). Incubate at 35-37°C for 24 hours. Streak plates of Lipovitellin Salt Mannitol Agar Base with a loopful of culture from positive (turbid) tubes. Incubate at 35-37°C for 24-48 hours. Opaque, yellow zones around the colonies are positive evidence of Lipovitellin + lipase activity (opaque) and mannitol fermentation (yellow) (6).

**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**

Basal medium : Red coloured clear to slightly opalescent gel After addition of 2% Egg Yolk Emulsion : Pink coloured opaque forms in Petri plates

**Reaction**

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

**Cultural Response**

M627: Cultural characteristics observed with added egg yolk emulsion, after an incubation at 35-37°C for 24-48 hours .

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lipase activity	
<i>Staphylococcus aureus</i> ATCC 25923	50-100	good to luxuriant	≥70%	yellow colonies with yellow	positive, iridescent sheen on	

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				opaque zone around the colonies	the colony surface and medium	
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**Reference**

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Seyfried P. L., Tobin R. S., Brown N. E. and Ness P. F., 1985, Am. J. Pub. Health 75:1071.
3. Klap N. A., and Vesley D., 1988, Appl. Environ. Microbiol., 52:589.
4. Covert T. C. and Scarpino P.V., 1987, Abstr. Annu. Meeting, American Soc. Microbiology, Atlanta, Ga. ASM, Washington, D.C.
5. Charoenca N. and Fujioka R. S., 1995, Water Sci. Technol. 32:11.
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**Storage and Shelf Life**

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