Mannitol Lysine Agar

M1071

Mannitol Lysine Agar is used for selective isolation of *Salmonella* species other than *Salmonella* Typhi and *Salmonella* Paratyphi A.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Yeast extract	5.000
Beef extract	2.000
Sodium chloride	4.000
Mannitol	3.000
L-Lysine hydrochloride	5.000
Sodium thiosulphate	4.000
Ferric ammonium citrate	1.000
Brilliant green	0.0125
Crystal violet	0.010
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 49.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta (1). One of the most important criteria in the identification of *Salmonella* species is the production of hydrogen sulphide. *Salmonella* Typhi and *Salmonella* Paratyphi A can be differentiated from the rest of the

Salmonella due to their inability to form hydrogen sulphide.

Mannitol Lysine Agar, formulated as described by Inoue et al (2) is used for the selective isolation of *Salmonella* species other than *Salmonella* Typhi and *Salmonella* Paratyphi A from different foods and faeces. Mannitol Lysine Agar may be used directly with the specimen or from an enrichment culture (3). Enrichment can be carried out in Modified Semisolid RV Medium (M1482). Mannitol Lysine Agar does not depend upon lactose fermentation and is therefore recommended for investigating lactose fermenting Salmonellae like *Salmonella* Arizonae . Further tests should be carried out for confirming *Salmonella* species.

Peptic digest of animal tissue, beef extract, yeast extract provide essential nutrients for the growth of *Salmonella* . Mannitol is the fermentable carbohydrate in the medium while L-lysine is the amino acid. Salmonellae grow as large purple colony with black center because of H_2S production. Mannitol is fermented by organisms and the resulting acid stimulates lysine decarboxylation. This elevates the pH due to production of amines and promotes blackening. Sodium thiosulphate and ferric ammonium citrate help in H_2S production. Atypical *Salmonella* strains do not produce H2S and form grey colonies. Brilliant green dye in the medium inhibits gram-positive and majority of gram-negative organisms.

Mannitol Lysine Medium should be used in conjunction with Brilliant Green Agar, Modified (M016) or Bismuth Sulphite Agar (M027). Mannitol Lysine Medium can be directly inoculated with the specimen or the specimen can be first enriched in Modified Semisolid RV Medium Base (M1482). Atypical *Salmonella* will form a characteristic bulls eye due to less H_2S production, which gets concentrated in the centre of the colony. *Salmonella* colonies will form purple black colonies. Presumptive *Salmonella* should be confirmed by biochemical tests.

Quality Control

Appearance
Light yellow to greenish yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Yellow with purple coloured tinge clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.9% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
M1071: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	
Salmonella Paratyphi B ATCC 8759	50-100	luxuriant	>=50%	purple with black centre
Salmonella Typhi ATCC 6539	50-100	None-poor	0-10%	colourless with purple tinge, may have black centres
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	>=50%	purple with black centre
Salmonella Enteritidis ATCC 13076	50-100	luxuriant	>=50%	purple with black centre
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	

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

1. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company
2. Takao Inoue et al, 1968, Jap. J. Vet. Sci., 30.
3. Aspinall S. T., Hindle M. A. and Hutchinson D. N., 1992, Eur. J. Clin. Microbiol. Inf. Dis., 11:936.

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