



## Mannitol Lysine Agar

M1071

### Intended Use:

Recommended for selective isolation of *Salmonellae* other than *Salmonella* Typhi and *Salmonella* paratyphi A.

### Composition\*\*

Ingredients	Gms / Litre
Peptone	10.000
Yeast extract	5.000
HM peptone B #	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

# Equivalent to Beef extract

### Directions

Suspend 49.02 grams in 1000 ml purified/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. Cool to 45-50°C.

### 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.

Peptone, HM peptone B, 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<sub>2</sub>S 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<sub>2</sub>S production. Atypical *Salmonella* strains do not produce H<sub>2</sub>S 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<sub>2</sub>S 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.

## Type of specimen

Clinical samples - Faeces

## Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). After use, contaminated materials must be sterilized by autoclaving before discarding.

## 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

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> ATCC 25922	≥10 <sup>4</sup>	inhibited	0%	
<i>Salmonella Paratyphi B</i> ATCC 8759	50-100	luxuriant	≥50%	purple with black centre
<i>Salmonella Typhi</i> ATCC 6539	50-100	None-poor	0-10%	colourless with purple tinge, may have black centres
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	≥50%	purple with black centre
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	luxuriant	≥50%	purple with black centre
<i>Staphylococcus aureus</i> ATCC 25923	≥10 <sup>4</sup>	inhibited	0%	

## 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. Lippincott 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.

Revision : 02/ 2017

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