

Technical Information

Mannitol Lysine Agar

Product Code: DM 2071

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

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	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 (25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Salmonella infections in human 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 produce hydrogen sulphide.

Mannitol Lysine Agar, formulated by Inoue et al ⁽²⁾ is used for the exclusive 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 (DM2482). 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 H₂S production. Mannitol is fermented by organisms and the resulting acid stimulates lysine decarboxylation. This increases the pH due to production of amines and promotes blackening. Sodium thiosulphate and ferric ammonium citrate help in H₂S production. Atypical Salmonella strains do not produce H₂S and form grey colonies. Brilliant green dye in the medium inhibits the growth of gram-positive and gram-negative organisms.

Mannitol Lysine Medium should be used in conjunction with Brilliant Green Agar, Modified (DM1016) or Bismuth Sulphite Agar (DM1027). Mannitol Lysine Medium can be directly inoculated with the specimen or the specimen can be first enriched in Modified Semisolid RV Medium Base (DM2482). Atypical Salmonella will form a characteristic bull's eye due to less H₂S 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.

Methodology

Suspend 49.02 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Quality Control

Physical 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 range 6.60-7.00

Cultural Response/ characteristics

DM 2071: 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	50-100	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	50-100	inhibited	0 %	

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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.

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