Mannitol Lysine Agar

M1071

Intended Use:

For selective isolation of Salmonellae other than *Salmonella* Typhi and *Salmonella* Paratyphi A.

Composition**

Ingredients	g / L
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 H2S 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 H_2S 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.

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.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.Presumptive Salmonella should be confirmed by biochemical tests.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

Light purple to purple with green 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
Escherichia coli ATCC 25922 (00013*)	>=10^4	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 (00031*)	50-100	luxuriant	>=50%	purple with black centre
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	>=50%	purple with black centre
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

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.

4.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

5.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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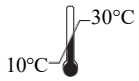
IVD *In vitro* diagnostic medical device



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE CE Marking

Do not use if package is damaged

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