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Mannitol Lysine Crystal Violet Brilliant Green Agar

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

REF: BS.1/MN01.100.0100	100 Gram	REF: BS.1/MN01.250.0250	250 Gram
REF: BS.1/MN01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

Salmonella are gram-negative facultative intracellular anaerobes that cause a wide spectrum of disease. Human Salmonella infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta (2). One of the major criteria in the identification of Salmonella species is the production of hydrogen sulphide (H2S). The species of Salmonella Typhi and Salmonella Paratyphi A are unbale to form H2S and can easily be differentiated from the rest of the Salmonella. Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB Agar) is based on the formula of Inoue et al. (1) for the selective isolation of Salmonellae species other than Salmonella Typhi and Salmonella Paratyphi A from faeces and foods. The medium may be inoculated directly with the specimen or from an enrichment culture (3). Enrichment can be carried out in Modified Semisolid RV Medium. MLCB Agar does not depend on lactose fermentation and is therefore recommended when investigating lactose fermenting Salmonellae such as Salmonella arizona. Further tests should be carried out for confirming Salmonella species.

METHOD PRINCIPLE

Peptic digest of animal tissue, beef extract and 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-black colonies with black centres due to hydrogen sulphide 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 H2S production. Mannitol Lysine Medium should be used in conjunction with Brilliant Green Agar, Modified or Bismuth Sulphite Agar. Mannitol Lysine Medium can be directly inoculated with the specimen, or the specimen can be first enriched in Modified Semisolid RV Medium Base. Atypical Salmonella strains that produce little or no hydrogen sulphide grow as mauve-grey colonies and may develop a central black `bulls eye'.To assist the detection of these atypical strains Brilliant Green Agar (modified) or Bismuth Sulphite Agar should also be used. Salmonella colonies will form purple black colonies. Presumptive Salmonella should be confirmed by biochemical tests.

MEDIA COMPOSITION

Item	Formula in g/L
Peptic digest of animal tissue	10
Yeast extract	5
Beef extract	2
Sodium chloride	4
Mannitol	3
L-lysine	5
Sodium thiosulphate	4
Ferric ammonium citrate	1
Brilliant green	0.0125
Crystal violet	0.01
Agar	15

pH 6.8 ± 0.2 at 25°C

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated people. Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Handle specimens and inoculated culture bottles as though capable of transmitting infectious agents. All inoculated culture bottles, specimen collection needles, and blood drawing devices should be decontaminated according to country requirement for waste disposal.

  S56: dispose of this material and its container at hazardous or special waste collection point.

  S57: use appropriate container to avoid environmental contamination.

  S61: avoid release in environment.

For further information, refer to the Mannitol Lysine Crystal Violet Brilliant Green Agar material safety data sheet.

MEDIA PREPARATION, STORAGE AND STABILITY

STORAGE AND STABILITY

BioScien Mannitol Lysine Crystal Violet Brilliant Green Agar should be stored between 10-30°C in a firmly 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 avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a 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 the stated expiry period.

PROCEDURE

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.

Deterioration

BioScien Mannitol Lysine Crystal Violet Brilliant Green Agar is Cream to yellow homogeneous free flowing powder. Prepared medium is Light amber coloured, opalescent gel with flocculent precipitate forms in tubes as slants. should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations

SPECIMEN COLLECTION AND PRESERVATION

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10,13). After use, contaminated materials must be sterilized by autoclaving before discarding.

TYPE OF SPECIMEN

Clinical samples
Food and Dairy samples
Environmental samples

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups
- Sterile plates
- Autoclave
- Incubator

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact the technical support.

PERFORMANCE CHARACTERISTICS

The following organisms are used by us as part of the quality assurance of the product. The total inoculum challenge for each test organism per bottle is 10 to 50 colony forming units (CFU's).

Test Organisms	Growth	Colony color
Escherichia coli (25922)	Inhibited	-
Escherichia coli (00013)	Inhibited	-
Salmonella Typhi (6539/-)	Luxuriant	colorless with purple tinge may have black centres
Salmonella Paratyphi B (8759)	Luxuriant	purple with black centres
Salmonella Typhimurium (14028)	Luxuriant	purple with black centres
Salmonella Typhimurium (00031)	Luxuriant	purple with black centres
Salmonella Enteritidis (13076)	Luxuriant	purple with black centres
Salmonella Enteritidis (00030)	Luxuriant	purple with black centres
Staphylococcus aureus subsp. aureus (25923)	Inhibited	-
Staphylococcus aureus subsp. aureus (00034)	Inhibited	-

REFERENCES

1. Takao Inoue et al, 1968, Jap. J. Vet. Sci., 30.

2. 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.

3. Aspinall S. T., Hindle M. A. and Hutchinson D. N., 1992, Eur. J. Clin. Microbiol. Inf. Dis., 11:936.

SYMBOLS IN PRODUCT LABELLING	
IVD For in-vitro diagnostic use	Number of <n> test in the pack
LOT Batch Code/Lot number	Caution
REF Catalogue Number	Do not use if package is damaged
Temperature Limitation	Consult Instruction for use
Expiration Date	
Manufactured by	