

## 53189 Mannitol Lysine Crystal Violet Brilliant Green Agar (Mannitol Lysine Agar; MLCB Agar) NutriSelect® Plus

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

### Composition:

Ingredients	Grams/Litre
Peptic digest of animal tissue	10.0
Yeast extract	5.0
Beef extract	2.0
Sodium chloride	4.0
Mannitol	3.0
L Lysine hydrochloride	5.0
Sodium thiosulphate	4.0
Ferric ammonium citrate	1.0
Brilliant green	0.0125
Crystal violet	0.010
Agar	15.0

Final pH 6.8 +/- 0.2 at 25°C

Store dehydrated powder between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Protect from moisture and light by keeping container in a low humidity environment.

Appearance(color): Light yellow & light beige & light brown  
 Gelling: Firm, comparable with 1.5% Agar gel  
 Color and Clarity: Light purple to purple with green tinge clear to slightly opalescent gel forms in Petri plates.

### 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:

*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 (H<sub>2</sub>S). The species of *Salmonella* Typhi and *Salmonella* Paratyphi A are unable to form H<sub>2</sub>S 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.



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 H<sub>2</sub>S 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.

Cultural characteristics after 18-48 hours at 35-37°C.

Organisms (ATCC/WDCM)	Inoculum (CFU)	Growth	Recovery	Color of colony
<i>Escherichia coli</i> (25922/ 00013)	≥10 <sup>4</sup>	-	0%	
<i>Salmonella</i> Typhi (6539/-)	50-100	+/-	0-10%	colorless with purple tinge may have black centres
<i>Salmonella</i> Paratyphi B (8759/-)	50-100	+++	≥50%	purple with black centres
<i>Salmonella</i> Typhimurium (14028/00031)	50-100	+++	≥50%	purple with black centres
<i>Salmonella</i> Enteritidis (13076/00030)	50-100	+++	≥50%	purple with black centres
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> (25923/00034)	≥10 <sup>4</sup>	-	0%	colorless

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

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

