L.mono Blood Agar Base

M1895

Intended Use

Recommended for the specific isolation and cultivation of *Listeria* species from food and environmental samples.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Sodium chloride	5.000
Lithium chloride	10.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50 grams in 1000 ml of purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated sheep blood and rehydrated contents of one vial of L. mono selective supplement (FD305). Mix well and pour into sterile Petri plates.

Principle And Interpretation

L. monocytogenes is a gram positive, facultatively anaerobic rod shaped bacteria. It can grow under refrigerated condition and therefore is a major concern to the food industry. The recovery of *Listeria* is very low from food and environmental samples, hence it requires enrichment and then further isolation. Various selective and differential media have been proposed for the detection of *Listeria* species in particular *L. monocytogenes* (2).

L.mono Blood Agar Base was developed by Johanson and Kankare (4) for the isolation of *Listeria* species. It uses Tryptone Soya Agar as a base with the addition of lithium chloride as a selective agent. This medium with the addition of 5% w/v sterile defibrinated sheep blood helps in the differentiation of haemolytic and pathogenic *Listeria* species which includes *L. monocytogenes, L. seeligeri* and *L. ivanovii* from non-haemolytic and non-pathogenic species which include *L. innocua, L. grayi* and *L. welshimeri.*

Tryptone, soya peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other growth requirements. Sodium chloride maintains osmotic balance. Lithium chloride, ceftazidime and Polymyxin B sulphate imparts additional selectivity to the medium.

L. mono forms 2 mm dull colonies with narrow haemolytic zones, *L. ivanovii* forms 2 mm dull colonies surrounded by wide haemolytic zone and *L. innocua* gives 2 mm colonies without haemolytic zone.

Type of specimen

Food and dairy samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Some organism may show poor growth due to nutritional variations.

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
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Basal medium :Light amber coloured clear to very slightly opalescent gel. After addition of 5%v/v sterile blood : Cherry red opaque gel forms in Petri plates

Reaction
Reaction of 5.0% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response

Cultural characteristics observed in aerobic atmosphere with added L.mono selective supplement (FD305) and 5%v/v sterile defibrinated blood, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Listeria monocytogenes ATCC 19112	50-100	good-luxuriant	>=50%	narrow haemolytic zone
Listeria monocytogenes ATCC 19117	50-100	good-luxuriant	>=50%	narrow haemolytic zone
Listeria monocytogenes subsp. serovar 1 ATCC 19111 (00020*)	50-100	good-luxuriant	>=50%	narrow haemolytic zone
Listeria ivanovii subsp. ivanovii serovar 5 ATCC 19119 (00018*)	50-100	good-luxuriant	>=50%	wide haemolytic zone
Listeria innocua ATCC 33090 (00017*)	50-100	good-luxuriant	>=50%	no haemolysis
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0 %	
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	none-poor	0 -10 %	
Proteus mirabilis ATCC 25933	50-100	none-poor	0 -10 %	

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,5).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Johansson, T., Kankare, M. (1996) Comparison of three selective plating media for the isolation of *Listeria monocytogenes* from fresh broiler cuts. In SLU (ed.) IUFoST Symposium of food Associated pathogens, 6-8 May, 1996, Uppsala, Sweden. Proceedings of the Symposium of Food associated pathogens, pp.228-229.
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.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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