



L.mono Blood Agar Base

M1895

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 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* (1).

L.mono Blood Agar Base was developed by Johanson and Kankare (2) 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, 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 2mm dull colonies surrounded by wide haemolytic zone and *L.innocua* gives 2mm colonies without haemolytic zone.

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.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Cultural Response <i>Listeria monocytogenes</i> ATCC 19112	50-100	good-luxuriant	>=50%	narrow haemolytic zone
<i>Listeria monocytogenes</i> ATCC 19117	50-100	good-luxuriant	>=50%	narrow haemolytic zone
<i>Listeria monocytogenes</i> ATCC 19111	50-100	good-luxuriant	>=50%	narrow haemolytic zone
<i>Listeria ivanovii</i> ATCC 19119	50-100	good-luxuriant	>=50%	wide haemolytic zone
<i>Listeria innocua</i> ATCC 33090	50-100	good-luxuriant	>=50%	no haemolysis
<i>Enterococcus faecalis</i> ATCC 29212	>=10 ³	inhibited	0 %	
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	none-poor	0 -10 %	
<i>Proteus mirabilis</i> ATCC 25933	50-100	none-poor	0 -10 %	

Storage and Shelf Life

Store at 10-30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1. Bille, J. (1990) Epidemiology of human listeriosis in Europe with special reference to the Swiss outbreak. In Miller, A.J., Smit, J.L. and Somtukti, G.A.(ed.) Foodborne Listeriosis. Elsevier, Amsterdam, pp.71-74.
2. 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.

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