Blood Agar Plate for Listeria

MP2096I

Intended Use

Recommended for isolation and confirmation of *Listeria* species based on haemolysis and CAMP test from food samples. The composition and performance criteria of this medium are as per the specifications laid down in ISO 11290-2-2017(E) .

Composition**

ISO 11290-1 Specification - Blood Agar

Ingredients	Gms / Litre
Enzymatic digest of animal tissues	15.000
Liver digest	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	9 g to 18 g
Sheep Blood	50ml
Final pH (at 25°C)	7.2±0.2

MP2096I- Blood Agar Plate for Listeria

Ingredients	Gms / Litre
Peptone #	15.000
HML extract ##	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	15.000
Sheep Blood	50ml
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Enzymatic digest of animal tissues

- Equivalent to Liver digest

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

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).

Blood Agar Base is recommended by ISO (2,3) for the isolation of *Listeria* species from food, animal feeds and environmental samples in areas of food production and food handling. This medium contains sterile defibrinated sheep blood helps in the detection of haemolytic pattern. It differentiates between pathogenic *Listeria* species, beta haemolytic which includes *L.monocytogenes, L.seeligeri and L.ivanovii* from non-beta haemolytic and non-pathogenic species which include *L.innocua, L.grayi* and *L.welshimeri*. This plates can also be used to perform CAMP test, on basis of which inter species can be differentiated. The CAMP test is performed using *S.aureus* and *Rhodococcus equi*.Other strains of *Listeria* such as *L.fleischmanii, L.marthii, L.rocourtiae* and *L.weihenstephanensis* show negative beta haemolytic reaction and negative reaction is observed when CAMP test is performed with *S. aureus* and *R. equi.*

Peptone, HML extract and yeast extract supplies nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients required by the organisms. Sodium chloride maintains osmotic balance.

Type of specimen

Pure isolates from food and animal feeds, environmental samples in the area of food production and food handling.

Specimen Collection and Handling

Processing: (3)

Initial Suspension: According to sample type, prepare initial suspension in an appropriate diluent.

Surface plating: Surface plating on HiCrome™ Listeria Ottaviani-Agosti Agar Plate (M1540I) of a specified quantity of the test sample for liquid products or of the initial suspension for other products and/or decimal dilutions if required.

Incubation: Incubation of the Petri dishes at 37 °C and examination after 24 h and after a further 24 h.

Confirmation: Confirmation of presumptive colonies of *L. monocytogenes* or *Listeria* spp. by means of appropriate morphological or biochemical tests i.e Hemolysis and CAMP Test.

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. 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 requirement.
3. Further serological and biochemical testing is required for complete identification.

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

Sterile Blood Agar for Listeria in 90mm disposable plates with smooth surface and absence of black particles/ cracks/ bubbles.

Colour

Cherry Red coloured medium

Quantity of medium

25ml of medium in disposable plate

SterilityCheck

Passes release criteria

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours. (Further incubate until satisfactory growth is observed)

Organism	Inoculum (CFU)	Growth	Recovery	beta-haemolysis	CAMP test w/ *S.aureus*	CAMP test w/ *R.equi*
Listeria monocytogenes ATCC 35152 (00109*)	50-100	good-luxuriant	>=70%	positive	positive	negative
Listeria monocytogenes ATCC 19112	50-100	good-luxuriant	>=70%	positive	positive	negative
Listeria monocytogenes ATCC 13932 (00021*)	50-100	good-luxuriant	>=70%	positive	positive	negative
Listeria monocytogenes ATCC 19111 (00020*)	50-100	good-luxuriant	>=70%	positive	positive	negative
Listeria ivanovii ATCC 19119	50-100	good-luxuriant	>=70%	positive	negative	positive
Listeria innocua ATCC 33090 (00017*)	50-100	good-luxuriant	>=70%	negative	negative	negative
Listeria seeligeri ATCC 35967	50-100	good-luxuriant	>=70%	weak positive	weak positive	weak positive
Listeria welshimeri ATCC 43549	50-100	good-luxuriant	>=70%	negative	negative	negative
Listeria grayi ATCC 19120	50-100	good-luxuriant	>=70%	negative	negative	negative

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

On receipt store between 2-8°C Use before expiry date on the label. 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 (4,5).

Reference

1. Bille, J. (1990) Epidemiology of human listeriosis in Europe with specila reference to the Swiss outbreak. In Miller, AJ., Smit, J.L. and Somtukti, G.A.(ed.) Foodborne Listeriosis. Elsevier, Amsterdam, pp.71-74.
2. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1 , Detection method ; ISO 11290-1:2017.
3. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp. - Part 2 , Enumeration method ; ISO 11290-2:2017.
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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