



Blood Agar Base for Listeria

M2096I

Intended Use

Recommended for testing haemolytic reactions of *Listeria* species from food, animal feeds and environmental samples in areas of food production and food handling. The composition and performance criteria of this media is as per the specifications laid down in ISO 11290-1:2017 and ISO 11290-2:2017.

Composition**

ISO 11290-1:2017 and ISO 11290-2:2017 Specification - Blood Agar Base for Listeria

M2096I -Blood Agar Base for Listeria

Ingredients	g / L
Enzymatic digest of animal tissues	15 g
Liver digest	2,5 g
Yeast extract	5 g
Sodium chloride	5 g
Agar	9 g to 18 g
Final pH (at 25°C)	7.2±0.2

Ingredients	g/ L
Peptone #	15.000
HML extract ##	2.500
Yeast extract	5.000
Sodium chloride	5.000
Agar	15.000
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

Suspend 42.50 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5-7% v/v sterile defibrinated sheep blood. 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).

Blood Agar Base is recommended by ISO (4,5) for the isolation of *Listeria* species from food, animal feeds and environmental samples in areas of food production and food handling . This medium with the addition of 5-7% w/v 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 medium 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

For food and animal feeds, environmental samples in the area of food production and food handling, follow appropriate techniques for sample collection and processing as per guidelines (2,3).

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. Haemolytic pattern varies with the source of blood used.

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 : Yellow coloured clear to slightly opalescent gel. After addition of 5-7% v/v sterile defibrinated blood: Cherry red coloured opaque gel forms in Petri plates.

Reaction

Reaction of 4.25% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed with added 5% w/v sterile defibrinated blood, 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/ <i>S.aureus</i>	CAMP test w/ <i>R.equi</i>
<i>Listeria monocytogenes</i> ATCC 35152 (00109*)	50-100	good-luxuriant	≥70%	positive	positive	negative
<i>Listeria monocytogenes</i> ATCC 19112	50-100	good-luxuriant	≥70%	positive	positive	negative
<i>Listeria monocytogenes</i> ATCC 13932 (00021*)	50-100	good-luxuriant	≥70%	positive	positive	negative
<i>Listeria monocytogenes</i> ATCC 19111 (00020*)	50-100	good-luxuriant	≥70%	positive	positive	negative
<i>Listeria ivanovii</i> ATCC 19119	50-100	good-luxuriant	≥70%	positive	negative	positive
<i>Listeria innocua</i> ATCC 33090 (00017*)	50-100	good-luxuriant	≥70%	negative	negative	negative
<i>Listeria seeligeri</i> ATCC 35967	50-100	good-luxuriant	≥70%	weak positive	weak positive	weak positive
<i>Listeria welshimeri</i> ATCC 43549	50-100	good-luxuriant	≥70%	negative	negative	negative
<i>Listeria grayi</i> ATCC 19120	50-100	good-luxuriant	≥70%	negative	negative	negative

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. 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, A.J., 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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Disclaimer :

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