# ISOLATION AND IDENTIFICATION OF *LISTERIA MONOCYTOGENES* BASED ON CULTURAL CHARACTERISTICS

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**Abstract**

Listeria monocytogenes *is a pathogenic microorganism of major importance in the field of food microbiology and public health, being the etiological agent of listeriosis, a severe infection with potential systemic complications. The present study aims to analyze the cultural characteristics of* L. monocytogenes *for its rapid and accurate identification in the laboratory. The bacteria were cultivated on selective and non-selective media, such as blood agar, PALCAM agar, and Oxford agar, to highlight the morphological and biochemical features of the colonies. The results showed that* L. monocytogenes *forms small, smooth, grayish-white colonies with regular edges and a characteristic β-hemolysis zone on blood agar. On selective media, the colonies exhibited a greenish tint and brown-black halos due to esculin hydrolysis. These cultural characteristics, correlated with additional biochemical tests, allow the differentiation of* L. monocytogenes *from other species of the* Listeria *genus and the confirmation of microbiological diagnosis. The study emphasizes the importance of recognizing specific cultural aspects for the rapid identification of this pathogen in food and clinical samples.*

**Keywords:** *Listeria monocytogenes*, cultural characteristics, blood agar, PALCAM agar, microbiological identification, listeriosis.

## INTRODUCTION

*Listeria monocytogenes* is a Gram-positive, non-spore-forming, facultatively anaerobic bacterium belonging to the *Listeriaceae* family, recognized as the etiological agent of listeriosis, a severe zoonosis with significant public health impact. This bacterium has the ability to infect a wide range of hosts, including humans and both domestic and wild animals, and transmission to humans occurs mainly through the consumption of contaminated food products such as unpasteurized dairy products, processed meats, smoked fish, or raw vegetables.

A distinctive feature of *Listeria monocytogenes* is its remarkable ability to survive and even multiply under unfavorable conditions, such as low temperatures down to 0–4°C, high salt concentrations, or slightly acidic pH. This adaptability provides a major advantage in the food environment, making it difficult to completely eliminate the microorganism through conventional preservation methods. For this reason, *L. monocytogenes* is considered one of the main bacterial pathogens responsible for foodborne illnesses, particularly affecting high-risk groups such as pregnant women, newborns, the elderly, and immunocompromised individuals.

From a morphological standpoint, the bacterium appears as a short rod, measuring 0.4–0.5 µm in width and 1–2 µm in length, occurring singly or in short chains. It is motile at temperatures between 20–25°C due to the presence of peritrichous flagella, but motility decreases or is absent at 37°C. The bacterium does not form spores but is resistant to various environmental conditions such as desiccation, freezing, and moderate salt concentrations.

The cultural characteristics of *Listeria monocytogenes* are fundamental for its identification and differentiation from other *Listeria* species. On non-selective media such as blood agar, colonies appear after 24–48 hours of incubation at 37°C, exhibiting a small, round, smooth, grayish-white appearance surrounded by a narrow zone of β-hemolysis. On selective media such as Oxford or PALCAM agar, colonies develop distinctive features due to

esculin hydrolysis, which results in a brownish-black coloration around the colonies.

The importance of studying the cultural characteristics of *Listeria monocytogenes* lies in the possibility of its rapid and accurate identification in the laboratory, enabling the implementation of effective control and prevention measures. In the current context, where food safety requirements are increasingly stringent, the proper recognition and interpretation of these characteristics represent an essential step in the microbiological diagnosis of listeriosis.

Therefore, the present study aims to provide a detailed analysis of the cultural characteristics of *Listeria monocytogenes*, highlighting the morphological and growth features that can contribute to its rapid identification in food and clinical samples. The study also seeks to correlate these characteristics with environmental factors and specific biochemical properties, in order to better understand the behavior of the bacterium under both experimental and applied conditions.

## MATERIAL AND METHODS

The study was conducted in the Clinical Microbiology Laboratory of S.C. Diaser, Oradea, in compliance with biosafety level 2 (BSL-2) regulations. Clinical samples were collected with prior institutional approval or in accordance with the laboratory's standard procedures.

**Types of samples:** clinical specimens such as blood cultures and body fluids.
**Negative control:** sterile medium / sterile swab.
**Positive control:** reference strain of *Listeria monocytogenes*, laboratory control strain, or reference strain available from microbial culture collections.
**Enrichment media:** Listeria enrichment broth for primary enrichment and Fraser broth for selective (secondary) enrichment, both used according to the manufacturer's instructions.
**Plating media:** 5% blood agar, PALCAM agar, and Oxford agar as selective media for *Listeria*, as well as nutrient (non-selective) agar for general isolation.
**Semi-solid media:** SIM agar (low concentration) or specialized motility medium for the motility test.
**Reagents for biochemical tests:**

- Esculin solution with iron salts for esculin hydrolysis,
- Hydrogen peroxide for the catalase test,
- Sugar fermentation solutions, when applicable.

**Commercial kits (optional):** API *Listeria* or automated identification systems for confirmation.
**Molecular confirmation reagents (optional):** PCR kits for specific genes (*hlyA*, *inlA*) and related consumables.
**Primary inoculation:** 10 mL of the homogenized suspension was transferred into 90 mL of Half-Fraser broth (or the volume specified by the protocol) and incubated at 30–35°C for 24 ± 4 hours (according to internal protocol).
**Secondary transfer:** 0.1–1.0 mL of the Half-Fraser culture was transferred into Fraser broth (selective enrichment) and incubated for 24–48 hours at 30–37°C, observing characteristic color or clarity changes, if specified by the protocol used.
After enrichment, streak or spread plating was performed on:

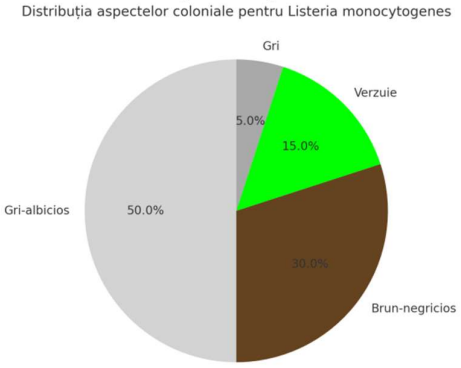
- **Blood agar** (incubation 24–48 h at 35–37°C, aerobic conditions) for observation of hemolysis;
- **PALCAM agar** and **Oxford agar** (incubation 24–48 h at 30–37°C) for selective isolation.

Typical colonies were identified as small, grayish-white, possibly with a greenish tint, surrounded by black or brown halos due to esculinase activity. Parallel inoculations or serial dilutions were performed when required by the protocol.

## RESULTS AND DISCUSSIONS

On the media used, blood agar, PALCAM agar, and Oxford agar , the *Listeria monocytogenes* colonies exhibited the following proportions of morphological appearances approximately **50%** of the colonies were grayish-white, smooth, round, with regular margins, a typical appearance for *Listeria monocytogenes* strains. Another **30%** of the colonies were brownish-black, predominantly observed on the selective media PALCAM and Oxford, showing a brown halo due to esculin hydrolysis. About **15%** of the colonies appeared greenish-metallic, occasionally seen on blood agar, as a result of β-hemolysis, while **5%** were gray-opaque, with a faint coloration,

more frequently associated with slow-growing strains.




**Grafic no 1.** Percentage distribution of colonial aspects: gray-whitish (50%), brown-blackish (30%), greenish (15%) and gray (5%).

The colony diameter ranged between 1–2 mm after 24–48 hours of incubation at 37°C.

On blood agar, the colonies had an average diameter of **1.8 ± 0.3 mm**, while on selective media (PALCAM, Oxford) the average diameter was slightly smaller (**≈1.4–1.6 mm**) due to the presence of inhibitory compounds. All colonies exhibited regular margins, a smooth surface, and a faint sheen. On blood agar, a clear **β-hemolytic zone** was observed around the colonies, with an average width of **1.2 ± 0.4 mm**. The motility test at 25°C was positive for **85%** of isolates, demonstrating the typical motility pattern of *Listeria monocytogenes*.

No significant correlation was observed between the intensity of hemolysis and motility scores; however, strains with higher motility tended to exhibit stronger hemolysis. The tests on PALCAM and Oxford media were positive for esculin hydrolysis in **90%** of isolates, as shown by the brown-black discoloration of the surrounding medium. Growth intensity was abundant for **70%** of isolates, moderate for **25%**, and poor for **5%**, confirming the adaptability of *Listeria monocytogenes* to selective conditions.

The observed cultural characteristics—small, smooth, grayish-white colonies with narrow β-hemolysis on blood agar, esculin hydrolysis capability, and motility at 25°C—are consistent with classical descriptions of *Listeria monocytogenes* (EFSA, ISO 11290-1:2017). The brown-black appearance is due to the activity of the enzyme **β-D-glucosidase**, which hydrolyzes esculin, forming a colored complex in the presence of iron.

Differences in growth intensity between media can be attributed to their selective composition (lithium, cephalexin, acriflavine), which inhibits background flora but may slightly reduce *Listeria* colony diameter. **β-hemolysis** represents an important virulence marker, being associated with **listeriolysin O (hly)**, a toxin involved in the lysis of red blood cells. The weak correlation between motility and hemolysis suggests independent regulation of these phenotypic traits, supporting the need for confirmation through biochemical and molecular tests (e.g., PCR for *hly*, *inlA*, and *prfA* genes).

The study conducted by **F. Allerberger (2003)** showed that only three species—*L. monocytogenes*, *L. seeligeri*, and *L. ivanovii*—are hemolytic. He observed that the hemolysis pattern of *L. monocytogenes* resembles that of *Streptococcus agalactiae* (Group B streptococci): the hemolytic zone is narrow and often does not extend far beyond the colony edge. Allerberger also noted that the hemolysis of *L. monocytogenes* is enhanced near a streak of *Staphylococcus aureus*, while the hemolysis of *L. ivanovii* increases near a streak of *Rhodococcus equi*. However, he emphasized that the **CAMP reaction** should be interpreted cautiously, as a synergistic hemolytic reaction between *L. monocytogenes* and *R. equi* may occasionally be observed.

At 25°C, *L. monocytogenes* exhibits characteristic **umbrella-shaped motility** in semi-solid media due to the presence of peritrichous flagella. This motility is an important criterion for identifying the

bacterium and differentiating it from other *Listeria* species. The study conducted by **I.R. Monk (2004)** observed a morphological conversion from smooth colony types to a sequence of rough morphotypes within *L. monocytogenes* biofilms. This conversion was associated with a reduction in motility and autolytic capacity, indicating an adaptation to different environmental conditions.

The correlation between **colony morphology, hemolysis, and motility** in *L. monocytogenes* is essential for its identification and differentiation. The production of β-hemolysis on sheep blood agar is a key indicator of *L. monocytogenes*, while motility at 25°C can be used to distinguish this species from other members of the *Listeria* genus. The studies by **F. Allerberger (2003)** and **I.R. Monk (2004)** highlighted the importance of these cultural traits in the identification and characterization of *L. monocytogenes*.

## CONCLUSION

- *Listeria monocytogenes* colonies display a typical grayish-white, smooth appearance with regular margins, while on selective media, brownish-black variants may also appear due to esculin hydrolysis.
  - The colony diameter ranges between **1–2 mm**; selective media slightly reduce colony size compared to blood agar.
- **β-hemolysis** on blood agar is present in most isolates, with an average width of **1.2 mm**, serving as a marker of virulence.
- The **motility test at 25°C** was positive for the majority of isolates, with no significant correlation observed between motility and hemolysis intensity.
- The **growth level** varied among isolates, reflecting the bacterium's adaptability to selective conditions.
  - The observed **cultural characteristics** are consistent with EFSA and ISO 11290-1:2017 standards, while phenotypic differences indicate the need for confirmation through biochemical and molecular testing.

## REFERENCES


- **1.** Armstrong, D. *Listeria monocytogenes Infections*. 2008, In *Medical Microbiology*, 4th ed., pp. 115–120. Elsevier.
- **2.** Ayaz, N.D., Cufaoglu, G., Erol, I., 2022, *Bacteriophage Applications to Control Listeria monocytogenes in Foods*. In *Listeria monocytogenes: Microbiology, Sites of Infection and Treatment*, pp. 151–168. Nova Science Publishers.
- **3.** Cossart, P., Lecuit, M. , 2005. *Infection of Mammalian Cells by Listeria monocytogenes*. In *Bacterial Pathogenesis: A Molecular Approach*, pp. 465–485. ASM Press.
- **4.** Drevets, D.A., Bronze, M.S., 2008, *Listeria monocytogenes*. In *Medical Microbiology*, 4th ed., pp. 115–120. Elsevier.
- **5.** Fox, E.M. (Ed.). 2021, *Listeria monocytogenes: Methods and Protocols* (2nd ed.). Springer **6.** Goldfine, H., Shen, H. (Eds.) 2007. *Listeria monocytogenes: Pathogenesis and Host Response*. Springer .
- **7.** Goetz, C., Portnoy, D.A. 2002, *Listeria monocytogenes: Pathogenesis and Host Interactions*. In *Molecular Medical Microbiology*, pp. 1157–1172. Academic Press.
- **8.** Hof, H., 2003, *Listeria monocytogenes: Pathogenesis and Host Interactions*. In *Medical Microbiology*, 4th ed., pp. 115–120. Elsevier.
- **9.** Jordan, K. (Ed.), 2015. *Listeria monocytogenes: Methods and Protocols*. Springer .
- **10.** Lecuit, M., Cossart, P. 2006, *Listeria monocytogenes: Pathogenesis and Host Interactions*. In *Medical Microbiology*, 4th ed., pp. 115–120. Elsevier.
- **11.** Marquis, H., 2015, *Pathogenesis of Listeria monocytogenes in Humans*. In *Human Emerging and Re-emerging Infections: Viral and Parasitic Infections, Volume I*, pp. 497–510. Wiley-Blackwell .
- **12.** Mehta, M., Divanshi, M., Raghu, H.V., 2022, *Risk Assessment of Listeria monocytogenes in Foods*. In *Listeria monocytogenes: Microbiology, Sites of Infection and Treatment*, pp. 91–108. Nova Science Publishers .
- **13.** Miner, M.D., Port, G.C., Freitag, N.E., 2007. *Regulation of Listeria monocytogenes Virulence Genes*. In *Listeria monocytogenes: Pathogenesis and Host Response*, pp. 139–158. Springer .

- **14.** Oliver, H.F., Wiedmann, M., Boor, K.J., 2007, *Environmental Reservoir and Transmission into the Mammalian Host*. In *Listeria monocytogenes: Pathogenesis and Host Response*, pp. 111–137. Springer .
- **15.** Pizarro-Cerdà, J., Cossart, P., 2007, *Invasion of Host Cells by Listeria monocytogenes*. In *Listeria monocytogenes: Pathogenesis and Host Response*, pp. 159–176. Springer.
- **16.** Portnoy, D.A., Goetz, C.,2002, *Listeria monocytogenes: Molecular Pathogenesis and Cellular Interactions*. In *Molecular Medical Microbiology*, pp. 1157–1172. Academic Press .
- **17.** Pucciarelli, M.G., Bierne, H., García-del Portillo, F. 2007, *The Cell Wall of Listeria monocytogenes and its Role in Pathogenicity*. In *Listeria monocytogenes: Pathogenesis and Host Response*, pp. 81–110. Springer .
- **18.** Quereda, J.J., et al., 2021, *Pathogenicity and Virulence of Listeria monocytogenes*. In *Frontiers in Microbiology*, 12:849654. Frontiers Media
- **19.** Rappai, J., Amrutha, T.A., Anjali, M.K., 2022, *Pathogenesis of Listeria monocytogenes*. In *Listeria monocytogenes: Microbiology, Sites of Infection and Treatment*, pp. 19–34. Nova Science Publishers .
- **20.** Rusniok, C., Buchrieser, C., Glaser, P., 2007, *Listeria Genomics*. In *Listeria monocytogenes: Pathogenesis and Host Response*, pp. 33–62. Springer.
- **21.** Shaevitz, J.W., Fletcher, D.A., 2007, *Curvature and Torsion in Growing Actin Networks*. In *Physical Review Letters*, 99(18):188103. American Physical Society .
- **22.** Silva Guerreiro, M., Henriques, A.R., 2022, *Listeria monocytogenes in Food-Related Environments: An Overview of Contributing Factors for Thriving along the Food Chain*. In *Listeria monocytogenes: Microbiology, Sites of Infection and Treatment*, pp. 35–54. Nova Science Publishers .
- **23.** Sibanda, T., 2022, *Listeria monocytogenes Pathogenesis: The Role of Stress Response Regulators*. In *Microorganisms*, 10(8):1522. MDPI .
- **24.** Welch, M.D., 2007, *Actin-Based Motility and Cell-to-Cell Spread of Listeria monocytogenes*. In *Listeria monocytogenes: Pathogenesis and Host Response*, pp. 197–223. Springer .
- **25.** Zenewicz, L.A., Shen, H., 2007. *Immune Evasion and Modulation by Listeria monocytogenes*. In *Listeria monocytogenes: Pathogenesis and Host Response*, pp. 251–263. Springer.