# Microbiology Focus

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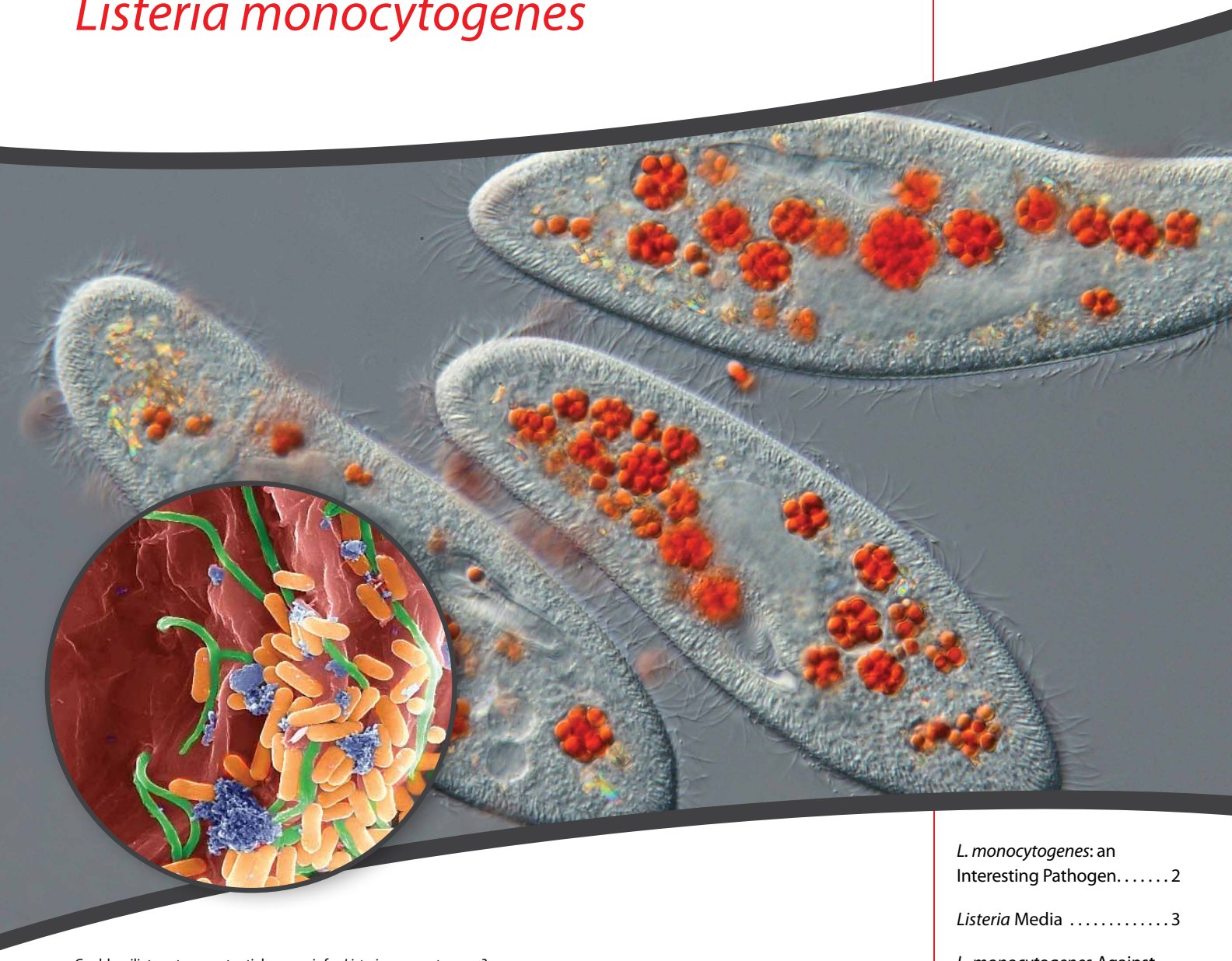
Fluka Analytical

## Interactions of *Listeria monocytogenes*



Could a ciliate act as a potential reservoir for *Listeria monocytogenes*?
Big image: *Paramecium caudatum* with colored yeast cells in the vacuoles.
Small image: ciliated protozoan cell (red) with cilia (green) and many extracellular *Listeria monocytogenes* cells (brown).



# *Listeria monocytogenes*: An Interesting Pathogen

By Rethish Raghu, University of Adelaide — *rethish.raghunadhanan@adelaide.edu.au*

## A Possible Reservoir of *L. monocytogenes*

*L. monocytogenes* is a Gram-positive, intracellular bacterial pathogen, facultatively anaerobic and rod-shaped. *L. monocytogenes* has been widely acknowledged both as an important hazard in the food industry and as a medically significant pathogen. *L. monocytogenes* is the causative organism of the disease listeriosis. Though the optimum growth temperature range for *L. monocytogenes* is between 30 °C - 37 °C, not only does growth occur between 3 °C - 45 °C, but the bacteria can also grow at temperatures between 1 °C - 4 °C. It is the adaptability of *L. monocytogenes* that suits it for survival and growth in processed, refrigerated foods.

Though *L. monocytogenes* is ubiquitous in the environment, this bacterium is predominantly found in decaying vegetation and soil. Following ingestion of *L. monocytogenes* by a susceptible person, the bacteria is apparently able to make the transition from a saprophytic lifestyle to a parasitic one that promotes bacterial survival and replication in host cells. *L. monocytogenes* is able to colonize various inert surfaces and can form biofilms on food-processing surfaces. The presence of *L. monocytogenes* in various environments such as farms, soil, water, silage produced from contaminated grasses and food processing facilities indicates that there are many opportunities for the bacterium to cause contamination during the process of food production. Its ubiquitous presence in various habitats explains the increasing cases of food-borne outbreaks in industrialized countries such as Australia, the U.S.A., and several in Europe.

Examples of contaminant origins include fresh vegetables that can be contaminated from the soil or from manure that was used as fertilizers. Animals may also carry the bacterium asymptomatically and contaminate foods of animal origin. Food items most linked to outbreaks include ready-to-eat meats, undercooked meats, cold cuts, pâté, salads and dairy products, especially soft cheeses and milk that is inadequately pasteurized or contaminated post-pasteurization.

Those most at risk from listeriosis include immunocompromised individuals such as diabetics, AIDS patients, those with renal failure, organ transplant patients, cancer patients and elderly adults. Diarrhea is an early symptom of infection. Advanced symptoms in these individuals manifest as septicemia or meningoencephalitis. Pregnant women, their fetuses and newborns are also at risk from this disease. The common fatal symptoms include pre-term labor, amnionitis, spontaneous abortion, stillbirth and early onset sepsis.

Various species of animals can be infected with *L. monocytogenes*, but clinical disease is rare. The bacterium can also live within the intestines of healthy animals without causing any infections. Though most instances of animal listeriosis are usually seen in ruminants, it can also occur in poultry and other birds, pigs, dogs, cats, domestic and wild rabbits, and many other small mammals. Infected ruminants experience encephalitis, septicemia and abortions. The course of disease in sheep and goats is rapid and death may occur 24 - 48 hours after onset of symptoms, whereas in cattle, the course of disease is less acute. Animals that excrete *L. monocytogenes* in feces are thought to be the primary cause of entry of this pathogen into food-processing plants. The proliferation of *L. monocytogenes* is promoted by high humidity and nutrient rich waste within certain food production plants. Hence, animal listeriosis poses a serious contamination risk for the food industry.

The major cause of bacterial mortality in the environment is suggested to be caused by feeding of bacteria by protozoans through phagocytosis. However, previous studies have clearly demonstrated that not all bacteria are digested as food source. Some types of bacteria survive within the protozoa in order to persist and utilize those protozoan cells as a host. Encapsulation of bacterial pathogens within protozoan cells can provide a protective effect against external stress, such as predation, starvation, chemical disinfectants, antibiotics and high temperatures. It is possible that protozoan cells are the link between bacteria that inhabits the environment and the bacteria that cause diseases in mammals such as humans. Hence it is crucial to better understand the role and ability of protozoan cells in allowing the intracellular survival and replication of *L. monocytogenes*. Although *L. monocytogenes* causes severe disease in human and animal hosts, this pathogen has no recognized animal reservoir. The question arises whether ciliated protozoans such as *Colpoda* could act as a potential reservoir for *L. monocytogenes* (see **Figure 1**).

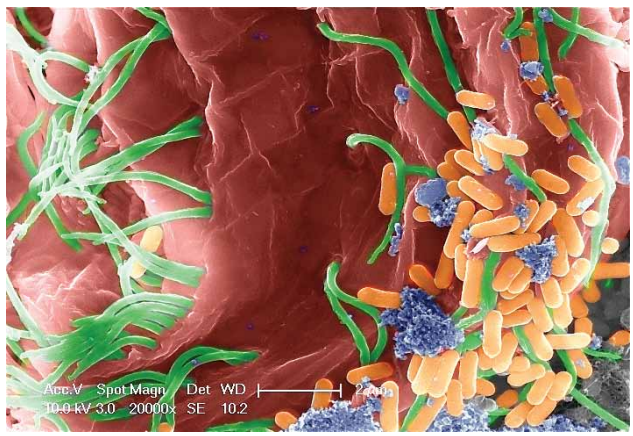



**Figure 1:** Inside a Ciliate's World: High magnification of the exterior of a *Colpoda sp.* ciliated protozoan cell (red). Cilia (green), many extracellular *Listeria monocytogenes* cells (brown), and co-culture debris (blue) are present. (Instrument used: Philips XL30 Field Emission Scanning Electron Microscope at Adelaide Microscopy. Color added through Adobe® Photoshop® CS5. Magnification: ×20,000)

# *Listeria* Media from Sigma-Aldrich®

Sigma-Aldrich developed and commercialized reliable media and biochemical tests for many pathogens, including *Listeria*, according to EN/ISO methodologies. The media contain the elements necessary to selectively grow, differentiate and identify *Listeria* in food substances according to recommended and established methods. The biochemical tests are designed to get quick, easy, and reliable results.

Common specific *Listeria* media are described in more detail and sorted in Tables 1 - 5.

| Cat. No. | Description | Pkg. Size | ISO Ref. |
|---|---|---|---|
| 91366 | Universal Pre-Enrichment Broth | 500 g | |

**Table 1:** *Listeria* Pre-enrichment Media

| Cat. No. | Description | Pkg. Size | ISO Ref. |
|---|---|---|---|
| 50595 | Buffered *Listeria* Enrichment Broth Base | 500 g | |
| 62351 | *Listeria* Selective Enrichment Supplement according to IDF/FIL | 5 vials | |
| 69198 | Fraser Broth, Base (see **Figure 2**) | 500 g | 11290-1:1996 |
| 18038 | Fraser Selective Supplement | 5 vials | |
| 90836 | Fraser Supplement | 10 vials | |
| F6672 | Fraser Secondary Enrichment Broth Base | 500 g | 11290-1:1996 |
| F2674 | Fraser Enrichment Supplement | 5 vial | |
| 62353 | *Listeria* Enrichment Broth according to FDA/IDF-FIL | 500 g | |
| 62351 | *Listeria* Selective Supplements according to IDF-FIL | 5 vials | |
| 59859 | PALCAM *Listeria* Selective Enrichment Broth, Vegitone (see **Figure 3**) | 500 g | |
| 91986 | PALCAM *Listeria* Selective Supplement according to Van Netten et al. | 10 vials | |
| 90554 | UVM *Listeria* Selective Enrichment Broth, modified I and II | 500 g | |

**Table 2:** *Listeria* Selective Enrichment Media

| Cat. No. | Description | Pkg. Size | ISO Ref. |
|---|---|---|---|
| 93395 | Tryptone Soya Yeast Extract Agar | 500 g | 11290-1:1996<br>11290-2:1998/ Amd 1:2004 |

**Table 3:** *Listeria* Purification Media

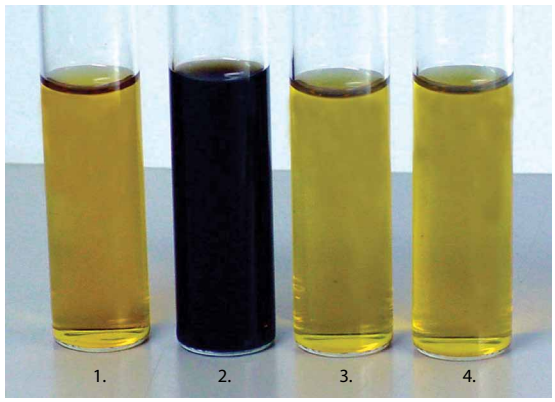



**Figure 2:** Fraser Broth (Fluka 69198): (1) Control, (2) *Listeria moncytogenes*, (3) *E. coli*, (4) *E. faecalis*

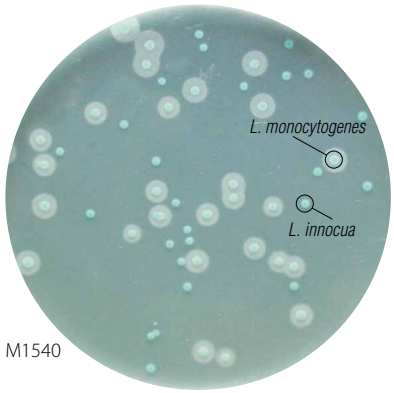



**Figure 3:** *Listeria* mono Differential Agar, Base (ALOA)

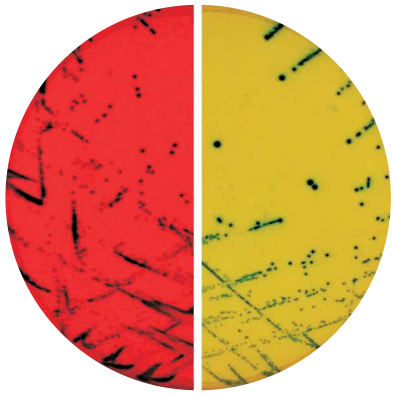


**Figure 4:** HiCrome™ *Listeria* Agar Base, modified

*L. ivanovii* (48 h incubation; blue colonies with red background),
*L. monocytogenes* (48 h incubation; blue colonies with yellow background)



| Cat. No. | Description | Features | Pkg. Size | ISO Ref. |
|---|---|---|---|---|
| 62355 | *Listeria* Selective Agar* | selective media | 500 g | |
| 62653 | LPM Agar* | selective media | 500 g | |
| 43963 | Moxalactam Supplement | | 5 vials | |
| 75805 | Oxford Agar | esculin hydrolysis, selective media | 500 g | 11290-1:1996 |
| 75806 | Oxford-*Listeria* Selective Supplement | | 10 vials | |
| 75977 | PALCAM *Listeria* Selective Agar | esculin hydrolysis, selective media | 500 g | 11290-1:1996 |
| 15776 | PALCAM *Listeria* Selective Agar, Vegitone | esculin hydrolysis, selective media | 500 g | |
| 91986 | PALCAM *Listeria* Selective Supplement according to Van Netten et al. | | 10 vials | |

**Table 4:** *Listeria* Identification Media (* not sold in USA)

| Cat. No. | Description | Features | Pkg. Size | ISO Ref. |
|---|---|---|---|---|
| B1676 | Blood Agar Base No. 2 | Lysis test | 500 g | |
| 7410 | Carbohydrate Consumption Broth | Fermentation ability | 500 g | |
| 53707 | HiCrome™ *Listeria* Agar Base, modified* (chromogenic media; see **Figure 4**) | β-glucosidase activity, rhamnose fermentation, selective media | 250 g | |
| 59688 | HiCrome *Listeria* Selective Supplement | | 5 vials | |
| 92302 | *Listeria* mono Confirmatory Agar, Base (chromogenic media) | Presence of phosphatidylinositol specific phospholipase C of *Listeria monocytogenes* and fermentation of α-methyl D-mannoside, selective media | 38.5 g<br>500 g | |
| 15895 | *Listeria* mono Enrichment Supplement II* | | 5 vials | |
| 92301 | *Listeria* mono Selective Supplement I | | 5 vials | |
| 91603 | *Listeria* mono Selective Supplement II | | 5 vials | |
| 77408 | *Listeria* mono Differential Agar, Base (ALOA, chromogenic media acc. ISO, see **Figure 2**) | Presence of phosphatidylinositol specific phospholipase C of *Listeria monocytogenes*, selective media | 500 g | 11290-2:1998/ Amd 1:2004 |
| 03708 | *Listeria* mono Enrichment Supplement I* | | 5 vials | |
| 92301 | *Listeria* mono Selective Supplement I | | 5 vials | |
| 91603 | *Listeria* mono Selective Supplement II | | 5 vials | |
| 55265 | *Listeria* Motility Medium | Motility test | 500 g | 11290-1:1996<br>11290-2:1998/ Amd 1:2004 |
| 80547 | Rhamnose Broth* | Fermentation ability | 500g | 11290-1:1996<br>11290-2:1998/ Amd 1:2004 |

**Table 5:** *Listeria* Confirmation and Differentiation Media (* not sold in USA)

# *Listeria monocytogenes* and Macrophages

By Sara Remuzgo-Martínez and Jose Ramos Vivas, Hospital Universitario Marqués de Valdecilla & Instituto de Formación e Investigación Marqués de Valdecilla-IFMAV — *ifimav.jvivas@fmdv.org*

## The Tricks of *Listeria monocytogenes* for Invasion, Distribution and Replication within the Human Body

*Listeria monocytogenes*, a Gram-positive, facultative intracellular pathogen, is responsible for severe foodborne infections in humans. *L. monocytogenes* is also of major veterinary importance having been recorded in several species of wild and domesticated animals, including farm ruminants such as cattle, sheep and goats.

*L. monocytogenes* has been isolated from various products including raw milk, cheese made from unpasteurized milk, soft cheese, ice cream, meat, poultry and fish. Ready-to-eat and ready-to-cook meat and poultry products are particularly at risk of infection with *Listeria*. The ability of the pathogen to survive at low temperatures, colonize surfaces in the form of biofilm-like structures, and resist various food-related stresses such as heavy metals and disinfectants is crucial for its persistence in the processing environment.

A variety of conventional and rapid methods are available for the detection and identification of *L. monocytogenes* in food samples and specimens from animal listeriosis (Liu, 2008) (see also Microbiology Focus 2.2, 2010).

In humans, this pathogen has the ability to cross the intestinal, placental, and blood-brain barriers (Cossart and Toledo-Arana, 2008). Listeriosis occurs primarily in immunocompromised individuals, causing septicemia, brain abscesses, meningitis and spontaneous abortion in pregnant women. Even if listeriosis is rare in humans, it is considered the most severe bacterial foodborne infection. More than 50% of the cases correspond to septicemia, and around 25% to Central Nervous System (CNS) infections.

The virulence factors involved in the successive steps of the cell cycle of the LM *listeria* infection cycle have been recently reviewed (Camejo et al., 2011; Mostowy and Cossart). Some virulence factors and modulators also assume an important role in bacterial resistance and evasion from host defense mechanisms. For *L. monocytogenes*, no link has been made between flagella and virulence, although the flagella are important for efficient invasion of some tissue culture cells (O'Neil and Marquis, 2006; Peel et al., 1988). *L. monocytogenes* produces five to six peritrichous flagella, although there is variation from strain to strain. Motility genes are down regulated at 37 °C *in vitro* (**Figure 5**). After detection of its target cells, *Listeria* must adhere and enter into these cells, delay phagosome maturation, resist lysozyme, escape into the cytoplasm, scavenge nutriments to replicate, and polymerase cellular actin to move and to infect other cells (**Figure 6**). The spatio-temporal control of virulence factors, as exerted by a number of protein and non-protein modulators, is vital for the adaptability of this pathogen to the specificities of the host intracellular environment and for the promotion of an efficient infection. Almost all the described virulence determinants are present in virulent strains (*L. monocytogenes* serovars 1/2a and 4b).

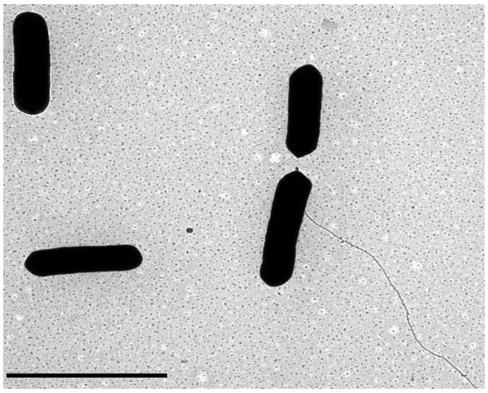


**Figure 5:** *Listeria monocytogenes* downregulates the expression of flagella at 37 °C. TEM microphotography of a clinical isolate showing only one single flagellum. Magnification ×20.000. Bar indicates 2 µm.

Innate immunity to *L. monocytogenes* is primarily mediated by two types of pattern recognition receptors, the Toll-like receptors (TLRs), and the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). In addition, there is some experimental evidence for the involvement of scavenger receptors and a TLR-9 independent cytosolic sensor system for bacterial DNA (Witte et al., 2012). Macrophages are key mediators in eliciting both innate and adaptive immune responses against *L. monocytogenes*. Monocytes produced in the bone marrow travel via the circulation to surrounding tissues, where they differentiate into macrophages. Macrophages perform multiple functions, including the phagocytosis and digestion of invading microbes, antigen presentation to T lymphocytes, and the production of cytokines that activate various other cell types. Macrophages phagocytose foreign particles and pathogens into membrane-bound compartments that undergo fusion events guiding their maturation. After phagosome closure, the phagosome resembles an early endosome that transitions into a late endosome and fuses with the lysosomes to degrade invaders.

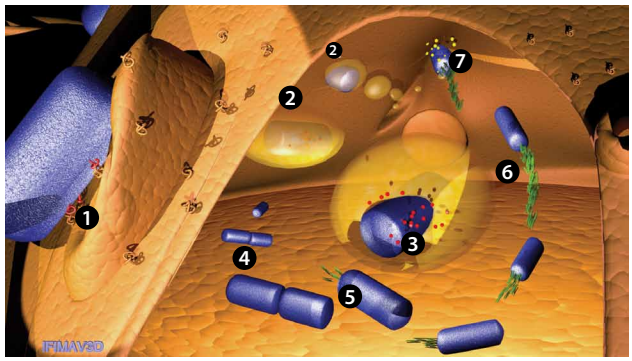



**Figure 6:** Stages in the Intracellular Life Cycle of *L. monocytogenes*. The drawing captures the different stages of *L. monocytogenes* infection.

(1) The bacterium is internalized by the host cell by means of cell adhesion proteins. (2) Once inside the host cell, the bacterium is enclosed by a phagosomal membrane. (3) It escapes the vacuole by secreting a pore-forming toxin, Listeriolysin, and the action of phospholipases which lyse the phagosomal membrane thus enabling the escape of the bacterium into the host cell´s cytosol. (4) Subsequent to its escape from the vacuole, replication occurs, and *L. monocytogenes* acquires actin based motility by generating a network of actin filaments (5). (6) Intracellular movement due to actin-polymerization mediated by ActA. (7) Bacteria use host cell actin to move and propel themselves into neighboring cells by pushing against the host cell membrane.

How *Listeria* enters into eukaryotic cells has been investigated in detail (Tilney and Portnoy, 1989). The bacterium is phagocytosed by normal macrophages and microglia (**Figure 7**). Internalization in non-phagocytic cells results from the tight apposition of the plasma membrane around the incoming microbe. The entry mechanism has been called the "zipper mechanism" as it involves the progressive interaction of bacterial surface ligands with their respective cellular receptors (**Figure 8**). Phagocytic cells internalize bacteria through a variety of opsonin-dependent and opsonin-independent mechanisms. In addition, *L. monocytogenes* can induce its own entry into epithelial cells by using invasion proteins such as internalin A and internalin B. After entry into cells, *L. monocytogenes* delay phagosomal maturation and targeting to the degradative pathway, and rapidly lyse the membrane of the acidified phagosome through the action of the enzyme listeriolysin O (LLO) working in concert with some phospholipases. The bacteria then reside freely in the cytoplasm, where they replicate and acquire F-actin-based intracellular motility based on expression of the ActA protein which recruit actin and polymerize this molecule, generating a network of branched filaments which can be easily visualized in fixed infected cells by phalloidin labeling. At this point, there is an increasing desire to understand the metabolic processes connected with pathogenic

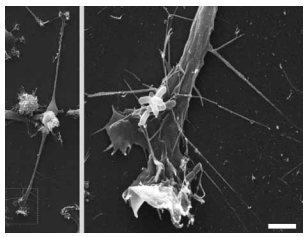


**Figure 7:** Phagocytosis of *Listeria* by Rat Macrophages (SEM microphotography). Original magnification ×2.000; ×8.000. Bar indicates 5 µm.

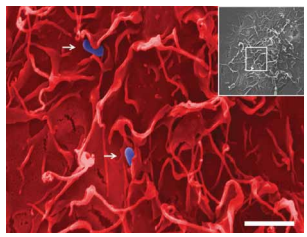


**Figure 8:** Pseudocolored SEM micro-photography. Entry of *L. monocytogenes* inside a Mouse Macrophage by a Zipper Mechanism. Original magnification, ×15.000; ×7330 (inset). Bar indicates 10 µm.



bacteria, especially for the metabolic interference between the intracellular *Listeria monocytogenes* and their host cells. It is recognized that the answer to these questions is crucial for the understanding of pathogenesis of infectious diseases caused by this and other intracellular bacteria (Joseph and Goebel, 2007). The polymerization process at one pole of the bacteria produces energy to propel bacteria which move in the cytoplasm (**Figure 9**). After moving freely in the cytoplasm, *L. monocytogenes* contact with the inner side of the plasma membrane. Bacteria push the membrane and induce the formation of protrusions to invade neighboring cells and generate a two membrane vacuole from which bacteria escape again, allowing a new cycle of replication in the second infected cell. This phenomenon of direct cell to cell spread allows the bacterium both to escape the harsh environment of the phagosome and to evade humoral defenses in the extracellular milieu. Macrophages also are important for eliciting both innate and adaptive immune responses. Importantly, activated macrophages are the principle effectors of a strong immune response against *L. monocytogenes*. *L. monocytogenes* growth inside activated macrophages is restricted and bacteria are actively cleared.

*L. monocytogenes* also is a bacillus with high tropism for the central nervous system (CNS) (Clauss and Lorber, 2008). *Listeria* is able to cause brain abscesses either with or without meningitis, although this mechanism has not been extensively studied. CNS listeriosis occurs either via bacterial migration through the axons of the cranial nerves, by invasion and replication inside of brain endothelial cells, and/or by infection of phagocytes, which ultimately reach the brain tissue (Drevets and Bronze, 2008). In brain tissue, microglia constitutes the monocyte-lineage immune effector cells (brain macrophages). During bacterial infection, microglial cells play an important role in defense by phagocytosing pathogens and removing cellular debris (Ousman and Kubes, 2012). To gain knowledge about the immune response elicited by *L. monocytogenes* in the brain, we used a rat ex-vivo organotypic nervous system culture as a model for *Listeria* infection. Brain sections were maintained alive for several weeks to study the infection process (**Figure 10**). A major aim of our work is to gain an understanding of the complex response of the CNS to bacterial insults. Hopefully, this will generate opportunities to develop innovative and neuroprotective therapies.

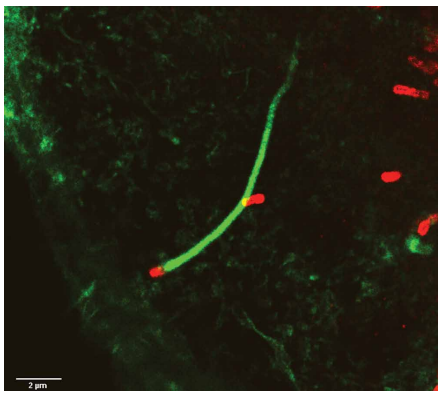



**Figure 9:** Confocal maximum projection image of a *Listeria* tail formed inside a rat primary microglial cell, obtained with a Nikon® A1R confocal scanning laser microscope equipped with a Nikon A1 digital camera, and 488nm, and 561nm lasers. Actin tails were visualized by labeling host actin with Atto-488 phalloidin (Sigma-Aldrich®). Bacteria were stained with a polyclonal anti-*Listeria* antibody (red). Original Magnification ×600. Bar, 2 µm.

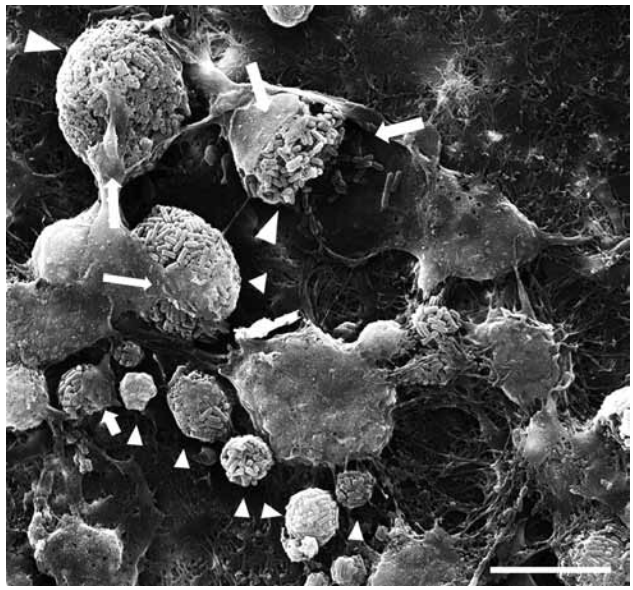


**Figure 10:** Scanning electron microscopy reveals how brain phagocytic cells (called brain macrophages or "microglia") are quickly recruited from deeper brain layers to the surface of the infected tissue. Picture shows the surface of the brain (asterisk), and a dead microglial cell fragmented into phagosomes of different sizes still filled with *L. monocytogenes* (arrowheads). New phagocitic cells arrive to engulf this dangerous cargo (arrows). Magnification: ×5.000. Scale bar: 10 µm.

### References


Camejo, A., Carvalho, F., Reis, O., Leitao, E., Sousa, S., Cabanes, D., 2011, The Arsenal of Virulence Factors Deployed by *Listeria monocytogenes* to Promote Its Cell Infection Cycle. Virulence 2, 379-394.

Cossart, P., Toledo-Arana, A., 2008, *Listeria monocytogenes*, a Unique Model in Infection Biology: An Overview. Microbes and Infection / Institut Pasteur 10, 1041-1050.

Drevets, D.A., Bronze, M.S., 2008, *Listeria monocytogenes*: Epidemiology, Human Disease, and Mechanisms of Brain Invasion. FEMS Immunology and Medical Microbiology 53, 151-165.

Joseph, B., Goebel, W., 2007, Life of *Listeria monocytogenes* in the Host Cells' Cytosol. Microbes and Infection / Institut Pasteur 9, 1188-1195.

Liu, D., 2008, Preparation of *Listeria monocytogenes* Specimens for Molecular Detection and Identification. International Journal of Food Microbiology 122, 229-242.

Mostowy, S., Cossart, P., Virulence Factors That Modulate the Cell Biology of *Listeria* Infection and the Host Response. Advances in Immunology 113, 19-32.

O'Neil, H.S., Marquis, H., 2006, *Listeria monocytogenes* Flagella Are Used for Motility, not as Adhesins, to Increase Host Cell Invasion. Infection and Immunity 74, 6675-6681.

Ousman, S.S., Kubes, P., 2012, Immune Surveillance in the Central Nervous System. Nature Neuroscience 15, 1096-1101.

Peel, M., Donachie, W., Shaw, A., 1988, Temperature-dependent Expression of Flagella of *Listeria monocytogenes* Studied by Electron Microscopy, SDS-PAGE and Western Blotting. Journal of General Microbiology 134, 2171-2178.

Tilney, L.G., Portnoy, D.A., 1989, Actin Filaments and the Growth, Movement, and Spread of the Intracellular Bacterial Parasite, *Listeria monocytogenes*. The Journal of Cell Biology 109, 1597-1608.

Witte, C.E., Archer, K.A., Rae, C.S., Sauer, J.D., Woodward, J.J., Portnoy, D.A., 2012, Innate Immune Pathways Triggered by *Listeria monocytogenes* and Their Role in the Induction of Cell-mediated Immunity. Advances in Immunology 113, 135-156.

# rRNA Probes System for Detection of *Listeria*

By Jvo Siegrist, Product Manager Microbiology — *ivo.siegrist@sial.com*

## Currently, Real-Time PCR is the standard for modern microbiology, but there are other, easier methods

Our HybriScan™ test system is able to detect *Listeria* ssp. and specifically *Listeria monocytogenes*. PCR is not needed, but nevertheless, the result is based on the genetic information, with the target molecule being ribosomal RNA. Each bacterial cell possesses several hundred copies of ribosomal RNA and therefore no PCR is needed. The test method, sandwich hybridization, is designed on a simple 96-well microplate with 12 strips of 8 wells. The test is not sensitive to the sample matrix and detects only living cells. No special expensive equipment is needed and the test is done in approximately 2 to 2.5 hours. A positive result is visible to the naked eye, but can also be read by a standard microplate reader to quantify the number of cells at 450 nm. With a pre-enrichment step (24 - 30 hours in Half Fraser and Fraser broth), a sensitivity of 1 CFU/25 g can be reached.

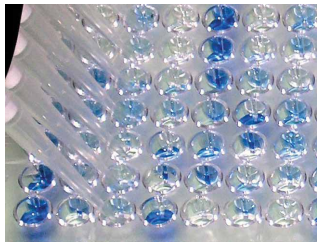


**Figure 11:** HybriScan Plate within the Chromogenic Reaction

The system was validated according to the German § 64 LFGB method. A 25 g sample was taken and enriched in 225 mL ONE-Broth for 24 hours at 30 °C. Then the broth was streaked out on PALCAM agar incubated for 48 hours at 37 °C and all *Listeria* appears as grayish-black, caved in colonies. The colonies are then confirmed as Gram-positive rods using biochemical differentiation with BD BBL CRYSTAL. As the HybriScan system missed three *L. monocytogenes* positive samples, single colonies were grown on PALCAM agar, sequenced by 16S rDNA, and the three undetected species turned out to be *L. innocua*, *L. seeligeri* and *L. welshimeri*. Therefore, the cultivation based method (§ 64 LFGB method) led to false positive results due to an incorrect biochemical identification (see more in **Table 6**).

| | Number of *Listeria monocytogenes* Positive Samples | | |
|---|---|---|---|
| **Food Category** | **n** | **§ 64 LFGB** | **HybriScan** |
| Meat products | 72 | 42* (39) | 39 |
| Fish | 36 | 15 | 15 |
| Milk products | 108 | 42 | 42 |
| Fruits and vegetables | 72 | 24 | 24 |
| **Sum** | **288** | **123* (120)** | **120** |

**Table 6.** HybriScan***D*** *Listeria monocytogenes* test validation (*subsequently identified as false positive result with cultivation based; § 64 LFGB method)

HybriScan***D*** *Listeria monocytogenes* has a relative accuracy of 99.0%, a relative specificity of 98.2%, and relative sensitivity of 100%. More about the HybriScan-System and the range of yeast and bacteria detection kits can be found at sigma-aldrich.com/hybriscan.

| Cat. No. | Description | Test/kit |
|---|---|---|
| **55661** | HybriScan***D*** *Listeria* | 96 |
| **49699** | HybriScan***D*** *Listeria monocytogenes* | 48 |
| **49712** | HybriScan***I*** *Listeria monocytogenes* | 96 |

**Table 7.** HybriScan *Listeria* Kits

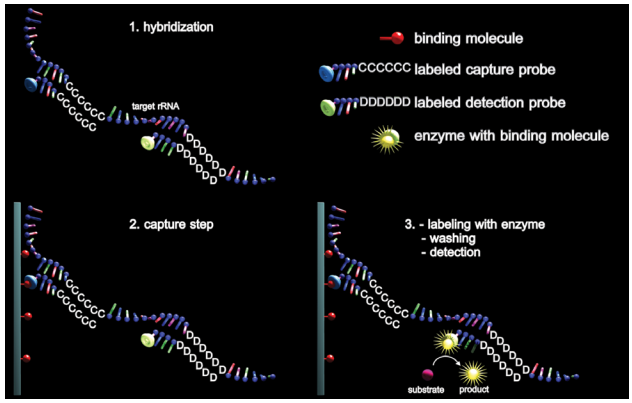



**Figure 13:** Schematic Illustration of the Sandwich Hybridization

## Did you know…

**where it is most probable to find *Listeria*?**

Here are the five most contaminated food types:

1. Canned and raw seafood
2. Fruit of all kinds
3. Foods that are refrigerated for long periods of time
4. Preserved and smoked meats
5. Root vegetables and soil-grown vegetables

**Figure 12:** Raw Seafood

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