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Review Article

Understanding the Nature, Physiology, Taxonomy, Diagnostic and the Federal Compliance Guidelines for Foodborne Pathogen *Listeria monocytogenes*

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Abstract

Invasive *Listeria monocytogenes* is a causative agent of a severe listeriosis infection that primarily affects immunocompromised people, pregnant women and occasionally healthy people. In addition, intrauterine infection of fetus resulted in dearth, or an actually ill infant with a septic disseminated from listeriosis. The association of listeriosis outbreaks with contaminated foods led to recognize that *L. monocytogenes* is a foodborne pathogen and that the intestine is the primary route of entry into the body. Currently, the incidence of listeriosis appears to be on increase worldwide, with the number of cases rising, especially in Europe and the annual endemic disease rate varies from 2 to 16 cases per million population.

Noninvasive *L. monocytogenes* strains appears to be normal residents in the intestinal tract of human. This may partially explain why antibodies to *Listeria* spp. are common in healthy people.

Understanding the sources of contamination and the nature of this foodborne pathogen, including, sources of contamination, mechanism of infection, disease symptoms, treatments, methods of isolation/detection in foods, and Federal compliance guidelines that provide information on sanitation, testing, and prevention of food cross contamination with *L. monocytogenes* are important factors for food safety to protect consumers from the severe illness of listeriosis

Keywords: *Foodborne Pathogens; Foodborne Illness; Listeria monocytogenes; Listeria Virulence Factors; Hemolysis; Listeriolysin O; Phospholipase A, B, and C; Listeriosis Illness; Febrile Listerial Gastroenteritis; Infantiseptica; Pyogenic Granuloma; Pregnant Women; Unborn Children; Elderly People; Immunocompromised Patients; Listeria Standard Detection Methods; Listeria Rapid Detection Methods; Hazard Analysis Control Points (HACCP); Good Manufacturing Plans (GMP)*

Introduction

Foodborne illness results from the consumption of food containing microbial agents such as bacteria, viruses, parasites or micro-toxins. Factors for foodborne illnesses are: Pathogen (microbes or micro-toxins), food vehicle (nutrients for the pathogen to grow), optimum conditions (PH, Temperature, water activity), and a susceptible person ingesting enough pathogen cell counts or pathogen secreted toxins. General symptoms of foodborne illness are flu-like symptoms (nausea, vomiting, diarrhea, abdominal pain, fever, and headache) that are usually disappeared in few days, but severe effects could occur such as joint inflammation or kidney failure and in most severe cases a person could die from foodborne illness.

The gram-positive bacterium *L. monocytogenes* is a ubiquitous, intracellular pathogen that invade body tissue and organs. The invasive *L. monocytogenes* has been implicated within the past decade in several outbreaks in the foodborne illness Listeriosis with a mortality rate of about 24%, mainly immunocompromised persons pregnant women, and their fetuses with symptoms of abortion, neonatal death, septicemia, and meningitis. The genus *Listeria* contains nine species including *L. monocytogenes*, which is pathogenic for both humans and animals and are widely found in the environment especially in soil, decaying vegetation, and water, plus they are part of the fecal flora

of mammals, including healthy human and animals. These nine species of the genus *Listeria* are *L. innocua L. ivanovii, L. monocytogenes, L. grayi, L. seeligeri, L. welshimieri, L. marthii, L. rocourtiae,* and *L. fleischmannii.* Only *L. monocytogenes* is the major human pathogen and there are 13 serotypes known for *L. monocytogenes* (1/2a, 1/2b 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7). From these 13 serotypes only serotypes 1/2a, 1/2b, and 4b were implicated in 90% of listeriosis outbreaks. Serotyping *L. monocytogenes* is based on antibodies that specifically react with "O" antigens and or "H" antigen on the bacteria cell surface. Serotyping methods are usually applied for epidemiological purposes.

History and mechanism of action

L. monocytogenes was first described by E.G.D. Murray in 1924 based on six cases of sudden death in young rabbits [1] and it was referred by the name *Bacterium monocytogenes* before it was changed into the genus *Listeria* in the year 1940 and named *Listeria monocytogenes* [2]. *L. monocytogenes* was not identified as a cause of foodborne illness until the year 1981. The first outbreak of listeriosis was occurred mostly for pregnant women and neonates in Halifax, Nova Scotia, and was involved in 41 cases with 18 deaths that was linked to contaminated coleslaw with *L. monocytogenes*-from sheep manure [3]. Since then, numbers of listeriosis cases have been reported, and *L. monocytogenes* became widely recognized by food industries [4] as an important foodborne pathogen. The largest listeriosis outbreak in United. States was occurred in the year 2011 that was affected 147 people causing 33 deaths and one miscarriage. It was occurred after eating contaminated cantaloupe from a single farm.

Since then Listeriosis poses as a very serious threat to the public, especially for pregnant women, their unborn children, elderly, and immunocompromised patients with approximately 2,500 cases per year in United States, causing a death rate of about 20 - 30% per year.

This Invasive *L. monocytogenes* that causes listeriosis illness with symptoms include septicemia [5], meningitis [6] encephalitis [7], corneal ulcer [8] pneumonia [9] and intrauterine or cervical infection in the case of pregnant women causing abortion in the second and third trimester. Surviving neonatal after birth suffer multiple symptoms such as gastroenteritis, infantiseptica [10] and pyogenic granuloma distributed over the infant body. Plus the infant may suffer from physical retardation.

Mechanism of infection [11,12] with invasive *L. monocytogenes* is initiated after the ingestion of less than 1,000 viable *Listeria* cells in contaminated foods. *Listeria* cells are colonized in the small intestine and enters cells lining of the gastrointestinal tract into the host blood circulations. Circulated immune defense cells such as monocytes, and macrophages phagocytose the *Listeria* cells in order to neutralize it in the phagolysosome compartment. Phagocytized *Listeria* cell in the phagolysosome compartment of monocytes and macrophages produce virulence factors listeriolysin O, and phospholipase A, B, and C in order to destroy the phagolysosome's membrane to escape from the immune cell defense into the host cell cytoplasm where it grow and spread from cell to cell and continue repeating the same mechanism (Figure 1).

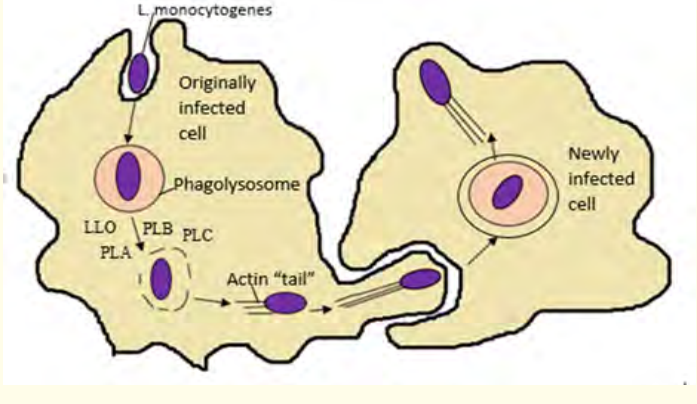


Figure 1: *Listeria monocytogenes uses virulence factors Listeriolysin O (LLO), phospholipase A (PLA), phospholipase B (PLB) and phospholipase C (PLC), to avoid the host's immune system and infecting new cells. [Stephanie McKee and Racha Lakrouf: Mechanism of pathogenicity 2005].*

Noninvasive *L. monocytogenes* is lacking these virulence factors and causing acute self-limited febrile gastroenteritis [13] in healthy persons without serious complication This cute Illness is typically occur in 24 hours after the ingestion of a large *Listeria* cell count (10^5-10^6) of this non-virulence *L. monocytogenes* in contaminated foods. This acute illness is usually last for two days with common symptoms include fever, watery diarrhea, nausea, headache, and pain in both joints and muscles.

L. monocytogenes should be consider etiologically noninvasive when microbial pathogenicity test failed in the detection of virulence factors that are present in invasive *L. monocytogenes*. Several incidents of febrile gastroenteritis from noninvasive *L. monocytogenes* was reported in United States from the ingestion contaminated ready-to-eat meats.

Source of infection and transmission

The main source of listeriosis are from contaminated foods such as milk and milk products (semi-soft cheeses, soft mold-ripped cheeses and butter), processed meats (hot dogs, and salami), seafood (smoked fish, and cooked shrimp), ready-to-eat food products, modified atmosphere packaged foods, raw vegetables, salads, and coleslaw. A small cell count (100 - 1000) of the invasive *L. monocytogenes* in contaminated food could cause listeriosis illness.

Babies can be born with listeriosis if their mothers eat contaminated food during pregnancy. The mode of transmission of invasive *L. monocytogenes* from pregnant woman to the fetus is either, through the Trans placental via the maternal blood stream or through ascending from a colonized genital tract [14] Infections during pregnancy can cause premature delivery, miscarriage or serious health problems for the newborn [15].

Immunocompromised, HIV, and cancer patients are highly susceptible for listeriosis illness from contaminated foods with the invasive *L. monocytogenes*, than healthy general population [16]. In addition, small percentage (2 - 6%) of healthy human are the carrier of *L. monocytogenes* as a source of infection via person to person contact. Infected healthy animals also can be a source for transmission via animal to person contact.

Treatment of patients

L. monocytogenes is susceptible for the treatment with most antibiotics and antibiotics of choice are ampicillin or penicillin in combination with gentamicin. In the case of patients with penicillin allergies, a mixture of trimethoprim and sulfamethoxazole are used in replacement of ampicillin or penicillin. It is important to highlight the immerging of antibiotic resistant strains of *L. monocytogenes* especially against cephalosporin and tetracycline has been reported.

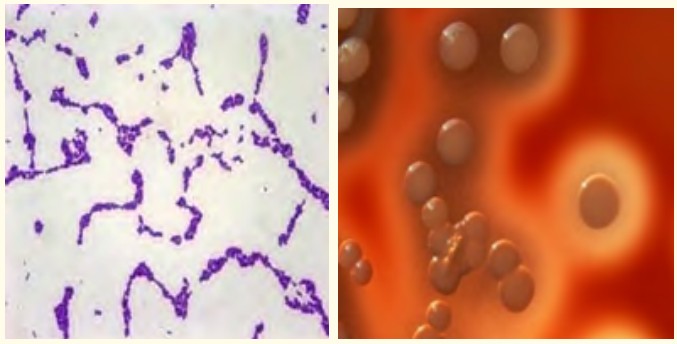
Morphology and Physiological properties of *Listeria monocytogenes*

L. monocytogenes is small, gram positive rods (Figure 2) which are sometimes arranged in short chains, nonperforming, catalase-positive, microaerophilic, salt tolerance (~10 % sodium chloride), 0.92aw (water activity), psychotropic growth at the range of 0 to 42^0C with optimum temperature at 37^0C and optimum PH for growth at the range of 4.4 - 9.4.

Listeria species are motile with flagella that are produced only at 30^0C but not at 37°C. Listeria species are not motile at 37^0C.

ß-Hemolytic activity on blood agar (Figure 2) has been used as a marker to distinguish between *L. monocytogenes* and other Listeria species, but it is not an absolutely reliable.

L. monocytogenes is characterized that it is fermentable for pentose sugar rhamnose and non-fermentable for pentose sugar xylose. Further biochemical characterization are necessary to distinguish between *Listeria* species (Figure 3).



A ***B***

Figure 2: *Listeria monocytogenes. Gram positive short rods (A) and B-hemolytic positive (B).*

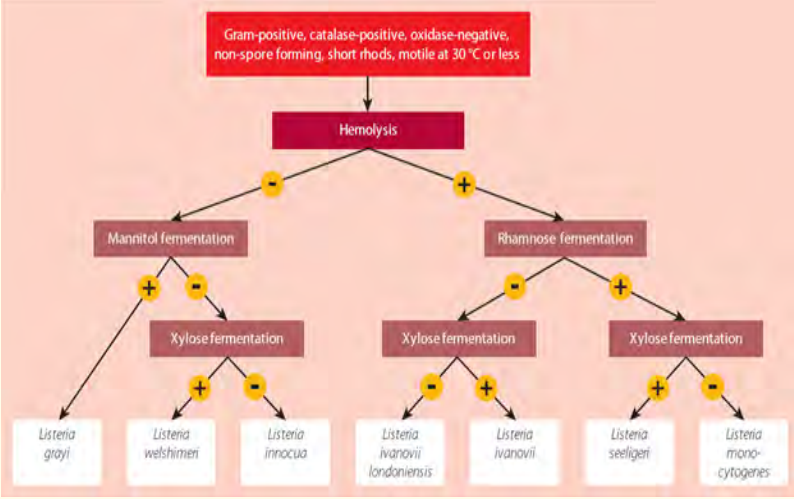


Figure 3: *Schematic of biochemical identification for Listeria spp. based on carbohydrates (sugars) fermentation and hemolysis tests. [Image source: Handbook of Listeria monocytogenes, 2008].*

Isolation and Identification of *Listeria monocytogenes*

L. monocytogenes is an important foodborne pathogen that is widely tested in foods, in environments, and in hospitals. Standard methods for identification (Figure 4) involved culturing on selective enrichments media for enumeration, following by plating on selective agar media for colonies characterization based on colony/cell morphology as presumptive testing, finally, following by sugar fermentation, and hemolytic property., as confirmatory testing This standard methods are reliable but lengthy in time (72 - 96 hours). This long testing time is not desirable for food manufacturers especially for food products with short shelf lives. As a result several rapid test methods were developed as an alternative methods for identification as a confirmatory testing. These reliable rapid test methods are based on antibodies such Enzyme-linked-immunoassay method (ELISA) and molecular biology methods such as DNA hybridization, Polymerase chain reaction (PCR) and Ribotyping.

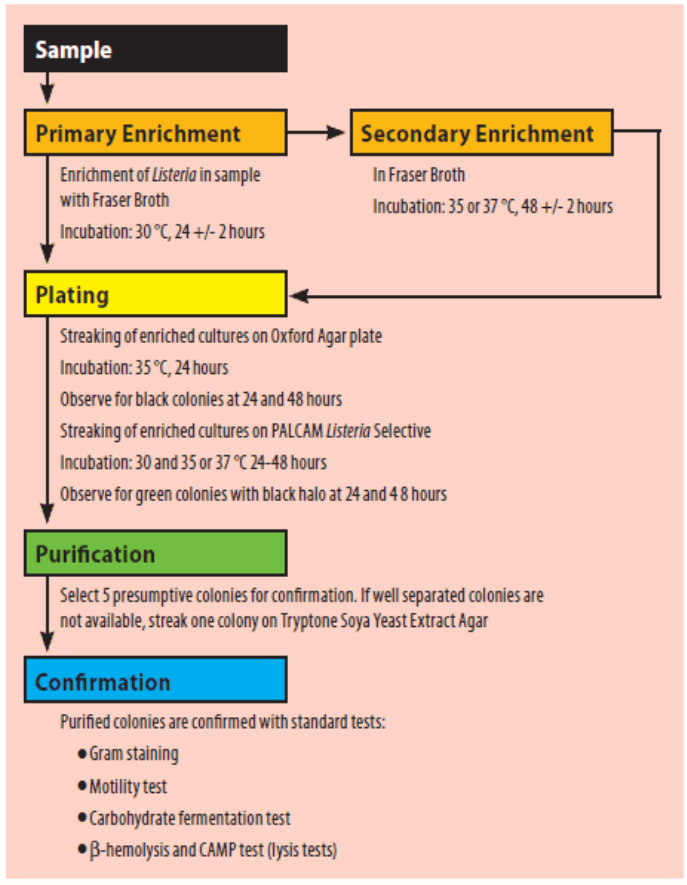


Figure 4: *Standard method for the detection and enumeration of Listeria monocytogenes. [ISO Protocol (EN-ISO 11290-1(1996)].*

Standard Methods for Identification

Enrichment broth and methods: Several enrichment broth for first and second enrichment process are used for the enumeration of *Listeria* species from food samples. These broth media are: University of Vermont broth (UVM), Brain heart infusion broth (BHI), Fraser both (FB) and Morpholine-propanesulfonic acid-buffered *Listeria* enrichment broth (MOPS-BLEB). These two-stages of enrichment process: are adopted for the enumeration of bacteria from food samples.

The first enrichment step is by adding 25 grams of food sample into 225 ml enrichment broth (10 % inoculum size), blended for 2 minutes, then transferred into sterile flask and incubated for 24 - 48 hours at 30^0C.

The second enrichment step an aliquot growth from first enrichment broth is transferred into the second enrichment broth containing selective agents such as acriflavin and nalidixic acid to enhance the growth of the target *Listeria* cells and inhibit competitive bacteria cells present in food samples. This second enrichment step is incubated for 12 - 24 at 30^0C

Selective agar media and methods: The two enrichment process, which are usually take about 30 - 72 hours, are followed by the presumptive identification of the enriched microorganisms. Selective agar media for isolation and identification of *L. monocytogenes* are MOX and PALCAM. These two media contains lithium chloride, acriflavin, colistin sulphate, cefotetan, cycloheximide or amphotericin and phosphomycin as selective agents to inhibit the growth of G (-) bacteria and suppress most of G (+) bacteria.

MOX agar selective medium contains the substrate aesculin (Figure 5) and the indicator ferrous ions for the identification of *L. monocytogenes* The endogenous enzyme ß-D glucosidase in *L. monocytogenes* cells hydrolyze the substrate aesculin producing black zones under and around *L. monocytogenes* colonies due to the formation of black iron phenolic compound resulted from the indicator ferrous ions (Figure 6). However, other microorganism's colonies can grow on MOX agar media such as some species from the genus *Enterococcus* and *Bacillus* that can utilize the substrate aesculin in the MOX agar media and give slight similar color appearance as *L. monocytogenes* colonies.

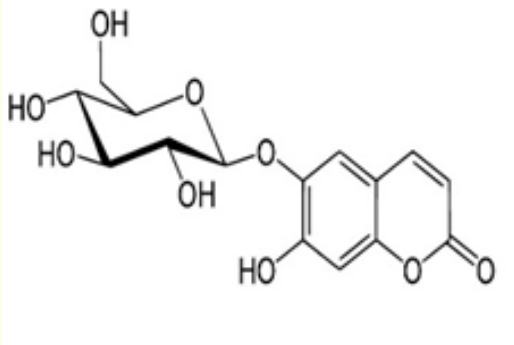


Figure 5: *The substrate aesculin (6, 7-dihydroxycoumarin) in L. monocytogenes selective agar media.*

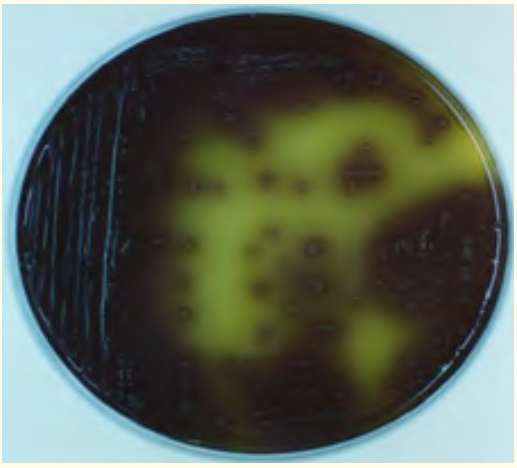


Figure 6: *MOX agar selective agar medium for L. monocytogenes. ß glucosidase enzyme from L. monocytogenes hydrolyze aesculin into β-D-glucose and esculetin. The end product esculetin is detected by the formation of a brown/black complex in the presence of iron salts.*

PALCAM agar selective medium also contains the same selective agents as MOX selective agar medium to inhibit the growth of G (-) bacteria and suppress most of G (+) bacteria. This PALCAM agar medium was designed to eliminate the interference from some species of genus *Enterococcus* and *Bacillus* in the detection of *L. monocytogenes* colonies. PALCAM selective agar medium contains esculin/ferrous iron, and mannitol/phenol red. The endogenous enzyme ß-D glucosidase in *L. monocytogenes* cells hydrolyze aesculin resulting in the formation of a green/black halo around *Listeria* colonies, but *L. monocytogenes* cells does not ferment the sugar alcohol mannitol However, contaminants from the genus *Enterococcus* and *bacillus* are capable to ferment the sugar alcohol mannitol into acid that is detectable by the formation of red to yellow colonies resulted from the indicator phenol red (Figure 7).

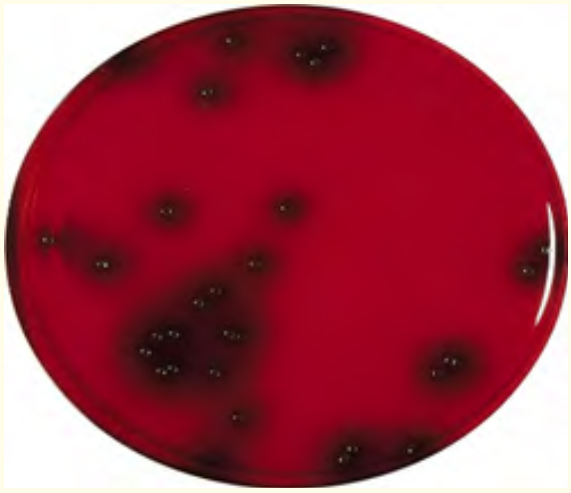


Figure 7: *PALCAM agar selective agar medium for L. monocytogenes. Colonies of L. monocytogenes cannot ferment mannitol and hydrolyze aesculin appear gray-green with a black halo. for the presence of L. monocytogenes. Colonies of contaminates that fermenting mannitol, such as genus from Bacillus, and Enterococcus may grow and appear yellow with a yellow halo.*

Biochemical methods: Confirmatory testing are required to identify *L. monocytogenes* colonies conclusively. Suspected colonies for *L. monocytogenes* from MOX or PALCAM selective agar media are tested first for purity (no microbial contamination) by streaking on tryptic soy agar and incubated at 30^0C. Single colonies, were examined for purity by oblique lighting, technique in which *L. monocytogenes* colonies appears reticulated with a distinct blue-green cast [17]. These distinct blue-green are subject for traditional biochemical testing, which is time consuming and takes a week for the differentiation of *Listeria* species including *L. monocytogenes* using sugars utilization test (Figure 3). Commercial test strips such as API *Listeria* (bio-Merieux, Marcy-Etoile, France) and Micro-ID™ (Remel, USA) have been extensively validated and are now incorporated into the standard biochemical methods [18,19].

CAMP test: The CAMP (Christie, Atkins, and Munch–Petersen) test [20] (Figure 8) is widely used for identifying the three hemolytic *Listeria* species (*L. monocytogenes*, *L. Ivanov* and *L. seeligeri*). CAMP-test method is based on using ß-hemolysin-producing *Staphylococcus aureus* and *Rhodococcus equi* as indicators The test is carried out by streaking β-hemolysin-producing *Staphylococcus aureus* strain and *Rhodococcus equi* in parallel to each other on a blood agar plate. Suspected colony for *Listeria* genus is streaked at right angles in between but not touching the two streaks of. The two indicators *Staphylococcus aureus* and *Rhodococcus equi.*

Hemolysis results from *L. monocytogenes* and to a lesser degree from *L. seeligeri* are enhanced in the vicinity of the indicator *Staphylococcus aureus*, but hemolysis result in the case of *L. ivanovii* is enhanced in the vicinity of the indicator *Rhodococcus equi*.

Currently, a variation of the original CAMP test using commercially available β-lysin discs is recommended by USDA as a method [21]. To enhance CAMP test for correctly differentiate between *L. monocytogenes* and *L. ivanovi* [22].

Figure 8: *CAMP test for L. monocytogenes. L. monocytogenes produce diffusible protein that acts synergistically with the beta-lysin produced by Staphylococcus aureus to produce arrow shape zone (as shown on left) of enhanced lysis of sheep erythrocytes.*

Alternative Methods for Identification

Many alternative rapid detection methods for the identification of *L. monocytogenes* have been developed based on immune assay and molecular biology assay methods. Both assay methods are capable in reducing detection times by more than 50%. Protocols; for these alternative methods can be initiated from a shorter primary or secondary enrichment broth and as a replacement to longer standard culturing methods, (selective agar media as a presumptive testing, and biochemical methods as a confirmatory testing).

Antibody based test methods:

Immuno- magnetic separation (IMS): This is a reliable method and easy to use to separate *L. monocytogenes* from other microorganisms in the culture medium for further identification in a short time [23]. This method is based on using anti-*Listeria monocytogenes* monoclonal antibodies to capture and concentrate *L. monocytogenes* from the growth of other microorganisms in primary or secondary enrichment broth (Figure 9). Separated *L. monocytogenes* by this IMS method can be used for Enzyme linked immune assay (ELISA) and for molecular based test methods.

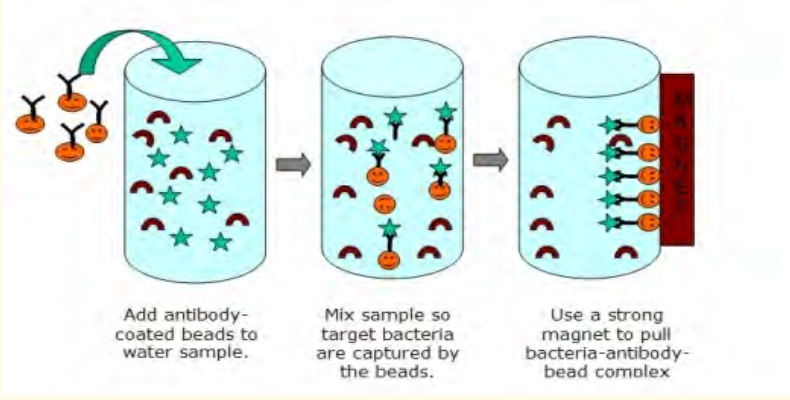


Figure 9: *Immuno- magnetic separation (IMS). Magnetic beads coated with antibodies for L. monocytogenes are added to the enrichment broth growth, then the mixture is subject to IMS, in which the bacteria-antibody-bead complex is separated from microbial population by using a strong magnet. [Image source: Lee Ji Young and Deininger RA 2004].*

Enzyme linked immune assay (ELISA): It is a biochemical method that couples immunoassay with enzyme assay [24]. Specific antibody against *Listeria monocytogenes* antigen are affixed to the surface of the walls of 96 well microtiter plates. The growth from primary or secondary enrichment broth is added to wells and incubated allowing *L. monocytogenes* to bind to the affixed antibodies. After washing step a second phase of specific antibodies against *L. monocytogenes* antigen is added to the well. Followed by the addition of nonspecific enzyme-labeled antibody. This nonspecific enzyme-labeled antibody is allowed to bind to the previously added second phase of specific antibodies against *L. monocytogenes*. In the final step enzyme substrate is added to the bound nonspecific enzyme-labeled antibody to convert the enzyme substrate into detectable colorimetric signal. This ELISA is reliable method with a result in 2 - 3 hours (Figure 10).

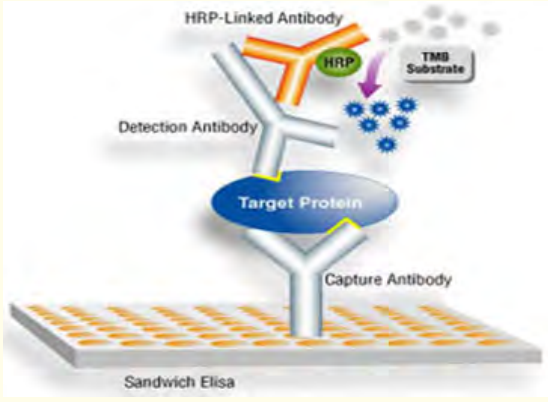


Figure 10: *Enzyme linked immune assay (ELISA). Add the bacteria cells (protein named antigen)- to the microtiter plate wells containing capture antibody, wash the plate to remove unbound antigen, A specific detection antibody is added to bind to the antigen (protein), add enzyme-linked antibodies as a detection antibodies, (non-specific binding), wash the plate to remove, unbound antibody-enzyme conjugates, Add a substrate(e.g. TMB) that react to the conjugated enzyme (e.g. HRP) and converted into a color, incubate and measure the absorbency of the plate wells to determine the presence and quantity of L. monocytogenes. [Image source: e.BioWorld.com].*

Molecular based methods

Identification of *L. monocytogenes* by using nucleic acid methods are extremely, sensitive, reliable for the identification and differentiation of *L. monocytogenes* from any other *Listeria* species and sub-species level in a short time.

DNA hybridization: The presence of a target sequence in *L. monocytogenes* can be detected by using an oligonucleotide probes that are complementary in sequence to the target DNA sequence and contains a label for detection. This test aims for the identification of *L. monocytogenes* by targeting probes to virulence factors DNA sequence in *L. monocytogenes*. Radioactive probes or florescence marker are commonly used for the detection of target virulence factors DNA sequence. Microtiter plates for DNA hybridization are convenient and highly sensitive and is specific approach for the detection of *L. monocytogenes* in a high-throughput 96-well (Figure 11). Commercially available DNA hybridization tests are routinely used for the detection *L. monocytogenes* in food samples have been extensively trialed for their sensitivity and accuracy [25].

Colony hybridization: It is a rapid method of isolating a colony containing a particular sequence or a gene (e.g. *Listeria* virulence factor) from a mixed microbial population. Colonies to be screened are first replica-plated on to a nitrocellulose filter disc that has been placed on the surface of the agar plate prior to inoculation. Master plate is retained for reference set of colonies. The filter bearing the colonies is removed and treated with alkali so that the bacterial colonies are lysed and the DNA they contain is denatured. The filter is then treated with proteinase K to remove protein and leave denatured DNA bound to the nitrocellulose. The DNA is fixed firmly by baking the filter at 80°C and labeled probe is hybridized to the target cell DNA and monitored by autoradiography (Figure 12).

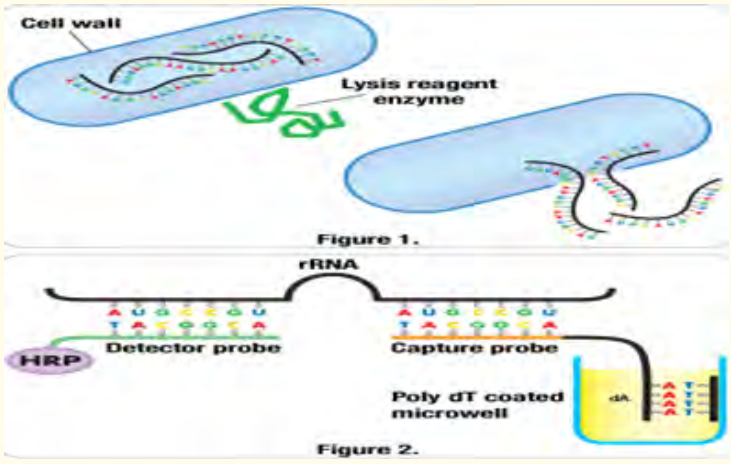


Figure 11: *DNA hybridization. The method is based on lyse bacteria cell (Figure 1) to extract genetic material, for the detection of target DNA via hybridization (Figure 2) using specific capture and detection probes. Capture DNA probe coated in microtiter plate is used to a target a virulence factor DNA sequence from lysed bacteria cells. The labeled detection probe provides with a marker (e.g. HRP) is added ,following by adding a substrate (e.g. TMB), incubate and measure the absorbency of the plate wells to determine the presence the virulence factor DNA sequence of L. monocytogenes. [Image source: NEOGEN].*

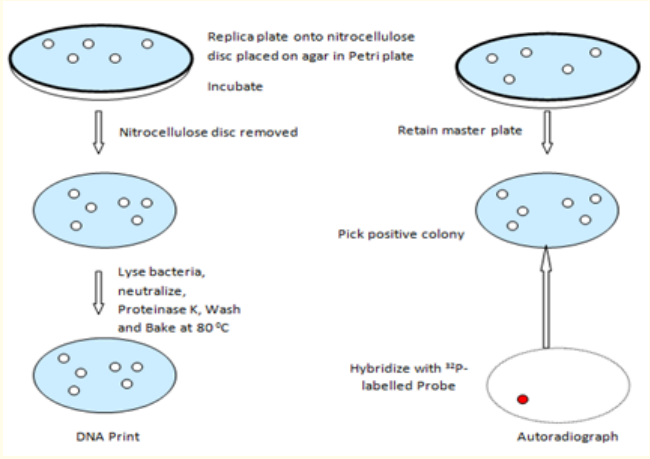


Figure 12: *Colony hybridization. Colony hybridization is applied to DNA released from microbial colonies. Colonies are transferred (blotted) to a nitrocellulose membrane. The DNA after denaturation is fixed to the filter and hybridized with a labelled (e.g. 32P) probe Master plate is retained for further testing a positive colony for L. monocytogenes. [Image source: nptel.ac.in/courses/102].*

Polymerase chain reaction (PCR): It is a fast and inexpensive technique used to amplify (copy) small segments of the target bacteria DNA [26]. In the case of *L. monocytogenes* the target is to amplify one of virulence factors DNA sequence to detect the presence of the invasive *L. monocytogenes* in food samples. The process require DNA template. (a virulence factor DNA extracted from Listeria cells), two DNA Primers that are complementary to the 3' and 5" prime ends of the DNA template (extracted target virulence factor DNA in *Listeria* cells), DNA polymerase enzyme to synthesizes new strands of extracted target virulence factor DNA, using the original extracted target

virulence factor DNA strands as templates,. dNTPs the DNA building blocks from which the DNA polymerase synthesizes a new DNA strand, and buffer solutions. This PCR processed in multiple cycles for the replication of the virulence factor DNA extracted from *Listeria* cells. The PCR cycling process is performed by automated machine called a thermocycler, which is programmed to alter the temperature of the reaction every few minutes to allow the target *Listeria* DNA template denaturing and synthesis (Figure 13). After PCR process the amplified target DNA can be detected by Gel electrophoresis.

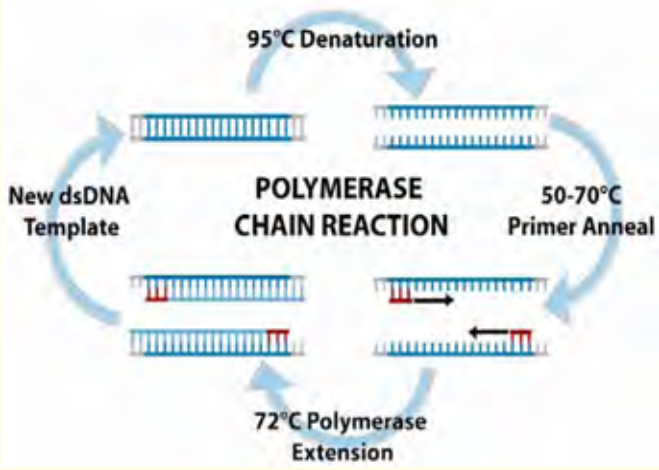


Figure 13: *Polymerase chain reaction (PCR). PCR is amplification technique for target DNA detection. The standard protocol involves raising the temperature of the reaction to 95°C to separate the double strand DNA (denaturation), then lower the temperature (50 - 70^{0}C) for primers to anneal (hybridize), and then raise the optimal DNA polymerase temperature (72°C) for primer extension (amplification) This process is repeated cyclically, creating many copies of the target sequence (e.g. L. monocytogenes virulence factors). [Image source: Thermo Fisher Scientific].*

Ribotyping: Bacteria cells have conservative ribosomal genes (rRNA) that are unique in sequence to each bacteria species. These unique 16S rRNA is used as a genetic fingerprint [27]. Therefore sequencing the particular 16S and comparing it to a database would yield identification of the particular bacteria species (e.g. *L. monocytogenes*). Ribotyping method require the digestion of bacterial DNA with specific restriction enzymes that cuts DNA at specific nucleotide sequence, resulting in fragments that are different in length. Those fragments are separated according to its size using Gel electrophoresis. Following separation in the gel matrix, the separated DNA fragments are transferred onto nylon membranes and hybridized with a labelled 16SrRNA probe to be visualized for analysis. In comparison to reference organisms (e.g. *L. monocytogenes*) in a computer database (Figure 14).

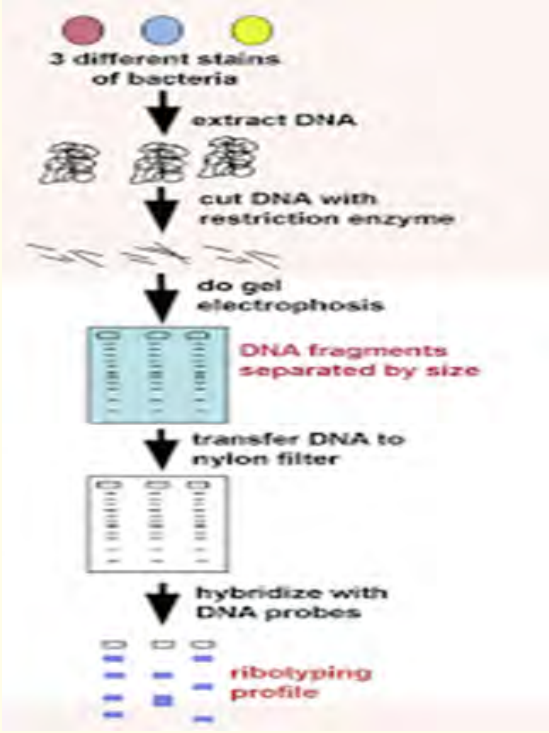


Figure 14: *Ribotyping. Ribotyping is a molecular genetic technique that takes advantage of unique DNA sequences to differentiate strains of bacteria. The extracted genomic DNA from bacteria cell is cut at specific sites by doing a restriction digest with restriction enzymes generating DNA fragments with different lengths. Because different strains of bacteria have different specific cut-site places for the restriction enzymes to cut DNA, each bacteria strain generates a unique pattern of DNA fragments that can be separated by electrophoresis, hybridized with labeled probe, fingerprinting and the bacteria name (e.g, L. monocytogenes.), can be identified from a data base. [Image source: Courses cit, Cornell edu].*

Acceptable limits of *Listeria monocytogenes* in Foods

Worldwide Scientific Organizations and Governments have accelerated their efforts in developing worldwide standard food regulations to ensure the global safe foods for consumers, and controlling public health threats from foodborne pathogens such as *L. monocytogenes* [28]. While some progress has been made in recent years, many global harmonization challenges remain and controlling threat from foodborne pathogen, and *L. monocytogenes* is in the front of these challenges. To eliminate such threat Zero tolerance policy for *L. monocytogenes* is adopted for ready to eat foods (RTE) and for any food categories that does support the growth of *L. monocytogenes*.

A zero tolerance policy according to the U.S. Food and Drug Administration (FDA), means the absence of *L. monocytogenes* in 25 gram food samples. For other foods that do not support the growth of *L. monocytogenes* FDA amended the regulatory limit to less than 100 CFU/gram food. Some countries such as Canada and Australia have a tolerance level of less than 100 CFU/g in ready-to-eat (RTE) foods or for any foods that do support the growth of *L. monocytogenes*.

Food Safety measure to prevent *Listeria infections*

The widespread presence of *L. monocytogenes* in the environment, and the fact that the vast majority of *Listeria* infections are the result of consuming contaminated foods or water, preventing illness and death from consuming food contaminated with *L. monocytogenes* became a food safety issue.

Hazard analysis and critical control points (HACCP): The definition of HACCP is a management system in which food safety is addressed through analysis and control of biological, chemical, and physical hazards from raw materials, manufacturing, distribution, retails and foods consumption [29].

The seven points of HACCP principals are:

1. Conducting hazard analysis (identify hazard).
2. Determining critical control points (CCPs).
3. Establishing critical limits.
4. Establishing monitoring procedures.
5. Establishing corrective actions.
6. Establishing verification procedures.
7. Establishing record keeping and documentation procedures.

These seven HACCP principals are currently implemented successfully worldwide in food industries, distribution facilities, retail operations [30], and in food service stores. In addition, consumers can also implement HACCP like practice in the home by following proper storage, handling, cooking, and cleaning procedures from the time of purchasing foods to the time of serving meals. There are regulatory steps to be taken by consumers to prevent the growth of foodborne pathogens and to ensure food safety.

These steps are:

1. Wash hand and sanitize food-contact surface often.
2. Keep raw meat, poultry, eggs, and seafood's away from ready to-eat foods.
3. Thaw food in the refrigerator or microwave, not on kitchen counter, (the danger zone for microbial growth is at the range of 40-145^{0}F).
4. Use food thermometer and cook at 165^{0}F or higher to kill harmful bacteria.
5. Refrigerate foods promptly and properly (cold temperatures slow the growth of foodborne pathogens

Good manufacture practice (GMP): GMP [31] is guidelines that are essential foundation for the development of successful HACCP plans. These guidelines provide guidance for food manufacturing, sanitation protocols, testing methods, and quality assurance in order to assist in reducing the risk of foodborne illness and to secure the production and distribution of safe foods for human consumption. Many countries follow GMP procedures and have created their own GMP guidelines which corresponded to their own legislations.

General GMP practices follow the following principals:

1. Maintain a clean and hygienic manufacturing areas.
2. Control environmental conditions to prevent cross contamination between products.
3. Develop manufacturing process that are clearly defined and controlled.
4. Validate all Critical control points (CCPs) to insure products safety and consistency.
5. Control manufacturing process, and evaluate/validate any process change.
6. Write production instructions and procedures clearly (production sheet) and record process data during manufacturing (good documentation practice).
7. Minimize the risk of contamination during distribution of food products.
8. Establish a system for quick recall of any unsafe food product distributed for sale.

Finally, it important to highlight that *L. monocytogenes* on wet surface has the ability to aggregate and grow to produce biofilm Biofilms in food processing environment will increase the risk of food contamination Food residue from unclean production line serve as nutrients for biofilm formations that are protected from sanitizers. Proper periodic sanitation with sanitizers containing enzyme and the application of hot water for both foods direct/non-direct contacts in production facility, and performing microbial testing are important factors to reduce foodborne illness including from *L. monocytogenes*.

Conclusion

L. monocytogenes is a ubiquitous, intracellular pathogen which has been implicated within the past decade as the causative organism in several outbreaks of foodborne disease listeriosis with a mortality rate of about 24% that infect mainly HIV patients, immunocompromised persons, pregnant women, and their fetuses with symptoms of abortion, neonatal death, septicemia, and meningitis.

Listeria spp., are small G (+) bacillus, arranged in small chains, motile at 20 - 25°C but not motile at 37^0C, and non-spore-forming. It is aerobic and facultative anaerobic, catalase-positive, oxidase negative, and ferments many carbohydrates without producing gas.

Strains of *L. monocytogenes* are always hydrolyses esculin, D-xylose negative and produce lecithinase. They are generally β-haemolytic and L-rhamnose positive. *L. monocytogenes* strains are divided into 13 serotypes based on somatic and flagellar antigens. These serotypes are 1/2a, 1/2b 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7, of these serotypes only 1/2a, 1/2b, and 4b were implicated in 90% of listeriosis outbreaks. The invasive *L. monocytogenes* organism that causing listeriosis illness has a multifactorial virulence factors, of thiol-activated hemolysin, listeriolysin O, and phospholipase A, B, and C. These virulence factors playing a crucial role in the organism's ability to multiply within host phagocytic cells and to spread from cell to cell.

Listeriosis is a serious infection caused by eating contaminated food with invasive *L. monocytogenes*. The bacteria is commonly found in soil, decaying plants, and water Animals can carry the bacteria causing meat and dairy products contamination Food products such as deli meats, hot dogs, smoked seafood, salads and ready-to-eat foods are at risk of such contamination. The largest listeriosis outbreak in United. States was occurred in the year 2011 after eating contaminated cantaloupe from a single farm and affected 147 people causing 33 deaths and one miscarriage.

Noninvasive *L. monocytogenes* is lacking these virulence factors and causing acute self-limited febrile gastroenteritis in healthy persons without serious complication This acute Illness is typically occur in 24 hours after the ingestion of a large number of noninvasive *Listeria* cell in contaminated foods and usually last for two days with symptoms include fever, watery diarrhea, nausea, headache, and pain in both joints and muscles.

Improved methods for enumerating and identification of *L. monocytogenes* in food samples are now available, including those that are based on the use of monoclonal antibodies, DNA probes, or the polymerase chain reaction (PCR). As continues advancing knowledge of molecular and applied biology of *L. monocytogenes* will increase, our progress in controlling foods from contamination and preventing consumers from foodborne illness.

Currently, worldwide food safety regulations and implementations of Hazard Analysis Control Point (HACCP), Good Manufacturing Practice (GMP) and proper sanitation protocols played an important rules in minimizing the incidence of listeriosis illness. However, *L. monocytogenes* remains an important cause of serious life threating to human and are responsible to multiple yearly outbreaks in United State and other countries.

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