Steps for molecular detection of *L. monocytogenes*

- Enrichment of sample
- Crude extraction of nucleic acid template from enriched sample
- First step PCR
- Second step PCR
- Preparation and running of gel
- Visualization of gel under UV transilluminator
- Interpretation

**Multiplex PCR for differentiation of *L. monocytogenes* and *L. innocua***

- *Listeria monocytogenes* is a pathogenic and *L. innocua* is non-pathogenic organism, but both the organism share a similar type biochemical characters.
- Conventional method of differentiation is based on haemolytic activity of *L. monocytogenes* on sheep blood agar. *Listeria innocua* is non-haemolytic.
- But differentiation based on this method is confusing and ambiguous. Haemolysis produced by *L. monocytogenes* generally has very narrow zone and thus may miss detection.

CIFT has developed a multiplex PCR, by which these two species can easily be differentiated on the basis of sizes of band on agarose gel and also genus *Listeria* can be differentiated from other bacteria and the process can be completed within a day.

Two bands of 938 and 267 bp: *L. monocytogenes*
Two bands of 938 and 749 bp: *L. innocua*
One band of 938 bp: Other species of *Listeria*
No band: Other bacteria

For further information contact:  
Dr. K.V. Lalitha, Head, MFP Division  
E-mail: kvlalithaa@gmail.com  
Tel. 0484-2666845 Extn. 384  
Publisher: Dr. T.K. Srinivasa Gopal, Director

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Dr. Sanjoy Das and Dr. K.V. Lalitha  
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**Listeria monocytogenes**

**The Facts**

- A foodborne pathogen of public health concern.
- Causes listeriosis evidenced by abortion, encephalomyelitis, arthritis, conjunctivitis, etc. both in human being and animals.
- Highest death rate among all food-borne infections.
- A food safety hazard and a zero-tolerant organism in ready-to-eat (RTE) food products. In seafoods meant for export, this organism should be absent in 25 g of sample.
- Ubiquitous in aquatic and terrestrial environments and can be found in a wide variety of foods, including meats, poultry, vegetables, dairy products, and seafood products.
- *Listeria* tends to form a biofilm and survives extremely well in the processing plant environment. Persistent *L. monocytogenes* contamination in processing plants represents a major concern for the industry and public health.
- A cold tolerant pathogen, which can survive or even multiply during refrigerated storage.
- Can resist drying and high salt concentration
- Can even grow at vacuum-packaged foods.

**Methods of detection**

- Conventional microbiological methods
- **Immunological methods** e.g. ELISA, Immunofluorescence, etc.
- Molecular methods e.g. PCR

**Conventional Method**

Uses cold enrichment and selective media containing a combination of antibiotics, which inhibit background flora. Some media contain chromogenic substrates for better identification. After isolation, the organisms have to be confirmed by using a series of biochemical tests.

**Disadvantages of conventional methods**

- Time consuming and laborious. It takes at least 7 days to complete.
- Requires skilled laboratory staffs.
- Difficult to differentiate *L. monocytogenes* and *L. innocua*.

**PCR-based methods**

PCR amplifies specific sequences of target DNA. The PCR process includes denaturation, amplification, annealing and extension. The whole process is repeated 25-30 times so that a single copy of DNA template can turn into millions of copies within 2-3 hrs. Gel electrophoresis is typically used to detect the amplified product. Multiplex PCR assay facilitate the differentiation of *Listeria* species in a single test.

**Advantages of PCR-based methods**

- Take less time. The presence of *L. monocytogenes* can be detected one day after receiving samples.
- Can easily differentiate *L. monocytogenes* (Pathogenic) and *L. innocua* (Non-pathogenic).
- More specific and sensitive.