

Listeria Genus Detection in Food Samples Using the New LightCycler foodproof Listeria Genus Detection Kit

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Introduction

The genus *Listeria* includes six species: *Listeria monocytogenes*, *L. grayi*, *L. innocua*, *L. ivanovii*, *L. welshimeri*, and *L. seeligeri*, characterized as gram-positive rod-shaped bacteria. Two are of higher importance due to their opportunistic pathogenicity, i.e., *L. monocytogenes* (human and animal pathogen) and *L. ivanovii* (animal pathogen). Listeriosis caused by *L. monocytogenes* is a severe human disease. The main clinical manifestations of listeriosis are meningoencephalitis, septicemia, and abortion. The mortality is up to 33% [1]. The *Listeria* bacteria are ubiquitous, and screening for the genus *Listeria* in environmental and food samples serves as an indicator of sanitation, and as an early warning of the potential presence of the pathogenic species such as *L. monocytogenes*.

The bacterium *Listeria* is typically transmitted to human beings through the ingestion of contaminated food such as raw milk, dairy products, meat, and raw vegetables. Most of these foods have relatively short shelf lives, and food industries need to speed up in-process control to prevent in-house contaminations. Therefore, the need for rapid, accurate, and sensitive methods for the detection of *Listeria* is a major food safety issue. Traditionally, this is accomplished through microbiological testing. The official methods in Europe and the U.S.A. require a combination of a nonselective and selective pre-enrichment of 25 g of the food sample for 48 hours or even 72 hours. To detect possible pathogens, samples are cultivated on a solid selective agar plate for an additional 24 hours. In total, the procedure requires at least three days [2, 3, 4]. These methods are very time consuming, and the interpretation of results demands considerable experience. The LightCycler foodproof *Listeria* Genus Detection Kit in combination with the dedicated sample preparation kits enables the users to detect one colony-forming unit (cfu) of *Listeria* in 25 g of sample within two days. In case of a positive result no confirmation is necessary. A negative result is confirmed by an Internal Control which is included in each reaction (Table 1).

Materials and Methods

Sample preparation and sensitivity

The pre-enrichment of a food sample (vanilla ice cream) is carried out for 24 hours in standard media according to

the Bacteriological Analytical Manual (BAM) conventional methods [2]. The culture was inoculated in six series with 10^4 cells/ml of *L. monocytogenes*, and serial dilutions were prepared with the corresponding non-spiked enrichment culture down to 10^3 and 10^2 cells/ml. Then the enrichment culture was additionally inoculated with approximately 10^8 cells/ml *Escherichia coli* as a background contamination to check for interference of background DNA.

ShortPrep foodproof II Kit

In less than 30 minutes the ShortPrep foodproof II Kit generates PCR template DNA from the enrichment culture. An aliquot of the enrichment culture (200 μ l) was centrifuged and the resulting cell pellet was resuspended in Resuspension Reagent. The bacterial cells were lysed by mechanical disruption and incubation at 95–100°C for 5 minutes. After brief centrifugation, the supernatant was used in PCR. For food samples with strongly inhibiting ingredients, the High Pure foodproof II Kit removes these substances.

High Pure foodproof II Kit

The cells of the pre-enrichment culture were pelleted by centrifugation, then lysed during a brief incubation with the Lysis Buffer and lysozyme supplied in the kit. After Proteinase K digestion and clearing of the lysis mixture by brief centrifugation, the sample was transferred to special glass fibers pre-packed in filter tubes. The DNA was bound selectively and was purified in two “wash-and-spin” steps to remove potential PCR inhibitors. Finally, DNA was released from the glass fibers by a low-salt elution buffer.

Each subsample of the six dilution series was processed in three replicates. Preparations of the nonspiked broth were processed as contamination control.



Matthias Kiehne

Table 1: Time frame of *Listeria* detection using the microbiological standard method and the LightCycler System

	Official method	LightCycler foodproof <i>Listeria</i> Genus Detection Kit
Pre-enrichment	48–72 hours	24–48 hours
Detection	24 hours	2 hours
Time to result	up to 4 days	max. 2 days

Specificity

To test the specificity for all *Listeria* species, 105 purified DNA extracts of the six *Listeria* species were investigated. Additionally, 56 purified and nonpurified DNA extracts of non-*Listeria* spp. strains were tested. The general feasibility of amplifying DNA extracts was shown by a consensus PCR system (conventional PCR followed by gel electrophoresis). Phylogenetically closely related bacteria strains (mainly gram-positive bacteria with DNA with low GC content) and strains of the same microbiological environment were used.

Table 2:
Comparison of different sample preparation kits

High Pure foodproof II Kit			
Cell count [cells/ml]	10 ⁴	10 ³	10 ²
Number of detected positive samples	18/18	18/18	18/18
Crossing point (mean value)	28.77	31.28	32.73
F _{max} * (mean value)	0.182	0.118	0.043
ShortPrep foodproof II Kit			
Cell count [cells/ml]	10 ⁴	10 ³	10 ²
Number of detected positive samples	18/18	18/18	17/18
Crossing point (mean value)	31.36	32.98	33.44
F _{max} * (mean value)	0.118	0.032	0.005

*Fluorescence maximum

Results

Sample preparation and sensitivity

Table 2 shows the sensitivity reached with the LightCycler foodproof *Listeria* Genus Detection Kit using the two sample preparation kits. With the High Pure foodproof II Kit 10² cells/ml were detectable, whereas the ShortPrep foodproof II Kit allowed the detection of 10³ cells/ml.

Table 3: Inclusivity testing of all six *Listeria* species

Species	Number of strains	Positive results
<i>L. grayi</i>	7	7
<i>L. innocua</i>	9	9
<i>L. ivanovii</i>	10	10
<i>L. seeligeri</i>	10	10
<i>L. monocytogenes</i>	60	60
<i>L. welshimeri</i>	9	9
Total	105	105

Specificity

In Table 3 the results for the inclusivity testing are shown. All 105 isolates from the six *Listeria* species were detected, and no false-negative results were noted. The exclusivity testing of the 56 non-*Listeria* species gave no false-positive results. The LightCycler foodproof *Listeria* Genus

Detection Kit is specific for the genus *Listeria* and allows detection despite a high background of other organisms often causing problems in food microbiology.

Discussion

The results clearly show that the detection of 10² cells/ml in an enrichment culture using the High Pure foodproof II Kit and 10³ cells/ml using the ShortPrep foodproof II Kit can easily be carried out with food samples. The testing of different *Listeria* isolates and of closely related or socialized non-*Listeria* species emphasizes the superior specificity of this PCR method.

The LightCycler foodproof *Listeria* Genus Detection Kit, in combination with the ShortPrep foodproof II Kit, is an easy-to-use and very rapid detection method. It can also be used with the High Pure foodproof II Kit for more difficult matrices, offering the user a very sensitive, specific, and reliable method for the investigation of various food samples, such as raw materials and half-finished or finished goods. It is faster than using conventional methods, and its ease of application makes it versatile in all food laboratories. ■

References

- Centers for Disease Control and Prevention – Listeriosis (<http://www.cdc.gov>)
- Hitchins A (1998) Detection and Enumeration of *Listeria monocytogenes* In: Foods Bacteriological Analytical Manual, 8th edition, revision A, AOAC Intl., Gaithersburg, U.S.A.
- Sparling P (1998) Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Egg, and Environmental Samples. In: Dey BP and Lattuada CP (eds) Microbiology Laboratory Guidebook, 3rd edition, revision 3a, FSIS/USDA, Washington, U.S.A.
- International Organization for Standardization: EN ISO 11290-1 and -2: Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: detection method (ISO 11290-1: 1996), Part 2: enumeration (ISO 11290-2: 1998)

Product	Pack Size	Cat. No.
LightCycler foodproof <i>Listeria</i> Genus Detection Kit	1 kit (96 reactions)	03 535 614 001
ShortPrep foodproof II Kit	1 kit (100 isolations)	03 358 062 001
High Pure foodproof II Kit	1 kit (100 isolations)	03 358 054 001

