Listeria monocytogenes Identification in Food of Animal Origin Used with Real Time PCR

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Abstract

The aim of this study was to follow the contamination of food with *Listeria monocytogenes* by using Step One real time polymerase chain reaction (RT PCR). We used the PrepSEQ Rapid Spin Sample Preparation Kit for isolation of DNA and SensiFAST SYBR Hi-ROX Kit for the real-time PCR performance. In 20 samples of food of animal origin with incubation were detected strains of *Listeria monocytogenes* in 9 samples (swabs). Eleven samples were negative. Our results indicated that the real-time PCR assay developed in this study could sensitively detect *Listeria monocytogenes* in food of animal origin without incubation. This could prevent infection caused by *Listeria monocytogenes*, and also could benefit food manufacturing companies by extending their product's shelf-life as well as saving the cost of warehousing their food products while awaiting pathogen testing results. The rapid real-time PCR-based method performed very well compared to the conventional method. It is a fast, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future.

Keywords: Real-time PCR, *Listeria monocytogenes*, detection kit, ready-to-eat food

1. Introduction

Listeriosis is a serious food-borne infection and although the incidence is low, mortality rates are on average approaching 30% and exceeding those of Salmonella and *Clostridium botulinum* infections and intoxications [1]. Out of 6 species that compose genus Listeria only *Listeria monocytogenes* is responsible for human listeriosis, while *Listeria ivanovii* is infecting other mammals [2]. Conventional methods for the detection of *L. monocytogenes* in food involve selective culture enrichment with subsequent culturing on selective media, followed by serological and/or biochemical tests for species identification [3]. Application of real-time PCR surmounts these shortcomings by removing the manipulation of the polymerase chain reaction (PCR) products after amplification, thus reducing the risk of false-positive results. Furthermore, results with better sensitivity and specificity can be obtained in an hour [4 - 6].

Considerable reagent cost is one of the key factors, beside standardization and validation issues [7-9], that influence wider routine adoption of real-time PCR-based tests in diagnostic laboratories. It is also important to consider that minimally processed vegetables are often used as ingredients to pre-pare salads, which may have longer refrigerated shelf lives, representing a risk of increasing of *L. monocytogenes* population. Most cases of listeriosis are caused by the ingestion of *L. monocytogenes* - contaminated ready-to-eat (RTE) foods that do not require heating prior to consumption [2]. Diagnosis of listeriosis and detection of *L. monocytogenes* is commonly based on classical culturing and serological identification methods that are laborious and time-consuming, requiring up to ten days for completion and often suffer from variability due to their dependence on the

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phenotypic characteristics of the bacteria [10]. Different PCR-based diagnostics for detection of *L. monocytogenes* have been increasingly applied; however, complex sample preparation methods and especially the use of gel electrophoresis endpoint detection have hampered the transition from research to routine use in food microbiology laboratories [11]. Application of real-time PCR surmounts these shortcomings by removing the manipulation of the PCR products after amplification, thus reducing the risk of false-positive results. Nowadays, with the second generation of PCR methods, in which a quantification of the target microorganisms is also possible, new applications have become available. Several quantitative PCR (qPCR) protocols have been recently published, highlighting that this method can be advantageously used to detect and quantify *L. monocytogenes* in food [12]. In a high-throughput setting a reduction in the reagent volume used in each reaction significantly reduces the cost of diagnosis.
The aim of this study was to follow the contamination of food of animal origin with *Listeria monocytogenes* by Step One real-time PCR.

2. Materials and methods

Food samples

A total of 20 samples of foods of animal origin were used in this study. The samples (5 pcs of Ipeľ sausage, 4 pcs of Čingovská salami, 4 pcs of Inovecká salami, 2 pcs of Smoked cheese, 5 pcs of sour cream) were analyzed for the presence of *Listeria monocytogenes*. Samples were obtained by taking swabs from the food of animal origin.

PrepSEQ Rapid Spin Sample Preparation Kit

Sample of 750 µL was loaded onto the spin column and microcentrifuged for 3 minutes at maximum speed. Supernatant was discarded and 50 µL of Lysis Buffer was added to the pellet. Samples were incubated for 10 minutes at 95 °C.

Measuring the concentration of DNA-UV-spectrophotometric quantification of DNA

The absorbance at a wavelength of 260 nm was measured and DNA concentration was calculated on base of the observation that double stranded DNA solution with a concentration of 5 μg.mL^{-1} has a density about 0.1. To determine the contamination of the protein preparation the additional measuring absorbance at 280 nm was used. Preparation is considered as uncontaminated if proteins A260/A280 are between 1.8 and 2.0. Spectrophotometric measurements were done using UV 1101 photometer (Biotech, UK).
SensiFAST SYBR Hi-ROX Kit: Thermal cycling conditions were as follows: 3 minutes of incubation at 95 °C, followed by 40 cycles of 5 sec. denaturation at 95°C and 15 sec. annealing and elongation at 60 °C. Data were collected during each elongation step.

Reaction mix composition

Sensi Fast sybr Hi-ROX Mix (1x)	10 µL
Forward Primer (400nM)	0.8 µL
Reverse Primer (400nM)	0.8 µL
ddH_2O	9.4 µL
Template	4 µL

Primers used for detection of *Listeria monocytogenes*:

- Division I: Primer D1 5' CGA TAT TTT ATC TAC TTT GTC A 3' and D2 5' TTG CTC CAA AGC AGG GCA T 3'.

PCR products were detected by monitoring the increase in fluorescence of the reporter dye at each PCR cycle. Applied Biosystems software plots the normalized reporter signal, ΔRn, (reporter signal minus background) against the number of amplification cycles and also determines the threshold cycle (Ct) value i.e. the PCR cycle number at which fluorescence increases above a defined threshold level.

3. Results and discussion

The most sensitive detection of *Listeria monocytogenes* was obtained using PrepSEQ^{TM} Rapid Spin Sample Preparation Kit and SensiFAST SYBR Hi-ROX Kit. A detection kit compatible with StepOne^{TM} Systems was less time-consuming than the other methods and relatively easy to use. Thus, the PCR-based detection of bacteria depends on the efficiency of DNA extraction procedure used to prepare the

template DNA. In our samples without incubation we could detect strain of *Listeria monocytogenes* in 9 out of 20 samples (swabs), as it is shown in Figure 1.

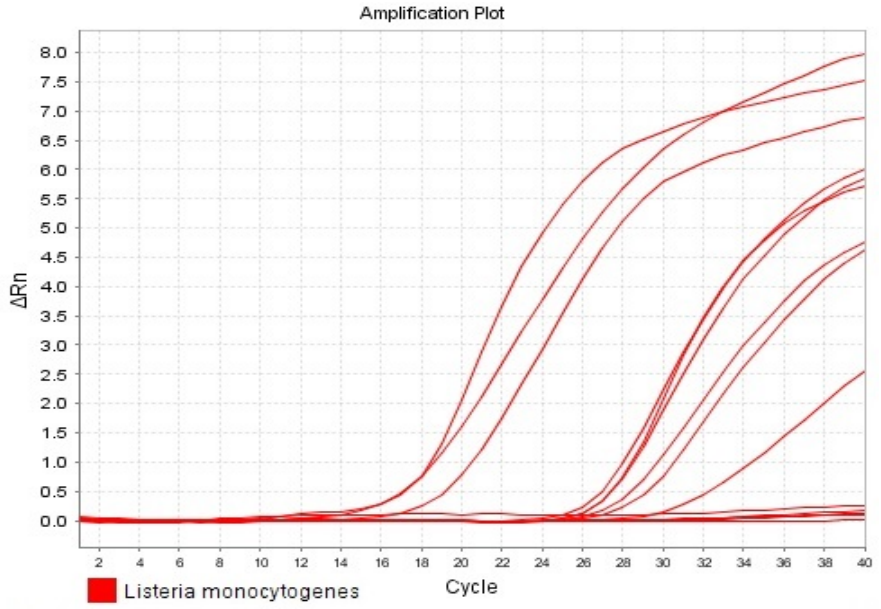

Figure 1. Contamination of food samples with *Listeria monocytogenes*

Eleven samples were negative. The (Ct) value of positive listeria samples was on average 23.32. The lowest value of positive listeria samples was found at 17.70 and the highest was at 30.04. Berrada et al. [13] performed a study on the presence or absence of *L. monocytogenes*, in 77 different types of salads served in restaurants in Valencia (Spain), by real-time PCR and conventional methods. Commercial kits based on real-time PCR and other molecular techniques are available for the identification of *L. monocytogenes* in foods including the BAX system, VIT technology and the LightCycler food proof *Listeria monocytogenes* detection kit [14]. Countries including the United States, Australia and New Zealand apply a zero-tolerance policy (absent in 25 g/ml of food) for *L. monocytogenes* in foods. All Association of Analytical Communities (AOAC) international approved *L. monocytogenes* detection tests, including culture, immunological and molecular-based tests, are required to detect 1CFU per 25 g food sample, therefore, all approved tests require culture enrichment [11, 15]. Many are based on the detection of virulence genes in *L. monocytogenes* and have been tested in a variety of foods. Some of the assays were quantitative and did not require enrichment but they were generally less sensitive than those including enrichment step [16]. Reported sensitivities range from 1000 CFU/ml [16] to 1–5 CFU per 25 ml/g [17]. Current conventional methods for *L. monocytogenes* detection and identification take between 5 and 10 days [13, 17]. Recently conventional and real-time PCR assays have been developed for the detection of *L. monocytogenes* in foods [17].

4. Conclusions

The decision to find "the most suitable" method for the detection and tracing of *Listeria monocytogenes* in food processing is difficult as information should not only relate to the presence of the pathogen, but also to useful information about transmission routes and types of strains correlating to raw-materials, mid-products, end-products and product environment. This will further contribute to better understanding the contamination and transmission routes of food-borne pathogens. The application of molecular methods can result in a better comprehension of the spread of a specific pathogen in a processing plant, thereby allowing the implementation of corrective actions to eliminate or decrease the risk

associated with its presence in the final product. Our results indicate that the Step One real-time PCR assay developed in this study could sensitively detect *Listeria monocytogenes* in ready-to-eat food. The rapid real-time PCR-based method performed very well compared to the conventional method. It is a fast, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future.

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