*Journal of Environmental Science and Health Part B* (2011) **46**, 697–702

ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601234.2011.594406

Taylor & Francis
Taylor & Francis Group

# The StepOne real-time polymerase chain reaction detection of *Salmonella* sp., *Salmonella enterica* ser. *typhimurium* and *enteritidis* in milk and meat

JAROSLAV POCHOP[1], MIROSLAVA KAČÁNIOVÁ[1], LUKÁŠ HLEBA[1], JADŽA LEJKOVÁ[1], MARTINA FIKSELOVÁ[2], SIMONA KUNOVÁ[2] and MACIEJ KLUZ[3]

[1]*Department of Microbiology, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*
[2]*Department of Food Hygiene and Safety, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*
[3]*Department of Biotechnology and Microbiology, University of Rzeszow, Rzeszow, Poland*

The aim of this study was to follow contamination of ready to eat milk and meat products with *Salmonella* spp. by using the StepOne real-time polymerase chain reaction (PCR). Classical microbiological methods for detection of foodborne bacteria involve the use of pre-enrichment and/or specific enrichment, following isolation of bacteria in solid media and the final confirmation by biochemical and/or serological tests. We used the PrepSEQ Rapid Spin Sample Preparation Kit for isolation of DNA and MicroSEQ® *Salmonella* spp. Detection Kit for pursuance of the real-time PCR (Applied Biosystems). In samples without incubation we detected strain of *Salmonella* sp. in 5 out of 25 samples (swabs), as well as in the internal positive control (IPC), which was positive in all samples. This StepOne real-time PCR assay is extremely useful for any laboratory equipped by real-time PCR. It is a fast, reproducible, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future. Our results indicated that real-time PCR assay developed in this study could sensitively detect *Salmonella* spp. in ready-to-eat food. This could prevent infection caused by *Salmonella*, 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.

**Keywords:** Real-time PCR; *Salmonella* spp.; detection kit.

## Introduction

Classical microbiological methods for detection of foodborne bacteria involve the use of pre-enrichment and/or specific enrichment, followed by the isolation of bacteria in solid media and a final confirmation by biochemical and/or serological tests.[1] Determining of bacterial viability is a key issue for the application of food risk management; thus a rational approach for detecting only viable bacterial cells by using molecular-based methods is necessary. However, the PCR does not distinguish among viable and dead bacterial cells. DNA from bacterial dead cells can serve as a template for the PCR many days after cell viability has been lost.[2–3] Raw (unpasteurized) milk is an important vehicle of transmission of pathogenic microorganisms such as *Salmonella* spp., and *Listeria monocytogenes*. Several outbreaks of foodborne illnesses following consumption of raw milk and milk-based products caused by Shiga toxin-producing *Salmonella* spp.,[4–5] have been reported in recent years, indicating the importance of this problem in safeguarding public health. In recent years, PCR-based methods have been reported as a rapid, specific and sensitive alternative, and have been increasingly used to identify several microbial species from food,[6–7] and clinical samples.[8–9] In addition, the multiplex PCR (mPCR) technique can conveniently detect multiple targets in the same amplification reaction.[10] Conventional multiplex PCR assays currently available for the detection of three target pathogens,[11] require agarose gel analysis, thus amplicon manipulations, leading to laboratory contamination and the consequent risk of false positive results. Several real-time PCR assays for single reaction are available for the detection of our target species.[12–15] The m-PCR allows the simultaneous amplification of more than one target sequence in a single PCR reaction, saving considerable time and effort, and decreasing the number of reactions to be performed in order to assess the possible presence of foodborne pathogens in a food sample.[10] Since its introduction,

Address correspondence to Miroslava Kačániová, Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra; E-mail: miroslava.kacaniova@gmail.com
Received September 17, 2010.

m-PCR has been successfully applied to many areas, including detection of foodborne pathogens.[16] However, some components of food and chemicals required for selective enrichment of cells may influence the effectiveness of the PCR and cause inhibitory effects.[17] Thus, the implementation of PCR-based assays as routine microbiology diagnostic tools, especially in testing laboratories with quality assurance programs, requires proper controls to verify the accuracy of the results obtained. An adequate strategy to assess the validity of the PCR results is the use of an internal amplification control (IAC), a non-target DNA sequence which is coamplified with the target sequence by the same set of primers.[18–19] Real-time PCR methods are often used for quantification of initial target DNA. Unfortunately, amplification efficiencies can be different from sample to sample due to the effects caused by inhibition of amplification, human failures or preparation errors. This implies that quantification, even with external controls, does not always represent a correct calculation of initial amount of target in each sample. To eliminate part of these drawbacks, different approaches of using an internal amplification control (IAC) in each real-time PCR have been described recently.[20–21] Especially real-time PCR methods such as 5′ nuclease Taqman PCR,[22–23] and PCR combined with molecular beacons,[16] have shown promising results due to the rapid, sensitive and specific detection of pathogens.[24] To detect *Salmonella* spp. and *E. coli* O157:H7, real-time PCR methods have been reported based on the amplification of species-specific genes, or genes related to pathogenicity or virulence. Recently, the detection of *Salmonella enterica* was accomplished by real-time Taqman PCR methods directed to the spaQ-gene,[25] the invA-gene,[20] and a molecular beacon-based method directed to the himA-gene.[20] To validate PCR results, both internal and external controls should be included with each assay to monitor assay performance. External controls monitor the use of instrumentation, the assay reagents, and ensure no DNA cross-contamination has occurred. External controls comprise the same reagents as used in the detection assay, but instead of a test sample, contain either known positive nucleic acid, that should always produce a PCR product, or no nucleic acid template, that should produce no PCR product. Internal controls (ICs) often involve the use of a separate PCR assay that is included in the same tube as well as the detection assay,[8,26] which amplifies a nucleic acid target sequence invariably present, such as an endogenous housekeeping gene, or an exogenous target sequence added with the PCR reagents. Examples of exogenous targets used for ICs are a fragment of the neomycin phosphotransferase gene or bacteriophage λ DNA.[27] The IC can be used to monitor the efficiency of each reaction,[28] providing assurance that amplification and detection are working effectively, with insufficient PCR inhibition to adversely affect the final result.[8,29] In a detection assay the IC should always give a positive PCR result and indicate that a negative test result is a true negative and not due to the inhibition of PCR. When introduced into an unprocessed food sample, an internal control can also serve to monitor the reliability of the complete procedure of nucleic acid extraction, amplification and PCR product detection.

The aim of this study was to detect *Salmonella* spp. by StepOne real-time PCR in ready-to-eat milk and meat products.

## Materials and methods

### *Food samples*

A total of 25 food samples were used in this study. Samples were obtained by taking of swabs from the inside of the food (Table 1). Observed milk and meat products were the aim of investigation. After sampling, procedure shown in Figure 1 was used.

### *Bacterial strains and DNA extraction*

As a pre-preparation step for the StepOne real-time PCR, DNA extraction was performed using DNA extraction method: PrepSEQ Rapid Spin Sample Preparation Kit (Applied Biosystems, USA).

### *General sample preparation protocol*

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

### *MicroSEQ® Salmonella spp. detection kit*

An assay to amplify the polymerase chain reaction (PCR), a unique microorganism-specific DNA target sequence and a TaqMan® probe to detect the amplified sequence were used. Strips of eight tubes each, containing assay beads compatible with StepOne™Systems were used. Samples of 30 $\mu$L to the lyophilized beads were loaded. MicroAmp® 48-Well Base and the MicroAmp® Cap Installing Tool were added to the tubes. A MicroAmp® Fast 48-Well Tray on the sample block of the StepOne System was used.

### *Real-time PCR*

TaqMan® probes labeled with both a fluorophore and a quencher dye were used in real-time PCR assays to detect amplification of specific DNA targets. FAM™, which has an emission of 520 nm, has become the most commonly used fluorophore for singleplex qPCR reactions. TAMRA™ will efficiently quench the fluorescence of FAM™, until the probe hybridizes to the target and is

**Table 1.** Source of ready-to-eat food samples used in this study.

| *Sample number* | *Source of food* | *Ct (threshhold cycle)* | | *Functional foods group* |
|---|---|---|---|---|
| | | *Salmonella spp.* | *IPC* | |
| 1 | Čingovská salami | Negative | 17.89 | Meat product |
| 2 | | Negative | 18.90 | |
| 3 | | 24.99 | 25.03 | |
| 4 | Inovecká salami | Negative | 22.47 | Meat product |
| 5 | | 16.99 | 17.87 | |
| 6 | | 16.49 | 22.49 | |
| 7 | Tourist salami | Negative | 19.06 | Meat product |
| 8 | | 17.62 | 18.91 | |
| 9 | | Negative | 22.51 | |
| 10 | | 17.99 | 19.04 | |
| 11 | Ipeľ sausage | Negative | 17.87 | Meat product |
| 12 | | Negative | 25.01 | |
| 13 | | Negative | 24.99 | |
| 14 | Sour cream | Negative | 22.49 | Milk product |
| 15 | | Negative | 22.53 | |
| 16 | | Negative | 18.89 | |
| 17 | Yogurt cream with vanilla Zvolen | Negative | 18.91 | Milk product |
| 18 | | Negative | 25.03 | |
| 19 | | Negative | 17.90 | |
| 20 | Strawberry yogurt cream with low fat | Negative | 22.49 | Milk product |
| 21 | | Negative | 18.88 | |
| 22 | | Negative | 22.46 | |
| 23 | Creamy yogurt quality Albert | Negative | 25.02 | Milk product |
| 24 | | Negative | 17.86 | |
| 25 | | Negative | 19.07 | |

cleaved by the 5′ exonuclease activity of the polymerase. We used three fluorophore detection chemistries that include FAM™ and VIC® dye-labeled TaqMan® MGB probe-based assays, VIC® and TAMRA™ dye-labeled probe-based assays and ROX™ as passive reference dye. Thermal cycling conditions were as follows: 2 minutes of incubation at 95°C, followed by 40 cycles of 1 sec. denaturation at 95°C and 20 sec. annealing and elongation at 60°C. Data were collected during each elongation step. PCR products were detected by monitoring of 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

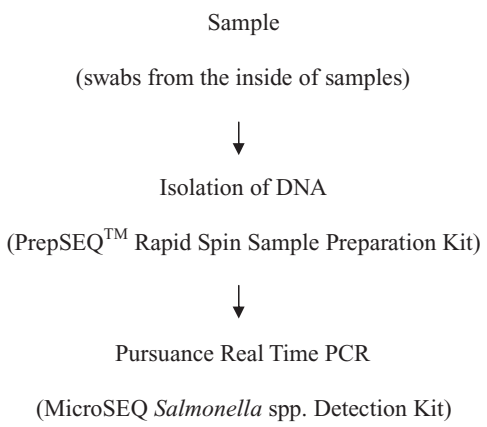


**Fig. 1.** Procedure used for processing the samples.

(Ct) value; i.e. the PCR cycle number at which fluorescence increases above a defined threshold level was used.

## Results and discussion

The most sensitive detection of *Salmonella* spp. was obtained using PrepSEQ™ Rapid Spin Sample Preparation Kit and MicroSEQ® Salmonella spp. A detection kitcompatible with StepOne™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 *Salmonella* spp. in 5 out of 25 samples (swabs), as it is shown in Figure 2, as well as in internal positive control (IPC), which was positive in all samples, as it is shown in Figure 3. The threshold value was 1.00 by salmonella samples and 0.05 by internal positive control (IPC). The (Ct) value of positive salmonella samples was on average 18.81 and IPC (Ct) value was on average 20.67, whereby the lowest value of positive salmonella samples was found at 16.49 and the highest value was at 24.99. The lowest IPC value was detected at 17.87 and the highest accomplish value was 25.02. Higgins et al.[24] noted that Ct values are generally a good indicator of the contamination level of the target organism, as well as the efficiency of the PCR assay. The Ct values observed for the Instagel protocol (27.2+1.4) are in agreement with the notion that it provided cleaner templates for PCR than the Bax-lysis buffer (30.5+1.1). The IPC was correctly amplified in PCR reactions containing colonies from both *Salmonella* spp. and non-*Salmonella* strains, being overall TET ΔRn values of 1.33 (0.35 S.D.) and 0.49 (0.12 S.D.), respectively. Moreover, non-template controls did not produce any FAM™ or TET-positive signal. The ΔRn results are in agreement with results of Hoorfar et al. [20] and indicate that the optimized PCR system is suitable to be used to specifically identify *Salmonella* spp. colonies. The multiplex real-time PCR

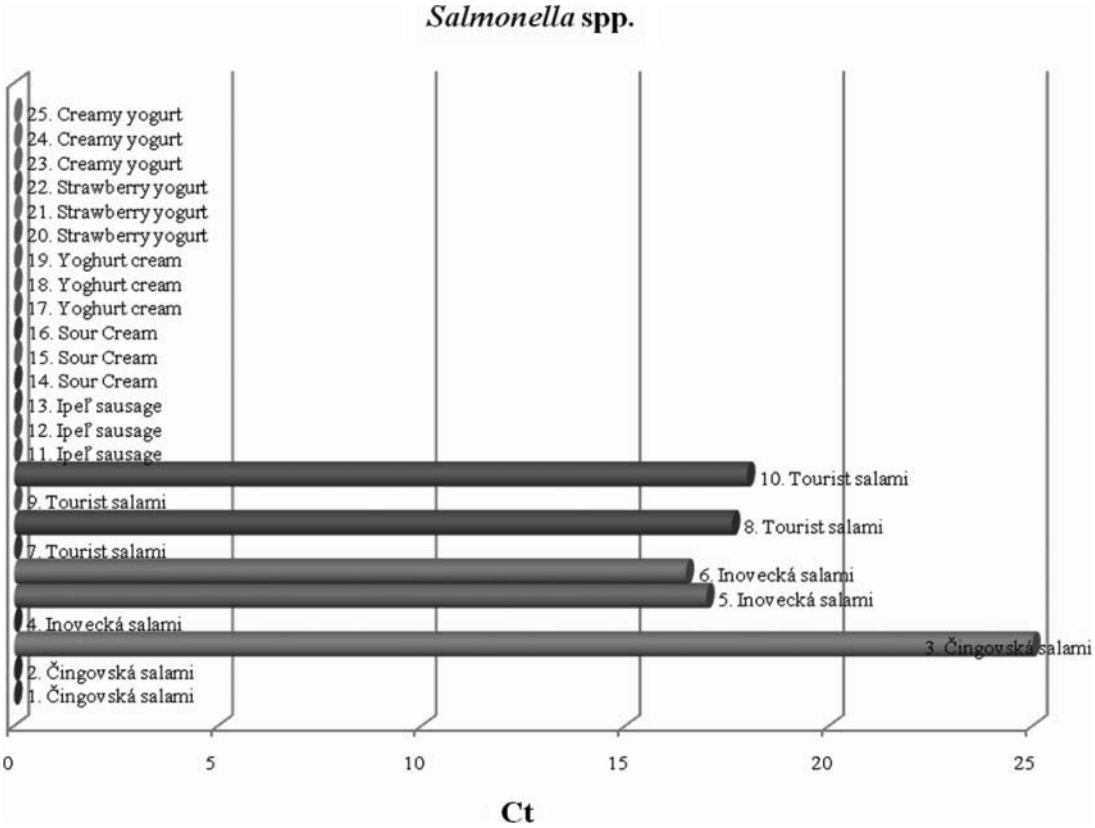


**Fig. 2.** Real-time PCR detection of *Salmonella* spp. (Ct- threshold cycle).

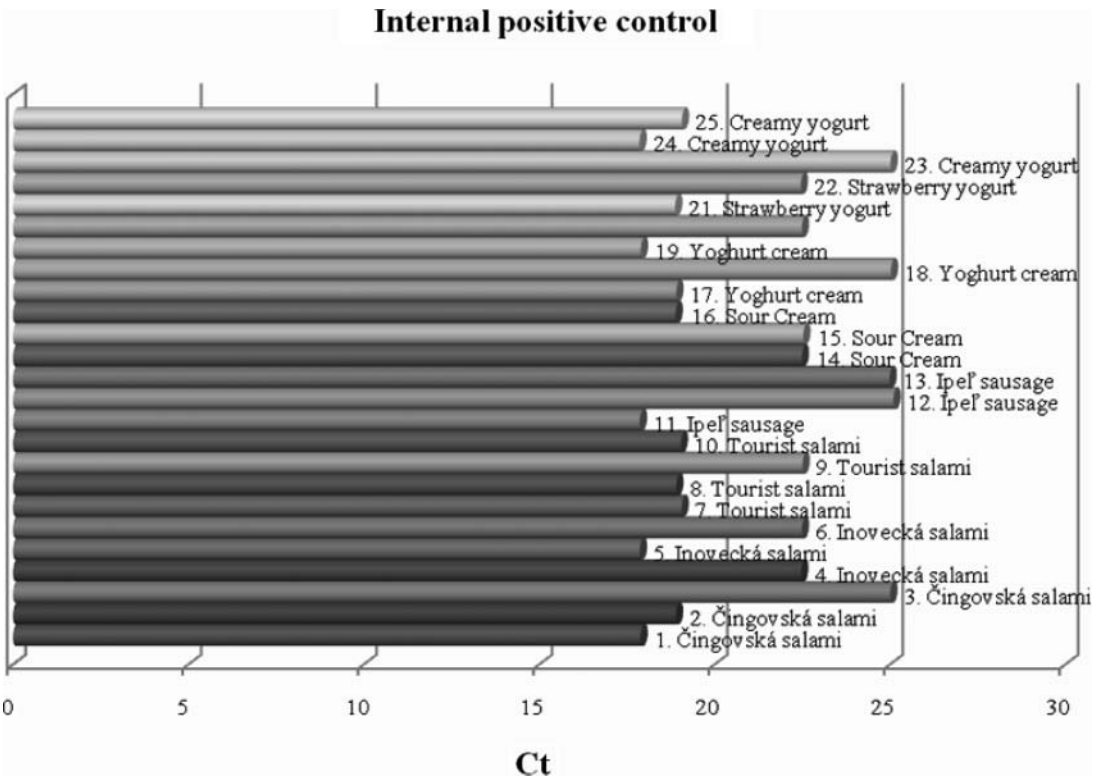


**Fig. 3.** Real-time PCR detection of internal positive control (Ct- threshold cycle).

developed in this study was the first technique to detect all *Salmonella* spp. possibly related with meats and to differentiate simultaneously *S.* Typhimurium from *S.* Enteritidis in meats. Previously, real-time PCR assays had been applied for *Salmonella* spp. and other food-borne pathogens.[15, 30–31] In the multiplex real-time PCR, *Salmonella* spp., *S.* Typhimurium and *S.* Enteritidis produced mean CT values of $13.00 \pm 0.94$, $18.29 \pm 2.23$ and $16.39 \pm 3.38$, respectively for the specificity of the multiplex real-time PCR. When $0.54 \pm 0.09$ log10 CFU/mL of *S.* Typhimurium and $0.65 \pm 0.07$ log10 CFU/mL of *S.* Enteritidis were spiked into meats, the multiplex real-time PCR assay could detect bacteria in the spiked beef but not in the spiked pork, both from a pre-enrichment condition. The multiplex real-time PCR assay detected bacteria that underwent a post-enrichment condition. Pre-enriched meats had CT values of 35.32 or more, while post-enriched meats had CT values of 14.41 to 22.23. When $1.45 \pm 0.21$ log10 CFU/mL of *S.* Typhimurium and $1.65 \pm 0.07$ log10 CFU/mL of *S.* Enteritidis were spiked into meats, the multiplex real-time PCR assay detected bacteria in all conditions (beef, pork, pre-enriched, and post-enriched). However, pre-enriched meats had CT values from 30.94 to 36.94, while post-enriched meats had CT values ranging from 14.52 to 22.06.[32] Coloured substances originally present in samples might interfere with the fluorescence signal detection of the PCR system. However, the procedure of re-growing the cultures in BHI and preparing cell lysates as DNA templates for BAX analysis can significantly dilute the effect of substances in sample matrices that might interfere with signal detection. Bennett et al.[33] reported that food constituents, such as organic and phenolic compounds, and fats might inhibit DNA amplification. In this study, *Salmonella* spp. could be detected by BAX-PCR from these particular food samples without difficulties. Our results were incongruent with the study by Bennett et al.[33] which demonstrated that the BAX system was optimized for the detection of meat and poultry products without any inhibition. In their study, *Salmonella* spp. could also be isolated from the BHI cultures of samples tested negatively by conventional culture method.

## Conclusion

The rapid real-time PCR-based method performed very well compared to the conventional method. It is a fast, reproducible, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future. Conventional PCR methods need amplification product separation by gel electrophoresis following by hybridization with a probe. These time-consuming protocols are replaced nowadays by more convenient and rapid real-time PCR assays. Our results indicate that the StepOne real-time PCR assay developed in this study could sensitively detect *Salmonella* spp. in ready-to-eat food. This could prevent many people from becoming infected with *Salmonella*. It could benefit food manufacturing companies by extending their product's shelf-life by several days and saving them the cost of warehousing their food products while awaiting pathogen testing results.

## Acknowledgments

This work was funded by Grant Agency VEGA 1/0372/09, APVV-VMSP-P-0111–09 and KEGA 430–014SPU-4/2010.

## References

[1] Reed, B.A.; Grivetti, L.E. Controlling on-farm inventories of bulk-tank raw milk: An opportunity to protect public health. J. Dairy Sci. **2000**, *83*, 2988–2991.

[2] Allmann, M.; Hofelein, C.; Koppel, E.; Luthy, J.; Meyer, R.; Niederhauser, C.; Wegmuller, B.; Candrian, U. Polymerase chain reaction (PCR) for detection of pathogenic microorganisms in bacteriological monitoring of dairy products. Res. Microbiol. **1995**, *146*, 85–97.

[3] Rodríguez-Lázaro, D.; Lombard, B.; Smith, H.; Rzezutka, A.; D'Agostino, M.; Helmuth, R.; Schroeter, A.; Malorny, B.; Miko, A.; Guerra, B.; Davison, J.; Kobilinsky, A.; Hernández, M.; Bertheau, Y.; Cook, N. Trends in analytical methodology in food safety and quality: monitoring microorganisms and genetically modified organisms. Trends Food Sci. Technol. **2007**, *18*, 306–319.

[4] Raggam, R.B.; Leitner, E.; Muhlbauer, G.; Berg, J.; Stocher, M.; Grisold, A.J.; Marth, E.; Kessler, H.H. Qualitative detection of *Legionella* species in bronchoalveolar lavages and induced sputa by automated DNA extraction and real-time polymerase chain reaction. Med. Microbiol. Immunol. **2002**, *191*, 119–125.

[5] Malorny, B.; Tassios, P.T.; Radstorm, P.; Cook, N.; Wagner, M.; Hoorfar, J. Standardization of diagnostic PCR for the detection of foodborne pathogens. Int. J. Food Microbiol. **2003**, *83*, 39–48.

[6] Mareček, J. The fungicides efficacy against *Fusarium graminearum, Fusarium culmorum* and *Microdochium nivale* in *in vitro* conditions. Agriculture **2005**, *51*(12), 656–663.

[7] Fikselová, M.; Šilhár, S.; Mareček, J.; Frančáková, H. Extraction of carrot (*Daucus carota* L.) carotenes under different conditions. Czech Journal of Food Sciences **2008**, *26*(4), 268–274.

[8] Hoorfar, J.; Cook, N. Critical aspects in standardization of PCR. In K. Sachse and J. Frey, Eds*., PCR Detection of Microbial Pathogens, Methods in Molecular Biology: Vol. 216*, Humana Press; Totowa, NJ, **2003**; 51–64.

[9] Malorny, B.; Paccassoni, E.; Fach, P.; Bunge, C.; Martin, A.; Helmuth, R. Diagnostic real-time PCR for detection of *Salmonella* in food. Appl. Environ. Microbiol. **2004**, *70*, 7046–7052.

[10] Elnifro, E.M.; Ashshi, A.M.; Cooper, R.J.; Klapper, P.E. Multiplex PCR: optimization and application in diagnostic virology. Clin. Microbiol. Rev. **2000**, *13*, 559–570.

[11] Aslam, M.; Hogan, J.; Smith, K.L. Development of a PCR-based assay to detect Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* in milk. Food Microbiol. **2003**, *20*, 345–350.

[12] Bohaychuk, V.M.; Gensler, G.E.; McFall, M.E.; King, R.K.; Render, D.G. A real-time PCR assay for the detection of *Salmonella* in a wide variety of food-animal matrices. J. Food Prot. **2007**, *70*(5), 1080–1087.

[13] Fujikawa, H.; Shimojima, Y. Estimation of viable *Salmonella* cell numbers in meat and meat product using real-time PCR. J. Food Hyg. Soc. Jpn. **2008**, *49*(4), 261–265.

[14] Higgins, J.A.; Jenkins, M.C.; Shelton, D.R.; Fayer, R.; Karns, J.S. Rapid extraction of DNA from *Escherichia coli* and *Cryptosporidium parvum* for use in PCR. Appl. Environ. Microbiol. *67*, 5321–5324.

[15] Rosenstraus, M.; Wang, Z.; Chang, S.Y.; DeBonville, D.; Spadoro, J.P. An internal control for routine diagnostic PCR: design, properties, and effect on clinical performance. Journal of Clinical Microbiology **1998**, *36*, 191–197.

[16] Tyagi, S.; Marras, S.A.E.; Vet, J.A.M.; Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. **1996**, *14*, 303–308.

[17] Kurowski, P.B.; Traub-Dargatz, J.L.; Morley, P.S.; Gentry-Weeks, C.R. Detection of *Salmonella* spp. in fecal specimens by use of real-time polymerase chain reaction assay. AJVR **2002**, *63*, 1265–1268.

[18] Abdulmawjood, A.; Roth, S.; Bulte, M. Two methods for construction of internal amplification controls for the detection of *E. coli* O157 by polymerase chain reaction. Mol. Cell. Probes. **2002**, *16*, 335–339.

[19] Holland, P.M.; Abramson, R.D.; Watson, R.; Gelfand, D.H. Detection of specific polymerase chain reaction product by utilizing the 5′ 3′ exonuclease activity of Thermus aquaticus DNA polymerase. Proc. Natl. Acad. Sci. U. S. A. **1991**, *88*, 7276–7280.

[20] Hoorfar, J.; Wolffs, P.; Rådström, P. Diagnostic PCR: validation and sample preparation are two sides of the same coin. Acta Pathologica, Microbiologica et Immunologica Scandinavica *2004*, *112*, 808–814.

[21] Mazurek, J.; Salehi, E.; Propes, D.; Holt, J.; Bannerman, T.; Nicholson, L.M.; Bundesen, M.; Duffy, R.; Moolenaar, R.L. A multistate outbreak of *Salmonella enterica* serotype *typhimurium* infection linked to raw milk consumption-Ohio. J. Food Prot. **2004**, *67*, 2165–2170.

[22] Holicka, J.; Guy, R.A.; Kapoor, A.; Shepherd, D.; Horgen, P.A. A rapid (one day), sensitive real-time polymerase chain reaction assay for detecting *Escherichia coli* O157:H7 in ground beef. Can. J. Microbiol. **2006**, *52*(10), 992–998.

[23] Sails, A.D.; Fox, A.J.; Bolton, F.J.; Wareing, D.R.; Greenway, D.L. A real-time PCR assay for the detection of *Campylobacter jejuni* in foods after enrichment culture. Appl. Environ. Microbiol. **2003**, *69*, 1383–1390.

[24] Higgins, J.A.; Ezzel, J.; Hinnebusch, B.J.; Shipley, M.; Henchal, E.A.; Ibrahim, S.S. 5′ nuclease PCR assay to detect *Yersinia pestis*. J. Clin. Microbiol. **1998**, *36*, 2284–2288.

[25] Hoorfar, J.; Ahrens, P.; Rådström, P. Automated 5′ nuclease PCR assay for identification of *Salmonella enterica*. J. Clin. Microbiol. **2000**, *38*, 3429–3435.

[26] Hoorfar, J.; Cook, N.; Malorny, B.; Wagner, M.; De Medici, D.; Abdulmawjood, A.; Fach, P. Diagnostic PCR: making internal amplification control mandatory. Journal of Clinical Microbiology **2004**, *96*, 221–222.

[27] Espy, M.J.; Uhl, J.R.; Sloan, L.M.; Buckwalter, S.P.; Jones, M.F.; Vetter, E.A.; Yao, J.D.; Wengenack, N.L.; Rosenblatt, J.E.; Cockerill, F.R.; Smith, T.F. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clinical Microbiology Reviews **2006**, *19*, 165–256.

[28] Lambertz, S.T.; Ballagi-Pordany, A.; Lindqvist, R. A mimic as internal standard to monitor PCR analysis of food-borne pathogens. Letters in Applied Microbiology **1998**, *26*, 9–11.

[29] Rodríguez-Lázaro, D.; Hernández, M.; D'Agostino, M.; Cook, N. Application of nucleic acid sequence based amplification (NASBA) for the detection of viable foodborne pathogens: progress and challenges. J. Rapid Methods Autom. Microbiol. **2006**, *14*, 218–236.

[30] Lee, S. H.; Byeong, Y. J.; Nabin, R. A multiplex real-time PCR for differential detection and quantification of *Salmonella* spp., *Salmonella enterica* serovar Typhimurium and Enteritidis in meats. J. Vet. Sci. **2009**, *10*(1), 43–51.

[31] Rossmanith, P.; Krassnig, M.; Wagner, M.; Hein, I. Detection of *Listeria monocytogenes* in food using a combined enrichment/real-time PCR method targeting the prfA gene. Res. Microbiol. **2006**, *157*(8), 763–771.

[32] Lantz, P.G.; Al-Soud, W.A.; Knutsson, R.; Hahn-Hagerdal, B.; Radstrom, P. Biotechnical use of polymerase chain reaction for microbiological analysis of biological samples. Biotechnol. Annu. Rev. **2000**, *5*, 87–130.

[33] Bennett, A.R.; Greenwood, D.; Tennant, C.; Banks, J.G.; Betts, R.P. Rapid and definitive detection of *Salmonella* in foods by PCR. Lett. Appl. Microbiol. **1998**, *26*, 437–441.