Analytical Biochemistry 545 (2018) 38–42

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Rapid detection of foodborne pathogen *Listeria monocytogenes* by strand exchange amplification

Meiling Zhang[a,1], Xiudan Wang[b,1], Lingzhi Han[a], Shuyan Niu[a], Chao Shi[c], Cuiping Ma[b,*]

[a] Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, PR China
[b] College of Marine Science and Biological Engineering, Qingdao University of Science and Technology, Qingdao 266042, PR China
[c] College of Life Sciences, Qingdao University, Qingdao 266071, PR China

ARTICLE INFO

Keywords:
Strand exchange amplification
Listeria monocytogenes
Isothermal nucleic acid amplification
RNA
One step

ABSTRACT

A strand exchange amplification (SEA) method to detect foodborne pathogen *Listeria monocytogenes* was developed. SEA is a novel nucleic acid amplification method that only requires one pair of primers. The specie-specific primers were designed by targeting the 16S rRNA gene and the amplification reaction was performed as short as 60 min at 61 °C. Notably, SEA method could not only detect genomic DNA but also detect RNA by one step without requiring extra reverse transcription. The result could be visualized by naked eyes so that water bath pot would be the only equipment needed. Moreover, culture fluids and bacteria colony could be successfully detected without any pretreatment and the method displayed good specificity and strong anti-jamming capacity. These features greatly simplified the operating procedure and made SEA method be potential for developing point-of-care testing (POCT) devices to detect viable *L. monocytogenes*.

Introduction

The gram-positive bacterium *Listeria monocytogenes* is a foodborne pathogen of global concern, which poses significant health problem to both human and domestic animals [1,2]. Despite the great efforts to decrease its incidence, *L. monocytogenes* is still an important cause of listeriosis [2,3]. Recently, an investigation of the World Health Organization (WHO) demonstrated that *L. monocytogenes* could still cause large numbers of illnesses and even deaths [4]. The mortality rate of *L. monocytogenes* is quite high compared to other foodborne pathogens, which is about 20–40% [2,5]. *L. monocytogenes* is ubiquitous in nature and easily contaminates vegetables, fruits, dairy products, meat and seafood [6,7], which significantly increases the risk of food poison [8]. Therefore, developing a rapid, specific and simple detection method of *L. monocytogenes* is of significant importance to food safety and human health.

The conventional detection method of *L. monocytogenes* is achieved by culture-based technique, which is time-consuming (about 4–7 days) [9]. In recent years, nucleic acid-based methods such as polymerase chain reaction (PCR) and immunoassay such as enzyme linked immunosorbent assay (ELISA) have been widely developed [10–12]. These approaches, however, usually require expensive equipment and are not available for point-of-care testing (POCT). Isothermal amplification provides a powerful method for nucleic acid amplification without the PCR thermocycling process and can realize detection of targets at a constant temperature [13,14]. Isothermal methods, such as nucleic acid sequence-based amplification (NASBA), and loop-mediated isothermal amplification (LAMP), have been developed to detect *L. monocytogenes* [15–17]. These methods could detect both DNA and RNA, but they need reverse transcription process when detecting RNA [18]. Here, a novel strand exchange amplification method with a simple reaction system was established by one-step detection of RNA which is more suitable for viability assays of *L. monocytogenes*.

Materials and methods

Materials and reagents

The SEA detection kit was purchased from Qingdao Navid Biotechnology Co., Ltd. (China). Ethidium bromide (EB), 20 bp DNA Marker, 6 × DNA Loading Buffer and SDS were provided by Sangon Biotech (Shanghai, China). Acrylamide and methylene diacrylamide were purchased from Sigma-Aldrich (St Louis, MO, USA). The bacterial strains including *L. monocytogenes, Staphylococcus aureus, Salmonella*

* Corresponding author.
E-mail address: mcp169@163.com (C. Ma).
[1] These authors contribute equally to this work.

https://doi.org/10.1016/j.ab.2018.01.013
Received 11 December 2017; Received in revised form 15 January 2018; Accepted 16 January 2018

Table 1
Sequences of nucleic acids used in this work.

Name	Sequence (5′-3′)
Listeria monocytogenes ([a] M58822.1 [b] 641–683)	GTCATTGGAAACTGGAAGACTGGAGTGCAGAAGAGGAGAGTGG
P1	GTCATTGGAAACTGGAAGACTG
P2	CCACTCTCCTCTTCTGCAC

The underlined portion was the same with primer P1. The dotted line sequence of target was complementary to the sequence of primer P2.
[a] GenBank accession number.
[b] The position of specific sequence in genomic DNA.

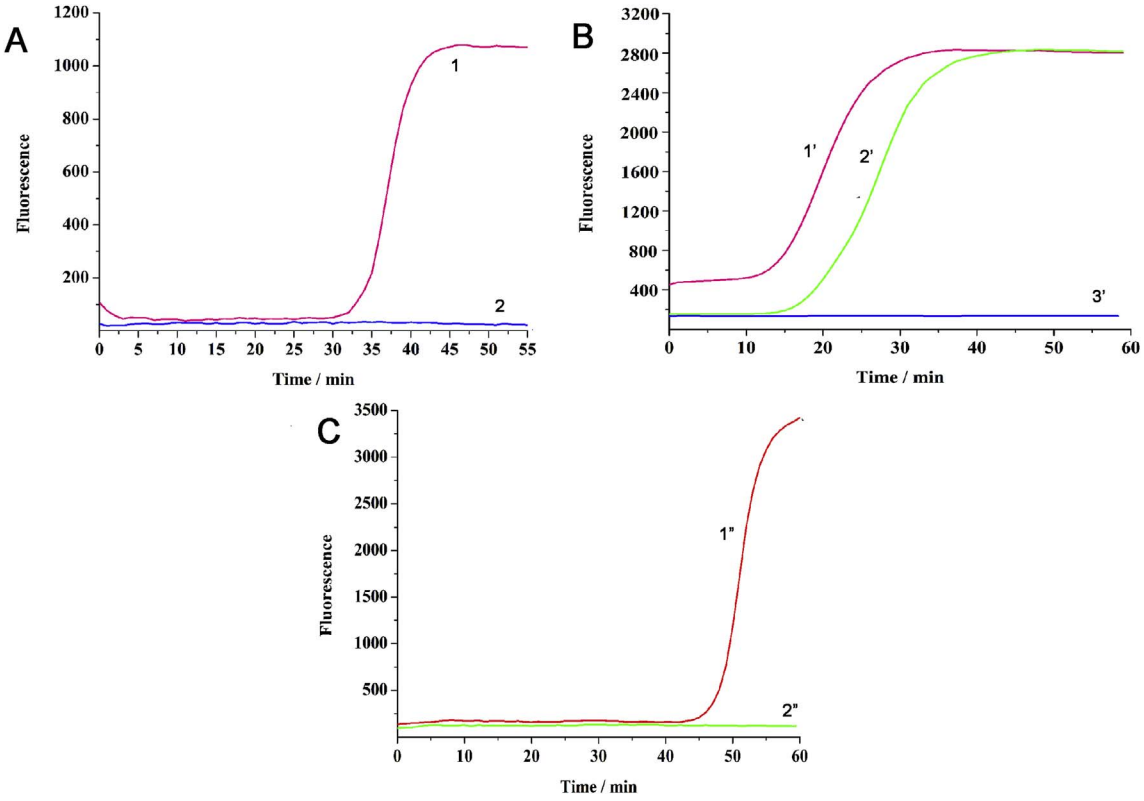

Fig. 1. The feasibility of SEA to detect *L. monocytogenes*. A represented that the targets were (1) 1.0×10^{-12} M genomic DNA and (2) no template control (NTC); B represented that the targets were culture fluids of *L. monocytogenes* diluted (1′) 100-fold and (2′) 1000-fold and (3′) NTC; C represented that the targets were (1″) bacteria colony of *L. monocytogenes* and (2″) NTC.

typhimurium, Vibrio parahemolyticus, Shigella castellani and *Escherichia coli* were saved by the laboratory.

Amplification reaction

The target was the hypervariable region from *L. monocytogenes* 16S rDNA. A pair of specific primers were designed by NUPACK software (http://www.nupack.org/) and synthesized by Sangon Biotech (Shanghai, China) and purified by HPLC (Table 1). The reaction of SEA was performed according to the manufacture's instruction. The fluorescence signal of SEA reaction was detected by CFX Connect™ Real-Time PCR System (Bio-Rad, CA, USA) at 1-min intervals. Gel images were recorded with ChampGel5000 system (Saizhi Innovation Technology Co., Ltd, Beijing, China). The reaction temperature was optimized at 55 °C, 57 °C, 59 °C, 61 °C, and 63 °C. The bacteria fluid and colony were then directly detected at the optimized temperature. The bacteria fluid cultured overnight was directly diluted 10-fold and 100-fold in water without RNase and DNase and 1 μL diluted sample was directly added to the SEA reaction as target. The bacterial colony was directly detected by adding a small amount of a colony into a PCR tube to trigger SEA reaction.

L. monocytogenes genomic DNA and total RNA extraction

The genomic DNA of *L. monocytogenes* was extracted using the bacterium genomic DNA extraction kits from Tiangen Biotech (Beijing, China) and the total RNA of *L. monocytogenes* was extracted with *TransZol* Up Plus RNA Kit from TransGen Biotech (Beijing, China) according to the manufacturers' instructions.

Results and discussion

The design of SEA to detect L. monocytogenes

The SEA is a novel isothermal nucleic acid amplification method which depends on the single-stranded denaturation bubbles of dsDNA at the reaction temperature. In this method, one pair of specific primers bind to the targets by invading to the bubbles, allowing DNA polymerase to extend [19]. The reaction requires a simple reaction system including a pair of primers and *Bst* DNA polymerase and can be

performed at a single temperature. Based on the innate reverse transcriptase activity of *Bst* DNA polymerase within 125-nt length [20], the specific targets of SEA can be DNA or RNA and it would be easy to find a short and unique sequence for infectious organisms with high mutation rate. So a pair of specific primers (P1 and P2) targeting the hypervariable V4 region of 16S rDNA were designed with amplification fragments of 43 bp (Table 1). According to Tm values of the primers, the reaction temperature was optimized using the SEA detection kit with *L. monocytogenes* genomic DNA as targets and 61 °C was chosen as the optimum reaction temperature (Fig. S1).

The feasibility of SEA detection

To demonstrate the feasibility of SEA detection method, genomic DNA of *L. monocytogenes* were detected. As shown in Fig. 1A, the fluorescence signal significantly increased with genomic DNA as targets compared with the NTC, indicating that SEA could effectively detect genomic DNA. The SEA detection was also directly performed for culture fluids and bacteria colony of *L. monocytogenes* without requiring additional DNA or RNA extraction. As shown in Fig. 1B and C, both culture fluid and bacteria colony of *L. monocytogenes* could successfully triggered the amplification process. Collectively, these results indicated SEA could realize real-time fluorescence detection of *L. monocytogenes* with genomic DNA, culture fluids and bacteria colony as targets.

Sensitivity of SEA to detect genomic DNA

The sensitivity of SEA detection method for *L. monocytogenes* was evaluated with genomic DNA of different dilutions as targets. As shown in Fig. 2A, the fluorescence signal showed good regularity with the increase of genomic DNA. The fluorescence signals significantly increased with targets DNA ranging from 1.0×10^{-13} to 1.0×10^{-10} M compared to that when the target was 1.0×10^{-14} M genomic DNA. The relationship between the threshold time value (T_t) and the concentration of genomic DNA (C_{LM}) was investigated in Fig. 2B. The T_t value increased linearly with the increasing negative logarithm (lg) of target concentrations in the range from 1.0×10^{-13} to 1.0×10^{-10} M. The correlation equation was found to be $T_t = -65.856 + 8.289(-\lg C_{LM})$, and the corresponding correlation coefficient (R^2) was 0.9984. The real-time fluorescence products were visualized by PAGE electrophoresis (Fig. 2C). There were 43-bp amplification products in lanes a–d as expected and the amounts of products were correlated with concentration of DNA targets, which further demonstrated that SEA could be well triggered by genomic DNA of *L. monocytogenes*. Previously, SEA could detect the lowest DNA concentration of 1.0×10^{-11} M [19]. Herein, with the optimization of the reaction temperature and the reasonable design of the primers, the SEA detection kit could detect as low as 1.0×10^{-13} M genomic DNA. So the sensitivity of SEA method was enhanced by 100-fold, which will greatly facilitate its practical application.

RNA detection by one step

RNA molecules are also important targets in clinical science and they have been considered more suitable than DNA for viability assays of *L. monocytogenes* [16]. According to our previous studies, *Bst* DNA polymerase possesses high innate reverse transcriptase activity within 125-nt length and SEA could realize one-step RNA detection [19,20]. So

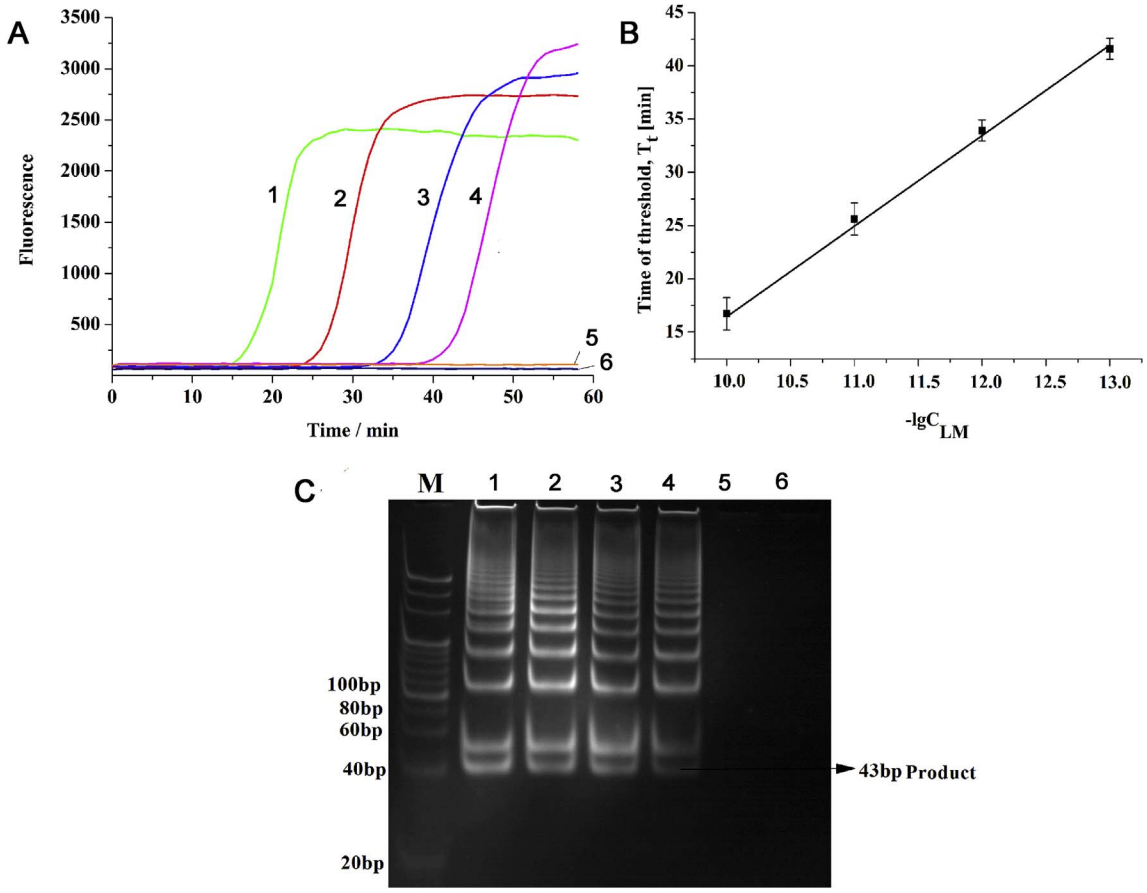

Fig. 2. (A) The real-time fluorescence curves for different concentrations of *L. monocytogenes* genomic DNA. 1–5 respectively represented from 1.0×10^{-10} to 1.0×10^{-14} M with 10-fold diluted; 6 represented NTC. (B) Relationship between the T_t values and the negative logarithmic values of the amount of genomic DNA targets (C_{LM}). Error bars showed mean standard deviations of three determinations. (C) The corresponding products of SEA reaction were visualized using PAGE electrophoresis.

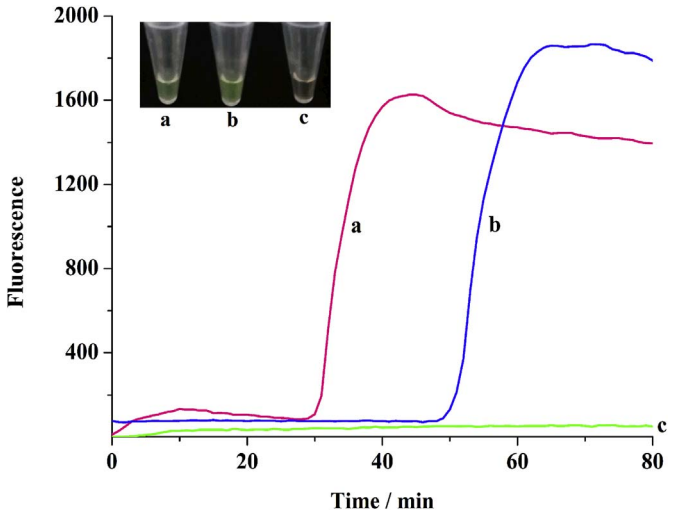

Fig. 3. Detection of different concentrations of *L. monocytogenes* total RNA by SEA method. Inset represented the corresponding colorimetric result for the amplification. **a-c** respectively represented the RNA concentration were 10 ng/μL, 1 ng/μL and 0 ng/μL.

the total RNA of *L. monocytogenes* was extracted (Fig. S2) and one-step detection of RNA was performed. As shown in Fig. 3, the fluorescence curves also significantly increased in the presence of the RNA targets. The result indicated that the SEA reaction could be directly triggered by the total RNA of *L. monocytogenes* without extra reverse transcription step. Besides, by the addition of the colorimetric reagent, the positive result turned green after amplification while the NTC remained the original orange color with no amplicons (Fig. 3 inset), making it possible to judge the results by naked eyes.

Specificity and anti-jamming capacity of SEA detection

The specificity of SEA detection was investigated to detect the culture fluids of *L. monocytogenes* and five other common foodborne bacteria. As shown in Fig. 4A, the fluorescence signal increased significantly for *L. monocytogenes,* while no change of fluorescence signal was observed for *S. aureus, S. typhimurium, V. parahemolyticus, S. castellani* and *E. coli.* Additionally, the anti-jamming capacity of SEA method was investigated in the mixture of the culture fluids of *L. monocytogenes* with that of five other foodborne bacteria (Fig. 4B). There was no shift in the fluorescence curves of pure *L. monocytogenes* and bacteria mixture, thus indicating the strong anti-jamming capacity of SEA in a complex matrix. Therefore, the specificity and anti-jamming capacity results further demonstrated the feasibility and reliability of SEA method to detect *L. monocytogenes*, making it advantageous in practical sample detection.

Traditional DNA based amplification methods might result in false-positive data due to the detection of unviable pathogens. Recently, viable detection of foodborne pathogens such as *L. monocytogenes* based on RNA amplification have been developed (Table 2). Reverse-transcription PCR (RT-PCR) and isothermal amplification methods including RT-LAMP and NASBA could detect viable foodborne pathogen with high specificity and sensitivity, but these methods require extra reverse transcription process or reverse transcription enzyme [15,16,18,21–24]. Particularly, RT-LAMP assay is extremely sensitive and can detect several copies of pathogens [18,24]. Just because of the high sensitivity of LAMP, carryover contamination and false positive result might be easily caused [25]. In the present study, SEA could realize one-step RNA detection based on the innate reverse transcriptase activity of *Bst* DNA polymerase within 125-nt length [19,20]. Although the sensitivity of SEA did not reach that of these methods, it has a much simpler reaction system with only one pair of primers and one enzyme. Considering the simple system, constant reaction temperature, visual detection of result and one step detection of RNA, SEA has promising potential for screening various numbers of foodborne and clinical pathogens in field or instrument-free conditions.

Conclusions

In this work, a strand exchange amplification method to detect foodborne pathogen *L. monocytogenes* was established. The SEA method could detect as low as 1.0×10^{-13} M genomic DNA, and the sensitivity was enhanced by 100-fold compared with previous study that will greatly prompt its generalization and application. Compared with traditional PCR method, our method required no extra reverse transcription process, making it more suitable for viable *L. monocytogenes* detection. Moreover, this method could be visualized by naked eyes so that the water bath pot would be the only equipment needed. Importantly, culture fluids and bacteria colony could be successfully detected without any pretreatment and the method displayed good specificity and strong anti-jamming capacity. With one-step, isothermal, and visualization, this method greatly simplifies the operating procedure and is a very useful amplification platform for developing POCT and lab-on-a-chip devices to detect *L. monocytogenes*, even other foodborne and clinical pathogens.

Acknowledgements

This study was financially supported by grants from the National Natural Science Foundation of China (31670868, 21675094) and the Shandong Province Natural Science Fund Major Basic Research Project (ZR2017ZC0123).

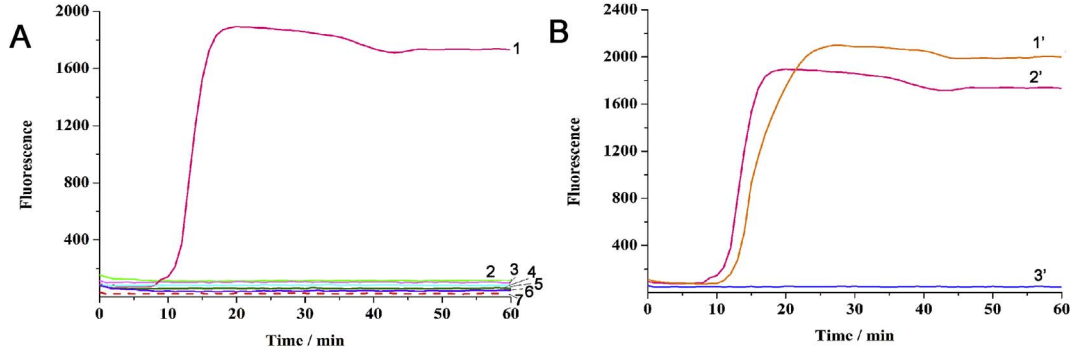

Fig. 4. (A) Specificity of the SEA method. 1–6 respectively represented that the targets were culture fluids of *L. monocytogenes, S. aureus*, *S. typhimurium*, *V. parahemolyticus*, *S. castellani* and *E. coli* diluted 1000-fold; 7 represented the NTC. (B) Anti-jamming capacity of the SEA method. 1′ represented that the targets were the culture fluids of *L. monocytogenes* diluted 1000-fold; 2′ represented that the targets were the mixture of the culture fluids of *L. monocytogenes, S. aureus*, *S. typhimurium*, *V. parahemolyticus*, *S. castellani* and *E. coli* diluted 1000-fold with the ratio of 1:1:1:1:1:1; 3′ represented the NTC.

Table 2
Comparison of different amplification methods.

Methods	Reaction time	Target	Number of primers	Number of enzymes	Extra reverse transcription enzyme[a]	Detection temperature	Detection limit	Reference
RT-PCR	130 min	RNA/DNA	2	2	+	94°C-60°C-72 °C	10-15 CFU/mL	[21,23]
RT-LAMP	95 min	RNA/DNA	6	2	+	63 °C	90 pg/μL	[18,24]
NASBA	95 min	RNA	2	3	+	42 °C	400 CFU/mL	[15,16]
SEA	60 min	RNA/DNA	2	1	–	61 °C	10^{-13} M	This work

[a] '+' Extra reverse transcription enzyme was added in the reaction system; '-' Extra reverse transcription enzyme was not added in the reaction system.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ab.2018.01.013.

References

[1] K.J. Allen, E. Wałecka-Zacharska, J.C. Chen, K.P. Katarzyna, F. Devlieghere, E. Van Meervenne, J. Osek, K. Wieczorek, J. Bania, *Listeria monocytogenes* – an examination of food chain factors potentially contributing to antimicrobial resistance, Food Microbiol. 54 (2016) 178–189.

[2] S. Alhogail, G.A.R.Y. Suaifan, M. Zourob, Rapid colorimetric sensing platform for the detection of *Listeria monocytogenes* foodborne pathogen, Biosens. Bioelectron. 86 (2016) 1061–1066.

[3] R.W. Armstrong, P.C. Fung, Brainstem encephalitis (rhombencephalitis) due to *Listeria monocytogenes*: case report and review, Clin. Infect. Dis. 16 (1993) 689–702.

[4] M.D. Kirk, S.M. Pires, R.E. Black, M. Caipo, J.A. Crump, B. Devleesschauwer, D. Döpfer, A. Fazil, C.L. Fischer-Walker, T. Hald, A.J. Hall, K.H. Keddy, R.J. Lake, C.F. Lanata, P.R. Torgerson, A.H. Havelaar, F.J. Angulo, World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis, PLoS Med. 12 (2015) e1001921.

[5] J.M. Farber, P.I. Peterkin, Listeria monocytogenes, a food-borne pathogen, Microbiol. Rev. 55 (1991) 476–511.

[6] T. Møretrø, S. Langsrud, *Listeria monocytogenes*: biofilm formation and persistence in food-processing environments, Biofilms 1 (2004) 107–121.

[7] L.V. Jørgensen, H.H. Huss, Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood, Int. J. Food Microbiol. 42 (1998) 127–131.

[8] L.A. Becker, S.N. Evans, R.W. Hutkins, A.K. Benson, Role of ς(B) in adaptation of *Listeria monocytogenes* to growth at low temperature, J. Bacteriol. 182 (2000) 7083–7087.

[9] V. Jasson, L. Jacxsens, P. Luning, A. Rajkovic, M. Uyttendaele, Alternative microbial methods: an overview and selection criteria, Food Microbiol. 27 (2010) 710–730.

[10] M. Magliulo, P. Simoni, M. Guardigli, E. Michelini, M. Luciani, R. Lelli, A. Roda, A rapid multiplexed chemiluminescent immunoassay for the detection of *Escherichia coli O157:H7, Yersinia enterocolitica, Salmonella typhimurium, and Listeria monocytogenes* pathogen bacteria, J. Agric. Food Chem. 55 (2007) 4933–4939.

[11] L. Somer, Y. Kashi, A PCR method based on 16S rRNA sequence for simultaneous detection of the genus *Listeria* and the species *Listeria monocytogenes* in food products, J. Food Protect. 66 (2003) 1658–1665.

[12] P. Scheu, A. Gasch, K. Berghof, Rapid detection of *Listeria monocytogenes* by PCR-ELISA, Lett. Appl. Microbiol. 29 (1999) 416–420.

[13] C. Ma, Y. Wang, P. Zhang, C. Shi, Accelerated isothermal nucleic acid amplification in betaine-free reaction, Anal. Biochem. 530 (2017) 1–4.

[14] C. Shi, M. Zhou, M. Pan, G. Zhong, C. Ma, Isothermal amplification detection of nucleic acids by a double-nicked beacon, Anal. Biochem. 496 (2016) 9–13.

[15] N. Cook, The use of NASBA for the detection of microbial pathogens in food and environmental samples, J. Microbiol. Meth. 53 (2003) 165–174.

[16] A. Nadal, A. Coll, N. Cook, M. Pla, A molecular beacon-based real time NASBA assay for detection of *Listeria monocytogenes* in food products: role of target mRNA secondary structure on NASBA design, J. Microbiol. Meth. 68 (2007) 623–632.

[17] M. Tang, S. Zhou, X. Zhang, J. Pu, Q. Ge, X. Tang, Y. Gao, Rapid and sensitive detection of *Listeria monocytogenes* by loop-mediated isothermal amplification, Curr. Microbiol. 63 (2011) 511–516.

[18] N. Nagatani, K. Yamanaka, M. Saito, R. Koketsu, T. Sasaki, K. Ikuta, T. Miyahara, E. Tamiya, Semi-real time electrochemical monitoring for influenza virus RNA by reverse transcription loop-mediated isothermal amplification using a USB powered portable potentiostat, Analyst 136 (2011) 5143–5150.

[19] C. Shi, F. Shang, M. Zhou, P. Zhang, Y. Wang, C. Ma, Triggered isothermal PCR by denaturation bubble-mediated strand exchange amplification, Chem. Commun. 52 (2016) 11551–11554.

[20] C. Shi, X. Shen, S. Niu, C. Ma, Innate reverse transcriptase activity of DNA polymerase for isothermal RNA direct detection, J. Am. Chem. Soc. 137 (2015) 13804–13806.

[21] P.G. Klein, V.K. Juneja, Sensitive detection of viable *Listeria monocytogenes* by reverse transcription-PCR, Appl. Environ. Microb. 63 (1997) 4441–4448.

[22] C. Chen, D.A. Ridzon, A.J. Broomer, Z. Zhou, D.H. Lee, J.T. Nguyen, M. Barbisin, N.L. Xu, V.R. Mahuvakar, M.R. Andersen, K.Q. Lao, K.J. Livak, K.J. Guegler, Real-time quantification of microRNAs by stem–loop RT–PCR, Nucleic Acids Res. 33 (2005) e179.

[23] E.A. Szabo, B.M. Mackey, Detection of *Salmonella enteritidis* by reverse transcription-polymerase chain reaction (PCR), Int. J. Food Microbiol. 51 (1999) 113–122.

[24] Y. Tang, Y. Diao, C. Yu, X. Gao, L. Chen, D. Zhang, Rapid detection of Tembusu virus by reverse-transcription, loop-mediated isothermal amplification (RT-LAMP), Transbound. Emerg. Dis. 59 (2012) 208–213.

[25] C. Ma, F. Wang, X. Wang, L. Han, H. Jing, H. Zhang, C. Shi, A novel method to control carryover contamination in isothermal nucleic acid amplification, Chem. Commun. 53 (2017) 10696–10699.