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Rapid Detection of *Listeria monocytogenes* Strains Isolated from Clinical and Non-Clinical Samples by Loop-Mediated Isothermal Amplification Method (LAMP)

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ABSTRACT

Aims Diagnosis of *Listeria monocytogenes* infections is critical for epidemiological study and prevention of diseases. This study aimed at identifying *Listeria monocytogenes* isolates, using Loop-Mediated Isothermal Amplification Method (LAMP).

Materials & Methods Listeria strains were obtained from clinical and seafood specimen. All *Listeria* strains were identified by standard microbiological and biochemical tests. The LAMP assay was performed at 65°C with a detection limit of 2.5 ng/µl for 46 min. Specific primers for the *hylA* gene were used to identify *L. monocytogenes*. The specificity of the assay was assessed, using DNA from *L. monocytogenes* ATCC 7644 and *L. ivanovii* ATCC 19119 and non-Listeria strains. Sensitivity of the LAMP assay was compared with polymerase chain reaction (PCR) method. Amplification LAMP products were visualized via calcein and manganous ions as well as agarose gel electrophoresis.

Findings A total of 191 samples were obtained, including clinical and food samples. Then, 21 (10.9%) isolates were recovered from specimens. The LAMP results showed high sensitivity (97.2%) and specificity (100 %). The LAMP assay was higher sensitive than of the PCR assay.**Conclusion** This data showed that this method could be used as a sensitive, rapid, and simple identification tool for diagnosis of *L. monocytogenes* isolates and it may be suitable for epidemiological study plans.

Keywords Identification; Listeria monocytogenes; Epidemiological Study; Polymerase Chain Reaction

CITATION LINKS

[1] Virulence characterization and genotypic analyses of Listeria monocytogenes isolates from food and processing environments in Eastern ... [2] Genotypic characterization, invasion index and antimicrobial resistance pattern in Listeria monocytogenes strains ... [3] Listeria monocytogenes, a food-borne ... [4] Pathogenicity islands and virulence evolution in ... [5] Deaths associated with bacterial pathogens transmitted commonly through food: Foodborne diseases active surveillance ... [6] Phenotypic and genotypic characteristics of Listeria monocytogenes isolated from dairy and meat ... [7] Methods used for the detection and subtyping of Listeria ... [8] Methods for the isolation and identification of Listeria ... [9] Rapid detection of Listeria monocytogenes in food using culture enrichment combined ... [10] Isothermal and sensitive identification of Streptococcus pneumoniae using loop mediated isothermal amplification ... [11] Development and clinical evaluation of sdaA loop-mediated isothermal amplification assay for detection of Mycobacterium tuberculosis ... [12] Loop-mediated isothermal amplification for direct detection of Mycobacterium ... [13] Development of a loop-mediated isothermal amplification assay for rapid detection of Burkholderia ... [14] Two methods for increased specificity and sensitivity in loop-mediated ... [15] Development of a loop-mediated isothermal amplification assay ... [16] Rapid and sensitive detection of Listeria monocytogenes by loop-mediated isothermal ... [17] A multiplex PCR for detection of virulence associated genes in ... [18] Listeria monocytogenes: Pathogenesis and host ... [19] Development and application of a simple loop-mediated isothermal amplification method on rapid detection of Listeria ... [20] Loop-mediated isothermal amplification (LAMP) for the detection of Listeria monocytogenes and major pathogenic ... [21] Prevalence and antimicrobial resistance profiles of Listeria monocytogenes in spontaneous abortions ... [22] Putative type II toxin-antitoxin systems in Listeria monocytogenes isolated from clinical, food ... [23] Identification, subtyping and virulence determination ... [24] DNA extraction and PCR methods for the detection of ... [25] Incidence of Listeria in Egyptian meat and ... [26] Rapid detection of Vibrio parahaemolyticus strains and ... [27] Development of a loop-mediated isothermal amplification assay for detection ... [28] A loop-mediated isothermal amplification method for the detection ...

Introduction

Listeria monocytogenes is a gram-positive, rod shape, non-sporulating, facultative intracellular bacterium that is ubiquitous in vegetable, food, and soil. Listeriosis is especially severe in pregnant, newborn and fetuses, and immunocompromised persons [1, 2]. Listeriosis symptoms are flu-like ones and may lead to severe health problems, such as septicemia, meningitis, meningoencephalitis, stillbirth, and spontaneous abortion [3, 4]. In the United States, listeriosis is responsible for 30% of food-borne death since 1996 to 2005 [5, 6]. The standard microbiological, biochemical, and immunological-based methods are performed for *L. monocytogenes* identification. However, due to needing a two-step enrichment and an additional isolation on a selective medium, these traditional approaches are time-consuming and difficult. Therefore, it is important to establish new molecular and rapid techniques to be replaced with the conventional methods [7, 8].

Molecular-based methods have been improved for rapid and accurate detection of *L. monocytogenes*; however, it usually needs to visualize products via gel electrophoresis; therefore, this procedure is time-consuming. Although real-time PCR method has been used to identify food-borne pathogens, it is uncommon due to its costly thermal cycler and technical expertise needs, as well as labor-intensive [9]. Recently, the loop-mediated isothermal amplification (LAMP) assay, a nucleic acid amplification technique, has been established. This method had been described as a quick, inexpensive, quite simple, and highly sensitive relies on an auto-cycling strand displacement DNA synthesis accomplished by the Bst DNA polymerase under isothermal situations [10]. Therefore, this assay is applied in the field of microbiological diagnosis [11]. In this method, the specificity of the reaction is high because it uses a set of 4 primers identifying different regions on the target DNA [12, 13]. The chief benefit of the LAMP over PCR is, which it does not want intricate thermal cycler and it amplification can be performed within 30minutes in a laboratory heating block or a water bath [14, 15].

One of the main virulence factor of *L. monocytogenes* is *hlyA*, which encodes listeriolysin O (LLO) that is necessary for invasion to host cell [16]. This gene is suitable for identification of this organism, using nucleic acid-based procedures [17]. The present study used a simple assay based on LAMP method for recognition of *L. monocytogenes* strains obtained from various clinical and seafood samples.

Materials and Methods

Bacterial Strains: A cross sectional study was performed in 12 months during May 2014 to June 2015. A total of 191 samples, including clinical and seafood specimens were collected from Tehran, Iran. For clinical specimens, briefly, 5ml of blood samples, vaginal swab, and 25g of the placental tissue were injected into 50ml, 10ml, and 225 ml of TSYEB broth media (Merck; Germany), respectively. The food samples comprised meat products like sausage, calf meat, seafood products, and dairy products like cheese and cream. Concisely, 25 g of each food samples were added to 225 ml of TSBYE Broth and homogenized in a stomacher. All of the TSYEB broth media were stored at 4°C and for 7-16 days till 6 months (cold enrichment method) [18]. Then, 100µl of the broth media were inoculated on PALKAM Agar (Merck; Germany) and Listeria Selective Agar (Himedia; India) and incubated at 37°C for 48h. Confirmation of suspicious colonies was performed, using standard microbiological and biochemical routine tests such as Gram staining, catalase and oxidase reaction, β-hemolysis on Sheep Blood Agar, Methyl Red and Voges-Proskauer (MR-VP) tests, Christie Atkins Munch Petersen (CAMP) test, sugars fermentation including Xylose, Rhamnose, Mannitol and Methyl-D-Mannopyranoside, nitrate reduction, urease, and umbrella motility.

Primers*:* A primer set was used based on published primer sequences for 8 regions in *hlyA* loci. (Table 1) [16]. A set of inner primer (FIP and BIP), outer primer (F3 and B3), and loop primers (LF and LB) were planned for the LAMP to target 8 separate regions.

Table 1) LAMP primer set for *L. monocytogenes* [16]

Primer type	Oligonucleotide Sequence (5′–3′)
FIP	CGTGTTTCTTTTCGATTGGCGTCTTTTTTTCATCC ATGGCACCACC
BIP	CCACGGAGATGCAGTGACAAATGTTTTGGATTTC TTCTTTTTCTCCACAAC
F3	TTGCGCAACAAACTGAAGC
B3	GCTTTTACGAGAGCACCTGG
LF	TAGGACTTGCAGGCGGAGATG
LB	GCCAAGAAAAGGTTACAAAGATGG

DNA Extraction: Genomic DNA was obtained from grown colonies in Luria-Bertani (LB) broth (Merck; Germany), using the DNA extraction Kit (Roche; USA) according to the manufacturer's instruction. UV-spectrophotometer Model UV-1700 (Shimadzu; Japan) was used to measure purity and concentration of extracted DNA. The DNA with high purity (1.8-2 A_{260}/A_{280}) was diluted in distilled water and kept at -20°C.

LAMP method: The optimization of LAMP reaction was performed, using amplification temperatures (65°C) and time intervals. LAMP ssay was carried out in a total volume of 25µl reaction mixture containing 2µl each of inner primers (FIP and BIP), 1µl each of outer primers (F3 and B3), 1µl each of loop primers (LF and LB), 1.5µl of dNTPs 10mM mixture (Dalian; China), 1.5µl $MgSO_4$ 50mM, 3µl betaein 3M (Sigma, St. Louis, MO, USA), 2µl of Thermobuffer 1x (New England Biolabs, Ipswich, MA; USA), 1µl (8 U) of Bst DNA polymerase (New

England Biolabs, Ipswich, MA, USA), 2µl template DNA, and 6µl sterile distilled water. Then, mixture was incubated at 65˚C in a water bath for 46min. Finally, the reaction was stopped by heating at 80˚C for 10min [16, 19].

Analysis of the LAMP Products: Primarily, the opacity obtained from the white sediment of magnesium pyrophosphate in the bottom of the tube was observed by a visual evaluation. To assess the best mode of observation, the products were stained with the fluorescent dyes, SYBR Safe® (Invitrogen™). The color of the solution turned green in the existence of LAMP products; however, it remained orange without any amplification. By electrophoresis in 1% agarose gels, the ladder like products was surveyed and visualized by a UV transilluminator [16].

PCR assay: LAMP sensitivity was determined in PCR assays with each F3 and B3 primer. PCR reaction was accomplished in a total volume of 25µL. PCR reaction mixture contained 2.5µl of 10 × PCR buffer,1.5 DNA template,1.5µl MgCl2, 0.5µl dNTPs, 1.25µl of each F3 and B3 primer, 0.5 IU of Taq DNA polymerase (Ampliqon; Denmark), and 16µl of sterile distilled water. The thermal pattern was performed by the DNA amplification instrument Master cycler gradient (Eppendorf; Germany) comprised of 95˚C for 3min, followed by 32 cycles of denaturation at 94°C for 2min, annealing step at 56°C for 30s and extension at 72˚C for 1min with a final extension step at 72°C for 10min. The amplified products were further analyzed by electrophoresis in 2% agarose gel for 40min and visualized under UV-Trans illuminator. The size of PCR products was 346bp, which was verified by electrophoresis. Moreover, 100bp DNA marker (Fermentase; USA) was used as a size reference for PCR method. *L. monocytogenes* ATCC 7644 was used as a quality control [16].

Specificity of LAMP method for diagnosis of *L. monocytogenes*: To characterize the specificity of the LAMP method, this assay was performed with DNA templates from *L. monocytogenes* ATCC 7644 and *L. ivanovii* ATCC 19119 and non-*Listeria* strains including *Bacillus cereus* ATCC 14579, *Enterococcus faecalis* ATCC 29219, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606, and *Klebsiella pneumoniae* ATCC 13883 [16, 20].

Determination of LAMP Sensitivity: By using a positive control (*L. monocytogenes* ATCC 7644), the sensitivity of the LAMP method was determined. The limitation of the LAMP procedure was measured with PCR method by using the equal sample at same concentration in triplicate. From overnight culture of *L. monocytogenes*, tenfold dilutions was prepared in LB broth. DNA extraction was done from a 100µl aliquot of each dilution. These prepared DNAs were usedto assess the sensitivity of PCR and LAMP recognition. Similarly, to the bacteria count, 100µl aliquots of proper dilutions were streaked on PALCAM agar plates (Merck; Germany) and grown colonies. After 48 h of incubation at 37°C, colonies were counted. [16, 20].

Usability of the LAMP method: The appropriateness of LAMP assay for diagnosis of *L. monocytogenes* was assessed by evaluating the LAMP assay results (191 clinical and non-clinical samples) versus microbiological–biotechnical and conventional PCR results [16].

Findings

Bacterial Isolates: In this study, 46 *L. monocytogenes* strains were used, which were collected in 6 years in the previous study [21]. Moreover, clinical samples, including blood, placenta tissue, and vaginal swabs were attained from patients with spontaneous abortions referred to the Shahid Akbarabadi Hospital, Tehran, Iran. Also, non-clinical sample were recovered from dairy and food products (Table 2). In the present study, 4 (3.8%) and 17 (19.5%) *L. monocytogenes* isolates were obtained from the clinical (N=104) and various foods (N=87) samples, respectively (Table 2). The most *L. monocytogenes* isolates (8%) were collected from the seafood products.

Table 2) Comparison of culture and LAMP method on *L. monocytogenes* strains isolated form clinical and food samples

Sample	Samples size	*L. monocytogenes* isolates	Culture	LAMP
From previous study	46	46	+	+
Blood	40	0	-	-
Vaginal swabs	40	0	-	-
Placenta tissues	20	0	-	-
Blood, vaginal swab, Placenta	4	4	+	+
Seafood samples	17	7	+	+
Different food samples	50	10	+	+
Sausage, cream, cheese, mozzarella	20	0	-	-
Total	237	67	+	+

Detection and confirmation of LAMP Products: The LAMP assay had been performed on a total of 67 *L. monocytogenes* isolates (46 and 21 isolates obtained from previous and this study, respectively). The lamp products were determined by both visual seeing of the color variation by naked eye and electrophoresis. As the outcomes of electrophoresis assay displayed, the specific ladder-like profile bands on the agarose gel (Figure 1), which showed the precise amplification of LAMP assay. All amplicons had also been specified by consideration straight by naked eye. By adding calcein and manganous ions, the color change from orange to green was also observed (Figure 2). The established conditions allowed visualization of the amplified products under UV light upon the adding of SYBR Safe® (Invitrogen™; Figure 3).

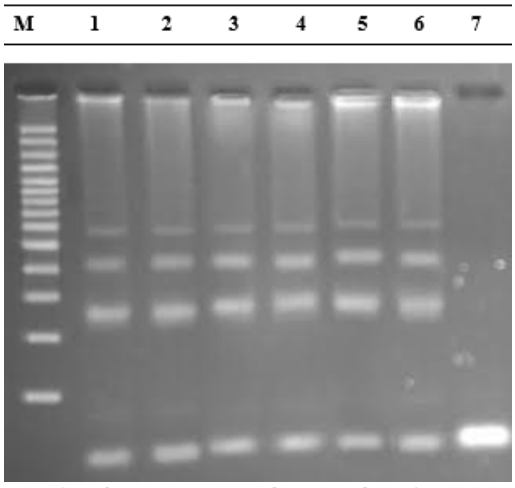

Figure 1) The LAMP result on identification of *L. monocytogenes* in the 5 selected isolated of *L. monocytogenes*; M; DNA molecular weight marker (1 kb, sigma), Lane 1: Positive control (*L. monocytogenes* ATCC 7644), Lane 2 and 3: strains isolated from clinical samples (Vaginal swab and placenta tissue), Lane 4: strain isolated from dairy samples, Lane 5: strain isolated from meat samples, Lane 6: strain isolated from animal samples, Lane 7: negative control*: L. ivanovii* ATCC 19119 .

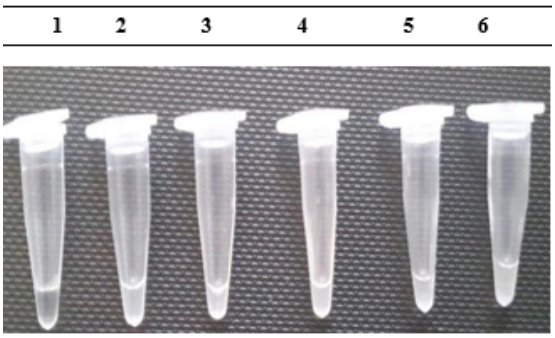

Figure 2) White precipitate of magnesium pyrophosphate. Tube 1; negative control, Tube 2; Positive control (*L. monocytogenes* ATCC 7644), Tubes 3, 4, 5, and 6; strains isolated from clinical, dairy, meat, and animal samples, respectively

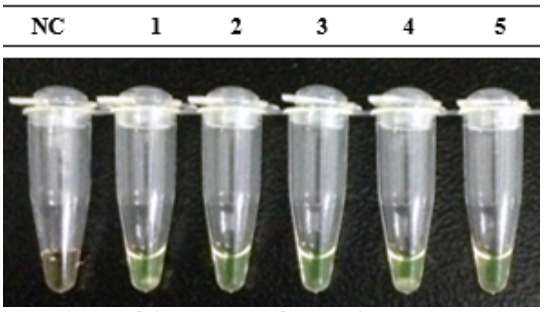

Figure 3) Amplification products of LAMP assays were visually detected by a fluorescence assay under UV. NC; negative control, Tube 1; positive control (*L. monocytogenes* ATCC 7644), Tube 2, 3, 4, and 5; *L. monocytogenes* strains isolated from the placenta tissue, dairy, meat products, and domestic animals samples, respectively.

Specificity determination of the LAMP assay: In assays with the LAMP primer, by using DNA from 67 *L. monocytogenes* strains obtained from various source, *L. ivanovii* (ATCC 19119) and five other non-Listeria strains, the specificity of primers set was determined. Amplification were discovered only by DNA from *L. monocytogenes*. When *L. ivanovii* or non-*Listeria* organisms were used in the LAMP assay, no reactions were detected. These consequences approved the specificity of *hlyA* sequence for diagnosis of *L. monocytogenes*.

Sensitivity determination of the LAMP Assay: Sensitivities of both PCR and LAMP methods for *L. monocytogenes* isolates with single colony cultures were obtained to be 2.1 CFU/reaction and 191 CFU/reaction, respectively. In all, the sensitivity obtained from the LAMP assay was 100-fold more than what was obtained the PCR method.

Assessment of the LAMP assay by direct detection of *L. monocytogenes* in various Specimens: To determine the efficiency of LAMP assay for *L. monocytogenes* diagnosis in various specimens, 191 specimens were examined by the PCR, LAMP, and standard microbiological–biotechnical tests. In the case of clinical and food source samples, 4 (3.8%) from 104 clinical samples and 17 (19.5%) from 87 food samples were *L. monocytogenes* positive (Table 2). The same result was obtained from both LAMP and PCR method. Therefore, the identification accuracy was 100%.

Discussion

Listeria monocytogenes is considered an opportunistic pathogen due to its capacity to cause a variety of serious infections such as meningitis, abortion, and septicemia particularly in susceptible persons, including pregnant women, newborns, and immunocompromised patients [22]. Contaminated food is considered the main source of illness in both sporadic and epidemics listeriosis. Thus, the improvement of quick and sensitive diagnostic tests

will be helpful for selecting suitable treatment regimens [23].

In the present study, out of 191 samples including 104 clinical and 87 non-clinical samples, 21 (10.9%) *L. monocytogenes* strains were recovered. The highest and the lowest prevalence of *L. monocytogenes* strains were isolated from the seafood products (N=17, 8%) and sausage, Mozzarella Cheese, Cheese, and cream (N=0, 0.0%), respectively. These data have compatible with study directed by Lotfollahi *et al*. [21]. Therefore, 14.3% of *L. monocytogenes* strains were obtained from the overall processed meat products. The findings of the present study are parallel with the Simon *et al*.'s study [24]. However, the results contrast with studies conducted by the Wang *et al*. and Ismaiel *et al*. [19, 25]. In these studies, the frequencies of pathogens were 4.7% and 5.3%, respectively. This difference may be due to the low number of investigated meat specimen.

The LAMP assay is easier to accomplish than several of the standardized methods because it needs only a water bath and the tube reaction components. LAMP also has the benefit of reduced reaction times [26]. The assay was confirmed to be a valuable and influential tool for rapid identification of *L. monocytogenes* strains.

In the previous study, which was done by Tang *et al*., only food samples were surveyed with LAMP method and here we used this method to identify clinical samples as well as food specimens.To the best of our knowledge, this is the first report of the detection of *L. monocytogenes* based on LAMP method in various clinical and food samples.

In the current study, LAMP assay was set up in both pure culture and direct detection of samples. In each sample, the LAMP method was used to detect *L. monocytogenes* strains. In comparison with molecular diagnostic assays, the established LAMP methods could be a fast and sensitive method for the detection of *L. monocytogenes*. Overall, time needed for DNA preparation, preparation of LAMP reaction mix, and identification was about 1h. In this method, a simple tool, water bath, was used for the reaction that high sensitivity and specificity (97.2% and 100%, respectively) detected by this method.

Our results are parallel with Wang *et al*.'s study that reported the specificity and sensitivity of the LAMP was 100% and 96.7%, respectively [14]. Tang *et al*. showed the LAMP assay was up to 100 fold sensitive than the PCR technique when bacteria were used as the template. [16]. The likely clarifications for the lower identification rate by PCR may be due to the low copy numbers of the *L. monocytogenes* templates or the presence of some specific inhibitors to the PCR affected the reaction sensitivity. Da Da Costa *et al*. reported that the preparation of a PCR or LAMP reaction mix is simpler than accomplishment a serological assay, as once the kit components are mixed and the operator only requires to add the DNA or culture specimens [20]. Certainly, quickness, easiness, and inexpensiveness of LAMP method will aid in the broad request for microbiological identification of *L. monocytogenes*. Therefore, Zhao *et al*., Liu *et al*. and Min *et al*. performed the LAMP method for detection of *Cronobacter* spp. (*Enterobacter sakazakii*) and *E. coli* O157, respectively [26-28].

Conclusion

In summary, the LAMP method was fast, very sensitive, simple, and low-cost for identification of *L. monocytogenes* from the food and clinical samples. Diagnosis of *L. monocytogenes* by LAMP method could facilitate investigation about contamination of *L. monocytogenes* in foods and other samples. So, we conclude that the LAMP method could be used for detection of *L. monocytogenes*, particularly for surveillance and epidemiological investigation.

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Conflict of interest: All authors declare that there are no conflicts of interest.

Authors' Contribution: Aleyasin N (First author), Introduction author/Methodologist/Original researcher/Statistical analyst/Discussion author (50%); Karimaei S (Second author), Introduction author/Discussion author (10%); Talebi M (Third author), Discussion author (10%); Kashanian M (Fourth author), Discussion author (10%); Irajian GH (Fifth author), Introduction author/Methodologist/Discussion author (20%).

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References

1- Jiang L, Chen J, Xu J, Zhang X, Wang S, Zhao H, et al. Virulence characterization and genotypic analyses of Listeria monocytogenes isolates from food and processing environments in Eastern China. Int J Food Microbiol. 2008;121(1):53-9.

2- Sadeghi Kalani B, Pournajaf A, Sedighi M, Bahador A, Irajian GR, Valian F. Genotypic characterization, invasion index and antimicrobial resistance pattern in Listeria monocytogenes strains isolated from clinical samples. J Acute Dis. 2015;4(2):141-6.

3- Farber JM, Peterkin PI. Listeria monocytogenes, a food-borne pathogen. Microbiol Rev. 1991;55(3):476-511.

4- Vázquez-Boland JA, Domínguez-Bernal G, González-Zorn B, Kreft J, Goebel W. Pathogenicity islands and virulence evolution in Listeria. Microbes Infect. 2001;3(7):571-84.

5- Barton Behravesh C, Jones TF, Vugia DJ, Long C, Marcus R, Smith K, et al. Deaths associated with bacterial

pathogens transmitted commonly through food: Foodborne diseases active surveillance network (FoodNet), 1996-2005. J Infect Dis. 2011;204(2):263-7.
6- Bahador A, Sadeghi Kalani B, Valian F, Irajian GR, Lotfollahi L. Phenotypic and genotypic characteristics of Listeria monocytogenes isolated from dairy and meat products. Avicenna J Clin Microbiol Infect. 2015;2(3):e26905.
7- Jadhav S, Bhave M, Palombo EA. Methods used for the detection and subtyping of Listeria monocytogenes. J Microbiol Methods. 2012;88(3):327-41.
8- Gasanov U, Hughes D, Hansbro PM. Methods for the isolation and identification of Listeria spp. and Listeria monocytogenes: A review. FEMS Microbiol Rev. 2005;29(5):851-75.
9- O'Grady J, Ruttledge M, Sedano-Balbás S, Smith TJ, Barry T, Maher M. Rapid detection of Listeria monocytogenes in food using culture enrichment combined with real-time PCR. Food Microbiol. 2009;26(1):4-7.
10- Ranjbar R, Afshar D. Isothermal and sensitive identification of Streptococcus pneumoniae using loop mediated isothermal amplification assay. Arch Pediatric Infect Dis. 2018;6(1):e61604.
11- Nimesh M, Joon D, Varma-Basil M, Saluja D. Development and clinical evaluation of sdaA loop-mediated isothermal amplification assay for detection of Mycobacterium tuberculosis with an approach to prevent carryover contamination. J Clin Microbiol. 2014;52(7):2662-4.
12- Iwamoto T, Sonobe T, Hayashi K. Loop-mediated isothermal amplification for direct detection of Mycobacterium tuberculosis complex, M. avium, and M. intracellulare in sputum samples. J Clin Microbiol. 2003;41(6):2616-22.
13- Mirzai S, Safi S, Mossavari N, Afshar D, Bolourchian M. Development of a loop-mediated isothermal amplification assay for rapid detection of Burkholderia mallei. Cell Mol Biol (Noisy-le-grand). 2016;62(10):32-6.
14- Wang DG, Brewster JD, Paul M, Tomasula PM. Two methods for increased specificity and sensitivity in loop-mediated isothermal amplification. Molecules. 2015;20(4):6048-59.
15- Ranjbar R, Afshar D. Development of a loop-mediated isothermal amplification assay for rapid detection of Yersinia enterocolitica via targeting a conserved locus. Iran J Microbiol. 2015;7(4):185-90.
16- Tang MJ, Zhou S, Zhang XY, Pu JH, Ge QL, Tang XJ, et al. Rapid and sensitive detection of Listeria monocytogenes by loop-mediated isothermal amplification. Curr Microbiol. 2011;63(6):511-6.
17- Rawool DB, Malik SVS, Barbuddhe SB, Shakuntala I, Aurora R. A multiplex PCR for detection of virulence associated genes in Listeria monocytogenes. Internet J Food Saf. 2007;9:56-62.
18- Goldfine H, Shen H, editors. Listeria monocytogenes: Pathogenesis and host response. New York: Springer Science & Business Media; 2007.
19- Wang L, Li Y, Chu J, Xu Z, Zhong Q. Development and application of a simple loop-mediated isothermal amplification method on rapid detection of Listeria monocytogenes strains. Mol Biol Rep. 2012;39(1):445-9.
20- Da Costa APR, De Lira Nunes M, Mendes-Marques CL, De Almeida AMP, Leal NC. Loop-mediated isothermal amplification (LAMP) for the detection of Listeria monocytogenes and major pathogenic serotypes. Am J Anal Chem. 2014;5(16):1057-64.
21- Lotfollahi L, Nowrouzi J, Irajian GR, Masjedian F, Kazemi B, Eslamian L, et al. Prevalence and antimicrobial resistance profiles of Listeria monocytogenes in spontaneous abortions in humans. Afr J Microbiol Res. 2011;5(14):1990-3.
22- Sadeghi Kalani B, Irajian GR, Lotfollahi L, Abdollahzadeh E, Razavi Sh. Putative type II toxin-antitoxin systems in Listeria monocytogenes isolated from clinical, food, and animal samples in Iran. Microb Pathog. 2018;122:19-24.
23- Liu D. Identification, subtyping and virulence determination of Listeria monocytogenes, an important foodborne pathogen. J Med Microbiol. 2006;55(Pt 6):645-59.
24- Simon MC, Gray DI, Cook N. DNA extraction and PCR methods for the detection of Listeria monocytogenes in cold-smoked salmon. Appl Environ Microbiol. 1996;62(3):822-4.
25- Ismaiel AAR, Ali AES, Enan G. Incidence of Listeria in Egyptian meat and dairy samples. Food Sci Biotechnol. 2014;23(1):179-85.
26- Zhao X, Wang L, Chu J, Li Y, Li Y, Xu Z, et al. Rapid detection of Vibrio parahaemolyticus strains and virulent factors by loop-mediated isothermal amplification assays. Food Sci Biotechnol. 2010;19(5):1191-7.
27- Liu X, Fang J, Zhang M, Wang X, Wang W, Gong Y, et al. Development of a loop-mediated isothermal amplification assay for detection of Cronobacter spp. (Enterobacter sakazakii). World J Microbiol Biotechnol. 2012;28(3):1013-20.
28- Min Z, Hongli J, Lifeng Z, Na W, Shaoqiang W, Xiangmei L. A loop-mediated isothermal amplification method for the detection of members of the genus Ranavirus. Arch Virol. 2013;158(10):2121-6.