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Evaluation of loop mediated isothermal amplification (LAMP) assay for rapid detection of *Listeria monocytogenes* from fish

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Abstract

A loop mediated isothermal amplification (LAMP) assay was optimised for rapid detection of *Listeria monocytogenes* from fish by targeting haemolysin gene and compared with conventional PCR and real time PCR (qPCR). All the assays were carried out using different DNA extraction methods like commercial kit, phenol-chloroform-isoamyl alcohol method and heat shock method. The analytical sensitivity of LAMP and qPCR was comparable and the detection limit was found to be 9.6×10^{1} CFU ml^{-1} from broth and 8×10^{2} CFU ml^{-1} from spiked fish whereas the detection limit of conventional PCR was found to be 9.8×10^{2} CFU ml^{-1} and 8×10^{4} CFU ml^{-1} from broth and fish respectively, when commercial kit was used for DNA extraction. The specificity of all these methods was 100% when compared with related bacterial species. The optimised LAMP assay when applied directly on 204 field fish samples gave an accuracy of 70.59% when compared to the gold standard while conventional PCR showed a lower accuracy of 52.94%. However, enrichment of LAMP negative samples for 6 h enhanced the sensitivity of detection to 100%. The optimised assay detected all negative fish samples by culture as negative hence giving detection specificity of 100%. Moreover, LAMP assay took the least detection time as compared to conventional PCR and qPCR. Thus, the optimised LAMP assay developed can be used as a sensitive, rapid and simple detection tool for the reliable detection of *L. monocytogenes* from fish.

Introduction

Listeria monocytogenes is a widely known food borne pathogen, which has the ability to persist in food processing environment acting as a source of contamination. It is widely distributed in the aquatic environment including marine, fresh and coastal waters, as well as in different types of seafood. The bacterium has been detected on fish surface, in the stomach lining, gills and intestine, with chances of contamination of flesh from these sources while processing. Seafood ranks first among the high risk, ready-to-eat food products responsible for human listeriosis (Dumen *et al.,* 2020), with majority of isolates belonging to serotype 1/2a followed by serotypes 4b and 1/2c (Basha *et al.,* 2019).

Pathogenic *L. monocytogenes* causes listeriosis, mostly affecting pregnant women, infants, elderly and immunocompromised individuals. Foodborne listeriosis is associated with a mortality rate of 20-50% and ranks among the most frequent causes of death due to foodborne illness, second to salmonellosis (Rossi *et al.,* 2008; Scallan *et al.,* 2011). In India too, there are several reports of prevalence of *Listeria* spp. in fish and fish handling areas which can substantiate fish and fishery products as vehicles of transmission of human listeriosis (Karunasagar and Karunasagar, 2000; Selvaganapathi *et al.,* 2018; Basha *et al.,* 2019).

According to FSSAI (2012), *L. monocytogenes* should be absent in 25 g of fish and frozen or canned meat and meat products. Detection of *L. monocytogenes* using conventional culture, biochemical and immunological based assays as well as by conventional PCR and qPCR is time consuming, laborious, and expensive. Thus, considering the public health significance of this pathogen, there is a need for the development of rapid, accurate and cost effective method of diagnosis to assess the prevalence of *Listeria* spp. in food of animal origin.

Loop mediated isothermal amplification (LAMP) method amplifies DNA under isothermal conditions (60-65^{0}C) based on the principle of auto cyclic strand displacement reaction by *Bst* DNA polymerase (Notomi *et al.*, 2000). The current study was done to optimise LAMP assay for the detection of *L. monocytogenes* in fish samples.

Materials and methods

Bacterial strains

L. monocytogenes strain ATCC 19115 was used as control for optimisation of LAMP assay. Gram positive strains; *Staphylococcus epidermidis* ATCC 49134, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 14506, *Listeria ivanovii* ATCC 19119, and Gram negative strains; *Salmonella* typhimurium ATCC 14028 , *Salmonella enteritidis* ATCC 13076 and *Escherichia coli* ATCC 10536 were used as negative controls in LAMP and PCR.

Genomic DNA extraction

Three different methods were used for the extraction of genomic DNA either from broth culture or from spiked fish/fish sample. The first method, commercial kit *viz.,* Ultraclean Microbial DNA Isolation Kit (MoBIO Laboratories) was used for extraction of genomic DNA from *L. monocytogenes* reference strains in BHI broth. However, DNA extraction from spiked fish/fish sample was done by using PowerFood™ Microbial DNA Isolation Kit (MoBIO Laboratories, USA). The second method was through conventional Phenol-chloroform-isoamyl (PCI) alcohol method as per Sambrook and Russel protocol (2000). Third was based on the heat shock DNA extraction method. In this method 1.5 ml of broth from respective dilutions was taken in 2 ml micro centrifuge tube. The culture was washed twice with nuclease free water after pelleting at 15,000 *g* for 15 min. Resuspended pellet was boiled and snap chilled for the extraction of DNA. DNA was stored at 4°C for use within a week.

Optimisation of LAMP assay

LAMP protocol was optimised by targeting haemolysin gene (*hly*A) of the standard strain *L. monocytogenes* ATCC 19115. Three sets of LAMP primers (Integrated DNA Technologies) (Table 1) were used for optimisation as described by Tang *et al.* (2011). Positive and negative controls were included and care was taken to prevent cross contamination. The LAMP reaction was carried out in a total reaction volume of 25 µl containing 2.5 µl of 10X ThermoPol Reaction Buffer (New England Biolabs, UK), 2 µl of Bst DNA polymerase (8U µl) (New England Biolabs, UK), 2 µl of 10mM dNTP mix (Thermo scientific Fermentas, Mumbai), 3 µl of 5M Betaine (Sigma, USA),1.5 µl of 100mM $MgSO_4$ (New England Biolabs, UK), 0.5 µl each of 0.2 µmol forward outer primer (F3) and backward outer primer (B3),1 µl each of 1.6 µmol forward inner primer (FIP) and backward inner primer (BIP),1 µl each of 0.8 µmol forward loop primer (LF) and backward loop primer (BF), 2 µl of template DNA and nuclease free water to make up the volume. The temperature-time-combination of 59, 60, 61, 62 and 63˚C for 30, 45 and 60 min at each temperature in a water bath was used. The reaction was terminated by heating the reaction mixture at 80°C for 10 min.

Table 1. Sequences of LAMP primers targeting haemolysin gene (*hly*A)

Primers	Sequence
hly – FIP	5'- CGTGTTTCTTTTCGATTGGCGTCTTTTTTTCA TCCATGGCACCACC - 3'
hly – BIP	5'-CCACGGAGATGCAGTGACAAATGTTTTGGA TTTCTTCTTTTTCTCCACAAC - 3'
hly - F3	5'-TTGCGCAACAAACTGAAGC – 3'
hly - B3	5'- GCTTTTACGAGAGCACCTGG – 3'
hly –LF	5'- TAGGACTTGCAGGCGGAGATG – 3'
hly – LB	5'- GCCAAGAAAAGGTTACAAAGATGG – 3'

Analysis and confirmation of LAMP products

Amplification was observed through colour change by adding 1 µl of SYBR Green I dye (1:1000) (Invitrogen, USA) to the finished LAMP reaction tube. In positive amplification, the original colour changed from orange to green under natural light, whereas no colour change was observed in negative. The amplified LAMP products were also subjected to gel-electrophoresis with 2% agarose in 1X TBE buffer and visualised under UV light in gel documentation system (Syngene, USA). Ladder like band pattern indicated positive reaction and no pattern was seen in negative reaction.

Optimisation of real time PCR (qPCR)

For optimisation of qPCR, primers targeting *hly*A gene as described by Barbau-Piednoir *et al.* (2013) were used. The qPCR amplification was carried out in LightCycler®96 Roche (Germany) with a pre-heated lid. A total reaction volume of 25 µl containing 12.5 µl LightCycler® 480 SYBR Green I master mix (Roche, Germany), 0.6 µl each of 250 nm (final concentration) of primer set containing forward and reverse primers, 2 µl DNA template and sterile nuclease free water to make up the reaction volume was used. The cycling conditions included an initial denaturation of DNA at 95˚C for 7 min, followed by 40 amplification cycles of 15 s at 95˚C (denaturing step) and 1 min at 60˚C (annealing and extension step), followed by one cycle of melt curve analysis at 95˚C for 1 min, 55˚C for 30 s and 95˚C for 30 s. The result of each reaction was expressed in cycle threshold (Ct). Samples which showed amplification before Ct value of 37 were considered as positive.

Optimisation of conventional PCR (PCR)

The PCR amplification was carried out in a total reaction volume of 25 µl containing 12.5 µl GoTaq®Green Master mix (final concentration 1.5 mM $MgCl_2$, 200 µM each dNTP and GoTaq® DNA Polymerase), 1 µl of 10 pmol µl^{-1} of each primer set, 2 µl template DNA and nuclease free water to make up the reaction volume. Primers targeting *hly*A (hemolysin) gene as described by Paziak-Domanska *et al.* (1999) were used. The cycling conditions included an initial denaturation of DNA at 94°C for 2 min followed by 35 cycles each of 30 s denaturation at 94°C, 1 min of annealing at 53°C and 1 min 30 s extension at 72°C, followed by final extension of 10 min at 72°C and hold at 4°C.The amplified PCR products were analysed by gel electrophoresis.

Comparative specificity evaluation of LAMP, qPCR and PCR assays

Evaluation of specificity in broth

Genomic DNA from standard strains of Gram positive and Gram negative bacteria mentioned earlier was extracted by kit method (MoBio Ultra clean Microbial DNA extraction kit, MoBIO Laboratories). Extracted DNA was used in LAMP, PCR and qPCR to analyse specificity of respective assays.

Evaluation of specificity in fish

The standard strains of Gram positive and Gram negative organisms were spiked @ 10^5 CFU ml^{-1} in fish samples individually and genomic DNA was extracted by kit method (PowerfoodTM Microbial DNA Isolation Kit, MoBIO Laboratories). Extracted DNA was used for LAMP, PCR and qPCR to analyse specificity of respective assay.

Comparative sensitivity evaluation of LAMP, qPCR and PCR assays

Evaluation of sensitivity in broth

An overnight grown culture of *L. monocytogenes* ATCC 19115 strain was serially diluted 10 folds in BHI broth, which was also evaluated for its count. Genomic DNA was extracted from different dilutions (containing *L. monocytogenesca* 10^1-10^8 CFU ml^{-1}) by three different DNA extraction methods *viz*., commercial kit, PCI method and heat shock method and used for amplification in LAMP, PCR and qPCR assays. The lowest detection limit of *L. monocytogenes* from broth was noted for the respective assays taking into account CFU ml^{-1}.

Evaluation of sensitivity in fish

Retail fish samples were collected and autoclaved for sterilisation. Sterilised samples were checked for sterility by plating onto BHI and PALCAM agar and the negative fish samples were used for sensitivity study. Tenfold serial dilution of the standard strain (containing *L. monocytogenes* @ 10^1-10^7 CFU ml^{-1}) was used to spike 9 g of sterile fish sample (in duplicates). Spiked sample was centrifuged at 1500 rpm for 5 min and the supernatant of each dilution was used for DNA extraction by three methods and then put through amplification by PCR, LAMP assay and qPCR.

Assessment of optimised LAMP assay for rapid detection of *L. monocytogenes* detection in field samples of fish

A total of 204 raw fish samples were collected from different retail markets of Punjab. About 25 g of fish sample was collected aseptically in a sterile container and transported to laboratory under chilled conditions. The collected fish samples were processed for isolation of *Listeria* spp. by conventional microbiological procedures and subjected to species identification by biochemical characterisation and PCR targeting the *hly*A gene, specific for *L. monocytogenes*. The same fish samples which were positive by bacteriological methods (culture) were then subjected to DNA extraction by all the three methods and then used for LAMP and other assays.

The fish samples which showed negative results on LAMP and qPCR assays were subjected to enrichment from one to twelve hours consecutively at 1 h interval. One ml of negative fish extract samples was inoculated in a tube containing 9 ml of BHI broth for 4 h of enrichment at 37ºC. The enriched sample was then processed for extraction of DNA using kit (PowerFood™ Microbial DNA Isolation Kit MoBio Laboratories) for detection of *L. monocytogenes* by optimised LAMP assay. Comparison of detection sensitivity and specificity between conventional culture method (with enrichment), LAMP and qPCR (before as well as after enrichment) was done.

Results and discussion

Optimisation of LAMP

LAMP optimisation protocol on different temperature-time combination showed reaction at 59, 60, 61, 62 and 63°C for 50 min, with typical ladder like pattern in the positive control and orange colour in negative control (NTC) (Fig. 1). In similar optimisation studies, the LAMP reaction was optimised at 65°C for 40 min (Tang *et al.,* 2011) and in another study at 63°C for 60 min (Shan *et al.,* 2012) targeting *hly*A gene. Differences in the temperature-time combination could be attributed to laboratory variations.

Optimisation of qPCR

The optimised protocol showed amplification in the form of amplification curve for Ct value at 12 (Fig. 2). The melting temperature of the product after amplification was 78°C during melt curve analysis. A similar study by Barbau-Peidnoir *et al.* (2013), targeting *hly*A gene using SYBR green qPCR, showed amplification with Ct value of 27 and melting curve in the range of 74-75°C. The same primers when used by Kedrak-Jablonska *et al.* (2015) gave Ct value of 13.98 to 16.28 with melting curve in the range of 76 to78°C. Singh *et al*. (2012) also optimised real time PCR for *L. monocytogenes* targeting *hly*A gene at 79.90±0.39°C.

Optimisation of PCR

Conventional PCR for detection of *L. monocytogenes* was optimised at an annealing temperature of 53°C using primers targeting *hly*A gene yielding amplicon size of 456 bp (Fig. 3).

Comparison of sensitivity of LAMP assay, PCR and qPCR from broth and spiked fish samples

As per the results of the study, LAMP as well as qPCR had a detection limit of 96 CFU ml^{-1} when done from broth with kit (MoBio DNA extraction kit) and conventional nucleic acid extraction method, whereas, conventional PCR showed a detection limit of 9.8×10^2 CFU ml^{-1} using same DNA extraction methods. When heat shock method of DNA extraction was used, sensitivity of LAMP assay and qPCR showed a reduced sensitivity of 9.8×10^2CFU ml^{-1} and that of conventional PCR was 9.8×10^3 CFU ml^{-1}. Thus LAMP

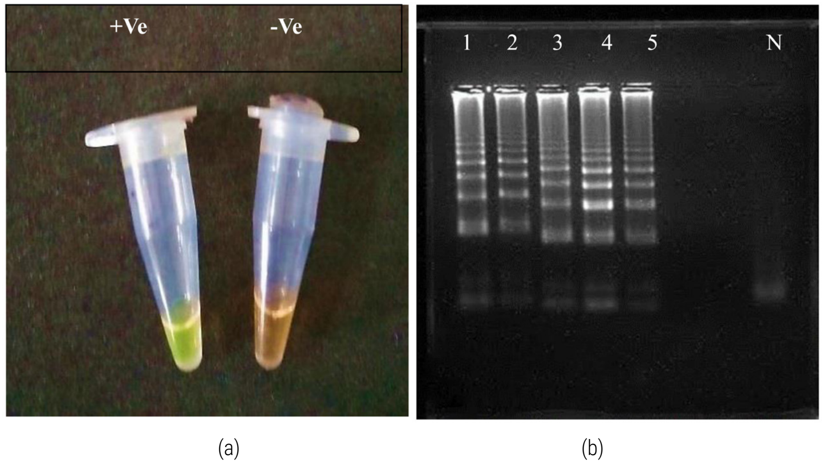


Fig. 1. (a). Colour change following addition of 1 µl of SYBR green I dye to the reaction tubes (b). Gel electrophoresis of optimised LAMP. Lane 1 - 59°C for 50 min, Lane 2 - 60°C for 50 min, Lane 3 - 61°C for 50 min, Lane 4 - 62°C for 50 min, Lane 5 - 63°C for 50 min, Lane N - NTC N, No template control

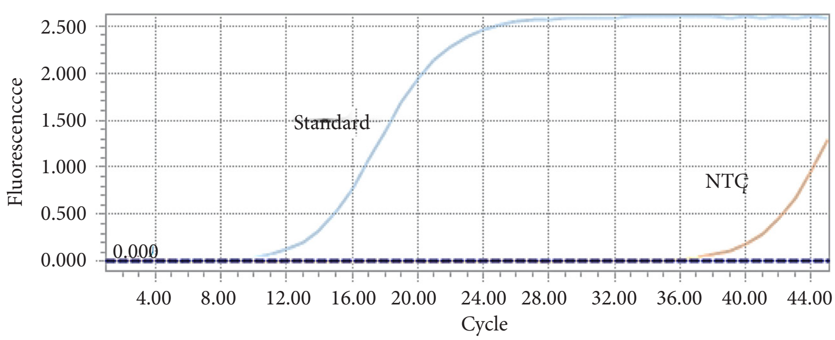


Fig. 2. Amplification curve of standard strain of *L. monocytogenes* in qPCR assay

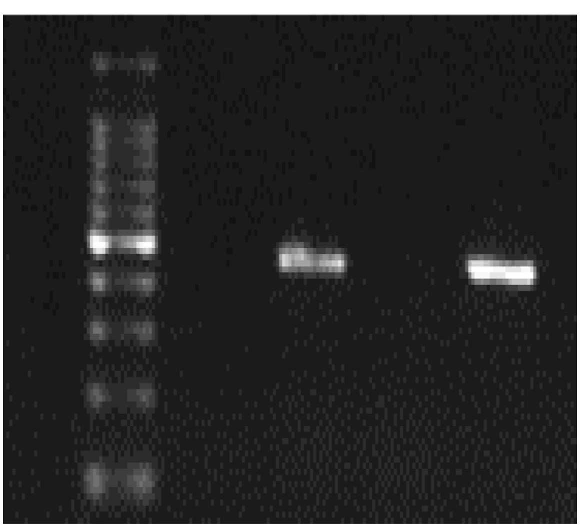


Fig. 3. Gel electrophoresis products of conventional PCR for hlyA gene

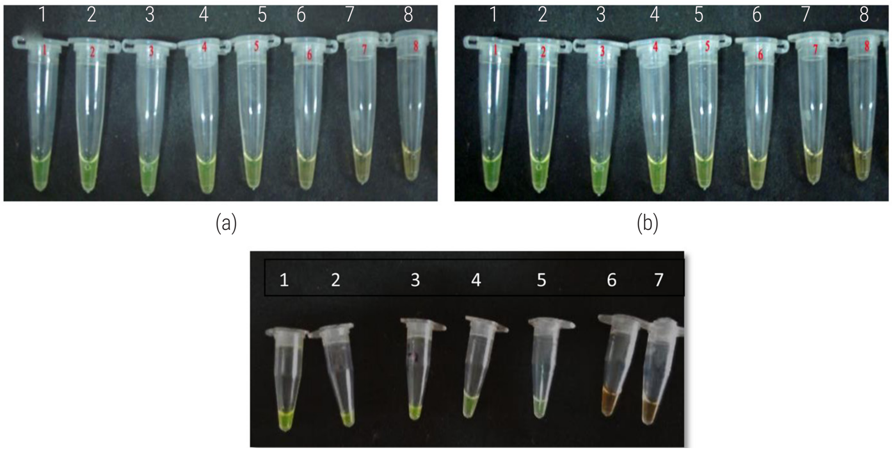
was found almost 10 times more sensitive than conventional PCR from broth (Table 2; Fig. 4). Taking detection time into consideration from broth using three molecular techniques into consideration, LAMP was found to be the quickest with a detection time of 50 min excluding DNA extraction time, whereas qPCR and conventional PCR took about 1 h 30 min and 3 h, respectively for detection (Table 3). Therefore, LAMP was found to be the quickest with sensitivity equal to real time PCR.

Application of LAMP for rapid detection from spiked fish sample also showed LAMP to be sensitive method with detection limit of 8×10^2, 8×10^3 and 8×10^4 CFU ml^{-1}, using Powerfood kit, conventional PCI and heat shock method of DNA extraction, respectively. Here also, qPCR performed equally well in comparison to LAMP (Table 4). When detection limit was combined with time for DNA extraction, LAMP was able to detect *L. monocytogenes* from fish rapidly in 1 h 50 min and 1 h 20 min using kit and heat shock method of DNA extraction, respectively (Table 4).

In previous studies also, LAMP has been reported to be sensitive method of detection both from pure culture as well as food samples (Tang *et al.*, 2011; Shan *et al.*, 2012). Comparing our results with the reports published in scientific literature regarding rapid detection of *L. monocytogenes* using LAMP and PCR, LAMP has been reported to be sensitive method of detection both from pure culture as well as food samples (Tang *et al.*, 2011; Shan *et al.*, 2012). Tang *et al.* (2011) reported detection limit of 2 CFU per reaction and 207 CFU per reaction for *L. monocytogenes* in broth using LAMP and conventional PCR, respectively. Shan *et al*. (2012) recorded detection

Table 2. Results of comparative sensitivity evaluation of LAMP, PCR and qPCR assay from broth using different DNA extraction techniques

DNA extraction method	LAMP		PCR		qPCR	
	CFU ml^{-1}	CFU per reaction	CFU ml^{-1}	CFU per reaction	CFU ml^{-1}	CFU per reaction
KIT	96	0.2	9.8×10^2	2	96	0.2
PCI	96	0.2	9.8×10^2	2	96	0.2
Heat shock	9.8×10^2	2.0	9.8×10^3	20	9.8×10^2	2.0



(a) (b)

(c)

Fig. 4. Sensitivity evaluation of LAMP from (a) Broth by kit, (b) Conventional method (phenol-chloroform-isoamyl alcohol) and (c) Heat shock method of DNA extraction. NTC - No template control
a: 1: 9.8 × 10-CFU g^{-1}, 2: 9.8× 10-CFU g^{-1}, 3: 9.8 × 10^4 CFU g^{-1}, 4: 9.8 × 10^3 CFU g^{-1}, 5: 9.8 × 10^2 CFU g^{-1}, 6: 96 CFU g– LDL^{-1}, 7: 10 CFU g^{-1}, 8 : NTC
b: 1: 9.8 × 10-CFU g^{-1}, 2: 9.8 × 10-CFU g^{-1}, 3: 9.8 × 10^4 CFU g^{-1}, 4: 9.8 × 10^3 CFU g^{-1}, 5: 9.8 × 10^2 CFU g^{-1}, 6: 96 CFU g – LDL^{-1}, 7: 10 CFU g^{-1}, 8 : NTC
c: 1: 9.8 × 10-CFU g^{-1}, 2: 9.8 × 10-CFU g^{-1}, 3: 9.8 × 10^4 CFU g^{-1}, 4: 9.8 × 10^3 CFU g^{-1}, 5: 9.8 × 10^2 CFU g-LDL^{-1}, 6: 96 CFU g^{-1}, 7 : NTC

Table 3. Comparison of detection time of LAMP assay, conventional PCR and qPCR from broth using different DNA extraction techniques

	Commercial kit method			Phenol-chloroform-isoamyl alcohol method			Heat shock method		
	LAMP	PCR	qPCR	LAMP	PCR	qPCR	LAMP	PCR	qPCR
Detection* time	50 min	2:10 h + 45 min	1:30 h	50 min	2:10 h + 45 min	1:30 h	50 min	2:10 h + 45 min	1:30 h
Time for DNA extraction	1 hrs	1 h	1h	26 hrs	26 h	26 h	30 min	30 min	30 min
Total detection time	1:50 hrs	3:55 h	2: 30 h	26:50 h	29 h	27:30 h	1:20 h	3 :25 h	2:00 h

* Detection time included amplification reaction + gel electrophoresis

Table 4. Results of comparative detection sensitivity and detection time of LAMP assay, conventional PCR and qPCR assay from spiked fish sample using different DNA extraction techniques

DNA extraction method	Detection limit (CFU ml^{-1})			Detection time (excluding DNA extraction method)			Time for DNA extraction			Total detection time
	LAMP	PCR	qPCR	LAMP	PCR	qPCR	LAMP	PCR	qPCR	
KIT method	8 $\times10^2$	8×10^4	8 $\times10^2$				1 h	1 :50 h	4 h	2:30 h
PCI	8×10^3	8×10^5	8×10^3	50 min	2:10 h+ 45 min	1:30h	26 h	26:50 h	29 h	27:30 h
Heat shock method	8×10^4	8×10^6	8×10^4				30 min	1:20 h	3: 30h	2:00 h

*Detection time include amplification + gel electrophoresis

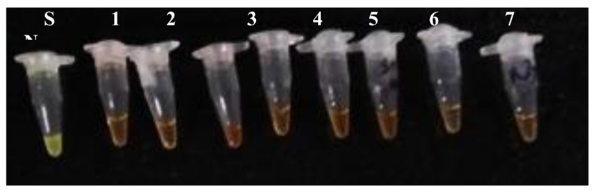
limit of LAMP assay for *L. monocytogenes* as 2.8×10^3 CFU ml^{-1} (6 CFU per reaction) and 56 CFU per reaction in case of conventional PCR from food samples. Rodrıguez-Lazaro *et al.* (2004) reported detection limit of 100 CFU g^{-1} from meat products using real time PCR targeting *hly*A gene of *L. monocytogenes.* Similar study was done by Han and Ge (2010) on *Vibrio vulnificus* from oysters which revealed a sensitivity of real time LAMP assay to be 6.4×10^4 CFU g^{-1} (116 CFU per reaction) without enrichment and found it 1000 fold more sensitive than conventional PCR (6.4×10^7 CFU g^{-1}).

Comparative Specificity evaluation of LAMP, qPCR and PCR assays from broth and spiked fish samples

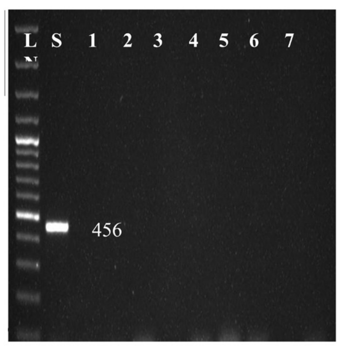
Specificity evaluation studies of LAMP, PCR and qPCR assay for rapid detection of *L. monocytogenes* from broth and spiked fish samples using all the three nucleic acid amplification methods were 100% specific with no false positive results (Fig. 5).The results were in agreement with findings published in relevant literature (Paziak-Domanska *et al.,* 1999; Wang *et al.,* 2012; Barbau-Peidnoir *et al.*, 2013; Kedrak-Jablonska *et al.*, 2015).Tang *et al.* (2011) and Shan *et al.* (2012) in their optimised LAMP protocol targeting *hly*A gene for the detection of *L. monocytogenes* and non-*Listeria* bacteria confirmed that *hly*A primer sequences were specific for *L. monocytogenes*.

Application of optimised LAMP protocol for rapid detection of *L. monocytogenes* in field samples of fish

Out of the 204 fish samples, 17 samples were positive for *L. monocytogenes* by culture method and 187 were negative. When these samples were subjected to direct detection by optimised LAMP protocol (without enrichment), out of 17 positive samples LAMP detected 12 samples positive for *L. monocytogenes,* hence giving detection sensitivity of 70.59% in comparison to gold standard culture method. The five culturally positive fish samples which were negative by LAMP assay turned positive after six hours of enrichment in BHI thus giving 100% detection sensitivity on including enrichment step. The specificity evaluation of LAMP on field fish samples gave 100% detection specificity in comparison to culture method (Table 5). Tang *et al.* (2011) in their study also reported sensitivity of 100% for LAMP assay for the rapid detection of *L. monocytogenes* from chicken samples. Similarly, Shan *et al.*



(a)



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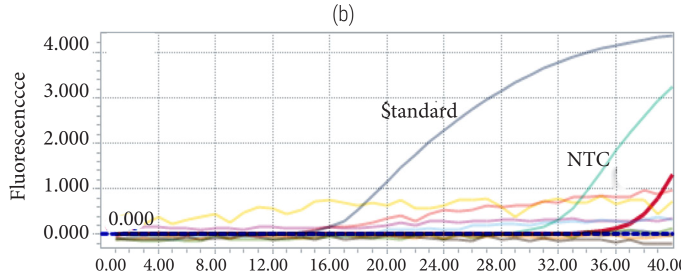

(c)

Fig. 5. (a) Specificity evaluation of LAMP from broth; (b) Specificity evaluation of conventional PCR assay from broth and (c) Specificity evaluation of qPCR assay from broth. S: Standard strain of *L. monocytogenes*; NTC: No template control; 1: *Listeria ivanovii* ; 2: *Staphylococcus epidermidis;* 3: *Enterococcus faecalis*; 4: *S. aureus*; 5, *Salmonella typhimurium;* 6: *Salmonella enteritidis*; 7: *Escherichia coli*

Table 5. Comparison of *L. monocytogenes* detection in field fish samples through isolation and LAMP

		L. monocytogenes positive samples by conventional method	*L. monocytogenes* negative samples by conventional method
Before enrichment	LAMP +ve	12	0
	LAMP −ve	5	15
	Total	17	15
After enrichment of 6 h in BHI broth	LAMP +ve	17	0
	LAMP −ve	0	15
	Total	17	15

(2012) reported a sensitivity of LAMP assay to be 100% from food samples of animal origin. The reason for lower detection rate in the present study could be ascribed to presence of bacteria below the detection limit of 8× 10^2 CFU ml^{-1} for LAMP whereas, the above quoted studies used enriched cultures from food samples for LAMP assay.

Taking the total detection time into consideration including enrichment of fish samples in BHI broth, LAMP assay was found to be the most rapid (7:50 h) in giving 100% sensitivity on field samples compared to qPCR and conventional PCR, which was 8 h 30 min and 16 h, respectively. Thus LAMP assay is a potential tool for the rapid detection of *L. monocytogenes* as compared to quantitative and conventional PCR assays. It exhibited high sensitivity and specificity with low sensitivity to inhibitory substances in biological samples. The optimised method required water bath to carry out the detection, therefore it can be used in field conditions with limited resources.

Rapid detection methods in food safety ensure time and quick detection of foodborne pathogens in food thereby ensuring health of the consumers. LAMP was optimised at 62°C for 50 min for the detection of *L. monocytogenes* in fish samples. The sensitivity of LAMP and qPCR was found to be 9.6×10^1 CFU ml^{-1} from broth and 8×10^2 CFU ml^{-1} from spiked fish, whereas the detection limit of conventional PCR was found to be 9.8×10^2 CFU ml^{-1} and 8×10^4 CFU ml^{-1} from broth and fish respectively, when commercial kit was used for DNA extraction. The optimised LAMP assay had 100% specificity. The accuracy of LAMP assay was 70.59% when tested in 204 field fish samples without enrichment. However, on enrichment of negative samples for 6 h, the accuracy of the assay increased to 100% and detection limit to < 8×10^1 CFU ml^{-1}. Moreover, LAMP assay took the least detection time as compared to conventional PCR and qPCR. The optimised assay could be applied to field samples for the rapid detection of the pathogens to ensure safety and quality.

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