J Food Sci Technol (May 2019) 56(5):2576–2583
https://doi.org/10.1007/s13197-019-03740-7

ORIGINAL ARTICLE

Development of a filtration-based LAMP–LFA method as sensitive and rapid detection of *E. coli* O157:H7

Jin-Hee Kim[1] · **Se-Wook Oh**[1]

Revised: 11 March 2019 / Accepted: 19 March 2019 / Published online: 1 April 2019

Abstract Rapid detection of low number of pathogenic bacteria in food is difficult. This study tested the filter-based loop-mediated isothermal amplification-lateral flow immunoassay (LAMP–LFA) method for rapid detection of pathogens in real food. *Escherichia coli* O157:H7 was inoculated on 25 g of beef and the homogenized sample was filtered with 0.45 µm cellulose nitrate filter, and concentrated *E. coli* was recovered and DNA was extracted and analyzed by LAMP. LFA reaction was performed by hybridization of digoxygenin-labeled LAMP amplicon and biotinylated probe. The sensitivity of the filtered sample was 100 times more sensitive than that of the unfiltered sample. The total reaction time used for detection from sample preparation to confirmation of *E. coli* was within 3 h. These results suggest that the LAMP–LFA method can be used in real food systems as point-of-care testing for *E. coli* O157:H7 in beef.

Keywords Loop-mediated isothermal amplification · Lateral flow assay · Filtration · *Escherichia coli* O157:H7

Introduction

Foodborne diseases present a major global health issue, and food safety is important for both consumers and producers in the food industry (Zhao et al. 2010). Shiga toxin-producing *Escherichia coli* is a foodborne pathogen that cause a range of symptoms in humans ranging from mild diarrhea to fatal complications, such as hemolytic–uremic syndrome (Wang et al. 2014). Beef and beef products are considered to be the main sources of *E. coli* infections in humans (Chapman et al. 2001). However, it is difficult to detect foodborne pathogens rapidly by conventional methods because the number of bacteria in food is very low (Dong et al. 2014).

To reduce food poisoning, particularly in the food industry, rapid pathogen detection methods for raw food materials and processed foods are required. These rapid detection methods must be sensitive enough for detection of pathogens at low concentrations that exist in and on foods. Although culture based methods are generally regarded as the gold standard for detecting foodborne pathogens, they are time-consuming (taking 3–4 days including biochemical testing) and labor intensive. To alternatives, nucleic-acid-based methods, such as real-time PCR, are widely used nowadays.

Loop-mediated isothermal amplification (LAMP) is another nucleic acid-based method that is known to be fast, accurate, and easy to perform (Lee et al. 2016). LAMP does not dissociate the DNA, but rather amplifies the DNA in 60 min under isothermal (60–65 °C) conditions using the autocycling strand displacement characteristics of large fragment *Bst* DNA polymerase (Techathuvanan et al. 2010, Yamazaki et al. 2011). Instead of a PCR thermocycler, DNA can be amplified using a low-cost temperature-holding device, such as a water bath. In addition, LAMP is 10–100 times more sensitive than PCR because of its low effect on inhibitors derived from foods (Techathuvanan et al. 2010; Zhao et al. 2010; Yamazaki et al. 2011). LAMP-amplified DNA can be detected by various techniques, such as electrophoresis, turbidity evaluation, or staining with a fluorescent dye (SYBR Green I) that

✉ Se-Wook Oh
swoh@kookmin.ac.kr

[1] Department of Foods and Nutrition, Kookmin University, Seoul 136-702, Korea

specifically reacts with dsDNA (Techathuvanan et al. 2010; Yongkiettrakul et al. 2014).

Lateral flow immunoassays (LFAs) have been developed extremely useful for rapid point-of-care testing (POCT) of foodborne pathogens, and they are based on the principles of specific binding between antibodies and bacteria. However, their detection sensitivity is very low and can detect only a high pathogen concentration (10^5 CFU/mL) (Shan et al. 2015). In the current study, instead of whole bacteria cells, an amplified DNA fragment was used as an epitope. After tagging the DNA amplicon with digoxygenin (DIG) and biotin, it can be detected by an anti-DIG antibody and visualized with a gold particle conjugated to streptavidin (Singh et al. 2015). Thus, a positive amplification result can easily be detected by the naked eye.

In general, for microbiological analysis, a 25-g analytical unit is used and should be pre-enriched at a 1:9 sample-to-broth ratio. Thus, if microorganisms are to be detected from a 25-g food sample, the sample would be diluted in 225 mL of broth, resulting in a potential 10-fold loss in sensitivity because 1-mL aliquots are normally used for detection methods, such as PCR and LAMP. Thus, microbial culture enrichment is routinely conducted to increase the bacterial concentration over the detection limit. This step requires > 10 h, making rapid detection of foodborne bacteria impossible. However, in several detection methods, microbial culture enrichment is now replaced with other concentration methods. Maheux et al. concentrated bacteria using a filter (Maheux et al. 2011). Filter-based methods have the advantage of not only concentrating bacteria but also removing the DNA amplification reaction inhibitors originating from food (D'Urso et al. 2009; Wolffs et al. 2006).

Many inhibitors of PCR and LAMP are present in complex food matrices, and generally, pathogens exist at very low concentrations. Because of these challenges, no reliable biosensors with high selectivity and sensitivity are yet available for rapid pathogen detection in real food systems. Therefore, in this study, we developed a filtration-based LAMP–LFA method for the rapid detection of *E. coli* O157:H7 present in beef at low numbers without microbial enrichment. *Escherichia coli* O157:H7 cells were concentrated by filtration instead of microbial enrichment culture, and the target DNA was isothermally amplified by LAMP and visually detected by LFA. This method enabled fast and sensitive detection of *E. coli* O157:H7 in a real food system.

Materials and methods

Bacterial strain

The bacterial strain used in this experiment was *E. coli* O157:H7 (ATCC 35150). Bacteria were maintained in 0.5 mL of 50% glycerol and stored at −80 °C. During experiments, bacterial cultures were grown in Tryptic Soy Broth (Difco, Flanklin Lakes, USA) at 37 °C for overnight.

Detection of *E. coli* O157:H7 from artificially contaminated beef

Beef was obtained from a local market (Seoul, Korea). Samples were artificially contaminated with *E. coli* O157:H7. The strain was serially diluted 10-fold (10^5–10^0 CFU/g) in 0.85% saline solution, and 100 µL of each dilution was inoculated on 25 g of beef and mixed well for uniform distribution. Briefly, 25 g of each beef sample was sliced and placed into a stomacher bag. Each sliced beef was inoculated by adding 100 µL of appropriately diluted inoculum. The inoculum was rubbed into one surface of each inoculated sliced beef by manually massaging (AVERY et al. 1994). For DNA isolation and bacterial quantification, 25 g were taken from the inoculated beef and mixed with 225 mL of 0.85% NaCl saline and homogenized in a Pulsifier (Macrogen, Seoul, Korea) for 1 min. Thereafter, 1 mL of each mixture was added in a 1.5-mL tube and centrifuged at 12,000 rpm for 2 min. DNA was extracted using a TaKaRa DNA extraction kit (Takara Bio Inc., Shiga, Japan), according to the manufacturer's protocol. To enumerate *E. coli* O157:H7 cells, serially diluted beef solution was plated on eosin methylene blue plates (EMB agar, Oxoid, Hampshire, UK) (Sagong et al. 2011; Kim et al. 2011). Plates were incubated at 37 °C for 24–48 h, and bacterial cells were counted.

Filter selection

Filtration was performed to concentrate *E. coli* O157:H7 cells. To select the most suitable filter, the recovery efficiency of the following five filters was tested: 0.45-µm cellulose nitrate filter (Whatman International Ltd., Maidstone, United Kingdom), 0.45-µm cellulose acetate filter (Toyo Roshi Kaisha, Ltd., Japan), 0.45-µm mixed cellulose ester filter (MCE, HYUNDAI Micro Co., Ltd., Korea), 0.45-µm polyethersulfon filter (PES, HYUNDAI Micro Co., Ltd., Korea), and 0.45-µm nylon filter (HYUNDAI Micro Co., Ltd., Korea). *Escherichia coli* O157:H7 was artificially inoculated on 25 g of beef. After adding 225-mL saline and homogenizing with a Pulsifier, the

solution was filtered through the five different filters under a vacuum. After filtration, each filter was disassembled and placed on EMB agar. After incubation at 37 °C for 24–48 h, bacterial counts were determined. The recovery ratio of *E. coli* O157:H7 was determined by comparison with inoculated microbial loads and recovered counts on EMB agar. All measurements were performed in five replicates.

Escherichia coli O157:H7 recovery from filters

Among the five tested filters, the cellulose nitrate filter showed the highest recovery ratio and was chosen as a suitable filter. After filtering *E. coli* O157:H7 with a cellulose nitrate filter, three different recovery methods with different times and reps were tested for the recovery of attached or embedded *E. coli* O157:H7 from the filter. In all cases, the filter was placed in a sterile bag (7.5 × 12.5 cm; Nasco, Fort Atkinson, WI, USA), and 2.25 mL of 0.85% NaCl was added. The filter was then vortexed for 15, 30, or 60 s; stomached (Laboratory Blender Stomacher 400; Seward, MO, USA) for 20, 50, 100, or 200 reps; or sonicated for 15, 30, or 60 s. After the recovery method was performed, 1 mL was taken from 2.25 mL of saline and plated on non-selective media, TSA, and selective media (EMB), respectively. The recovery ratio was determined using the bacterial cell counts on TSA plates and the initial inoculating bacterial count. The recovery percentage was defined as follows:

$$\text{Recovery rate } (\%) = \frac{\text{Number of bacteria recovered from the filter}}{\text{Initial inoculated bacteria } (\text{CFU/mL})} \times 100\%$$

The injured cell ratio was calculated as the percentage of cells that did not grow on the EMB plate but grew on the TSA plate.

Membrane filtration and isolation of DNA

Escherichia coli O157:H7 was cultured to give an initial bacterial concentration of 1.47×10^9 CFU/mL in TSB overnight and serially diluted with saline to a concentration of 10^6–10^2 CFU/mL; 100 µL of this diluted culture was inoculated on 25 g of beef. After mixing well, 225 mL of saline was added, and the beef was homogenized using a Pulsifier and subsequently filtered using a membrane filter (pore size, 0.45 µm) with a 47-mm diameter and vacuum pump (Welch, Ilmenau, Germany). After filtering the 225-mL solution, the filter was disassembled and put into 7.6 × 12.7-cm sterilized polyethylene filled with 2.25 mL of 0.85% NaCl saline. *Escherichia coli* O157:H7 cells were recovered or detached from the filter by stomaching for 200 reps. DNA was extracted using a TaKaRa MiniBEST Bacterial Genomic DNA Extraction Kit Ver.3.0 (TaKaRa, Dalian, China) from 1 mL of the 2.25-mL homogenate. The extracted DNA was used for LAMP and real-time PCR.

Primer design and conditions for the LAMP reaction

Primers

The Shiga-like toxin 2 (*stx*2) gene was selected as a target sequence for this method, as described by Zhao et al. (2010). The probe for *E. coli* O157:H7 was designed using Primer Explorer version 4 (http://primerexplorer.jp/lamp4.0.0/index.html) to target five distinct regions of the *stx*2 gene (GenBank Accession No. AF162758.1). Primer sequences are listed in Table 1.

LAMP operating condition

The LAMP reaction was performed in a final volume of 25 µL of the reaction mixture containing 0.8 µM of each of the FIP and BIP inner primers, 1.6 µM of each of the F3 and B3 outer primers, 1.4 mM dNTP mix (ABM Inc., Ontario, Canada), 0.8 M betaine (Sigma-Aldrich, USA), 6 mM $MgSO_4$ (Sigma-Aldrich, USA), 8 U of *Bst* DNA polymerase large fragment (Middleton, WI, USA), 1 × of the supplied buffer, and the template DNA extracted from inoculated samples. Hot-start LAMP was performed using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific Inc. Grand Island, NY, USA) as follows. Reaction mixtures without *Bst* polymerase were prepared on ice and then denatured at 94 °C for 2 min. Subsequently, *Bst* polymerase was added to the reaction mixtures, and LAMP was performed at 58–70 °C for 60 min. LAMP products were analyzed by electrophoresis on a 2% agarose gel. The reaction temperature and time were optimized to 63.5 °C for 45 min for the amplification of *E. coli* O157:H7 DNA.

LFA for detection of LAMP-amplified product

An oligonucleotide probe was designed to the sequence spanning the FIP and BIP regions and labeled with biotin at the 5′-end (Table 1). Five microliters containing 20 pmol of the labeled DNA probe was hybridized with the LAMP-amplified product at 63 °C for 5 min. An 8-µL sample of LAMP amplicons was transferred to a new tube and mixed with 120 µL of an LFA dilution solution (BoreDa Biotech, Gyeonggi, Korea) and placed on a sample pad of an LFA strip (BoreDa Biotech). LFA strips were immersed in the mixed solution, and DNA amplification was detected as positive (two lines) or negative (one line) after 5 min.

Table 1 Primer sequence sets for LAMP and real-time PCR detection of *E. coli* O157:H7

Species	Gene		Sequence (5′–3′)	5′ label	Reference or Genbank number
Escherichia coli	*Stx2* (for LAMP)	FIP	AGACGAAGATGGTCAAAACGCGCAGTTATTTTGCTGTGGA	5′ Dioxigenin	Zhao et al. (2010)
		BIP	CCGGGTTCGTTAATACGGCACGGGCACTGATATATGTGT		
		F3	TCGGTGTCTGTTATTAACCA		
		B3	TGGAAACCGTTGTCACA		
		Probe	CTACCGTTTTTCAGATTTT	5′ Biotin	AF162758.1
	Stx2 (for real-time PCR)	Forward	ATTAACCACACCCCACCG		Ibekwe et al. (2002)
		Reverse	GTCATGGAAACCGTTGTCAC		
		Probe	FAM-CAGTTATTTTGCTGTGGATATACGAGGGCTTG-TAMRA		

Real-time PCR detection of *E. coli* O157:H7

Primers and the probe used in real-time PCR were synthesized by IDT (San Diego, CA, USA), and the nucleotide sequences are shown in Table 1. Real-time PCR for *stx*2 (*E. coli* O157:H7) was performed to compare its detection limit with the LAMP–LFA method. The reaction mixture for real-time PCR was prepared by adding 10 µL of 2 × TaqMan® universal Master Mix (Applied Biosystems, Foster City, USA), 2 µL of DNA solution, 2 µL of both forward and reverse primers (500 nM), and 1 µL of the probe (250 nM) in a final volume of 20 µL with tertiary distilled water. Real-time PCR was performed on a 96-well microplate using the StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA). In the case of *E. coli* O157:H7, the reaction was performed at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Threshold cycle (Ct) values were analyzed using StepOne™ software (Applied Biosystems).

Statistical analysis

In this study, all experiments were performed in triplicate. Significant differences were assessed using analysis of variance at $P < 0.05$ for all parameters, and the mean comparisons were performed using Scheffe's test. Statistical analysis was performed by analysis of variance using SPSS (ver. 23.0, IBM Corp., Armonk, NY, USA).

Results

Membrane filter selection and recovery

To select the most suitable filter for the rapid determination of foodborne pathogens, *E. coli* O157:H7 was diluted to 10^0 CFU/mL (1–2 CFU/mL) in 225 mL of saline and filtered through five filters. The filters were incubated on EMB plates; after 24 h, characteristic black colonies were enumerated, and the recovery ratio was calculated (Table 2). Uninoculated beef was used as a control but did not form black colonies on EMB agar after incubation. The cellulose nitrate filter showed 96% recovery, and the filtration process took 100 s to complete. In all five repetitive tests, all filters successfully filtered without becoming clogged. The cellulose acetate filter showed 95% recovery but failed in one test as the filter became clogged. The nylon filter showed the lowest recovery ratio. Filtration time through the cellulose nitrate, cellulose acetate and polyethersulfone filters (filtration time of < 1 min) was significantly short ($P < 0.05$) than that through other filters. However, the polyethersulfone filter was lower in recovered cell (%) than the other two filters. The recovery efficiency of the filter was significantly higher ($P < 0.05$) in cellulose nitrate and cellulose acetate. The cellulose nitrate filter was similar in recovery efficiency to cellulose nitrate filter, but the production rate of injured cells was high (data not shown). If the percentage of injured cells is high, it may be a limit to detect low concentrations of bacteria. Therefore the cellulose nitrate filter showed the best performance and was selected for further filtration-based microbial concentration methods because it showed higher recovery ratio, speed, and reproducibility than other tested filters.

Table 2 Comparison of filter types for detecting *E. coli* O157:H7 in beef

Filter type	Pore size (μm)	Recovery of cells on the filter (%)	Filtration time	Probability of filtration
Cellulose nitrate	0.45	96 ± 16.7 a	100 ± 21 s b	5/5
Cellulose acetate	0.45	95 ± 30 a	55 ± 7 s b	4/5
Mixed cellulose ester	0.45	73 ± 45.6 ab	200 ± 42 s a	5/5
Polyethersulfone	0.45	84 ± 35.8 ab	49 ± 5 s b	5/5
Nylon	0.45	52 ± 30.3 b	234 ± 30 s a	5/5

Different lowercase letters indicate significant differences ($P < 0.05$) each % recovery and filtration time for each filter type

To detect the *E. coli* O157:H7 by molecular detection methods, such as PCR or LAMP, DNA must be isolated from *E. coli* O157:H7. DNA preparation of *E. coli* O157:H7 in a filter is very difficult, and it is more convenient to detach bacteria from the filter and then isolate the DNA. To identify the most efficient method for detaching *E. coli* O157:H7 from the cellulose nitrate filter after filtration, the recovery ratio was measured using three methods (vortexing, sonication, and stomaching) at different times (Table 3). The recovery ratio increased with increasing ($P < 0.05$) treatment time and frequency in all treatments. The percentage of injured cells in all treatments was not significantly different ($P > 0.05$). Vortexing, sonication, and stomaching methods recovered 41, 85, and 96% of cells from the filter, respectively. Finally, 200 cycles of stomaching were selected as the most efficient recovery method, which took approximately 60 s.

Optimization of temperature and time for LAMP

In LAMP, operating temperature and time are very important. To determine the optimal temperature and time combination for the LAMP assay, DNA was isolated from 10^6 CFU/mL of *E. coli* O157:H7, and LAMP was performed at different temperatures and times. LAMP amplicons showed a ladder-like pattern on an agarose gel due to the secondary structures of LAMP amplicons (Fig. 1). To determine the optimum temperature, reactions were conducted at 58–72 °C for 60 min in a thermocycler fixed at one temperature. LAMP amplicons of *E. coli* O157:H7 were detected at 58–66.9 °C. We confirmed the amplification of LAMP in the range of 58 °C and 66.9 °C. Of the five amplifiable temperatures, 63.5 °C were < 100 bp being the least and > 1500 bp the most. Therefore we used an amplification temperature of 63.5 °C. To optimize the reaction time, LAMP amplicons were obtained at 25, 30, 35, 40, 45, 50, and 60 min. It was thought that LAMP amplicons reached the highest concentration at 45 min (data not shown); therefore, 45 min was selected as the optimal operating time.

Comparison of detection limits in filtered and unfiltered samples

Ten-fold serial dilutions of DNA from *E. coli* O157:H7-inoculated beef were used as templates for LAMP and real-time PCR to determine the detection sensitivity (Table 4; Fig. 2). In real-time PCR analysis, the sensitivity of unfiltered beef was 10^3 CFU/g, and filtered beef showed a 37.04 Ct value at 10^2 CFU/g, thus improving the sensitivity by

Table 3 Comparison of methods for cell recovery from a cellulose nitrate filter

Filter type	Recovery method	Processing time/count	Recovery ratio (%)	Injured ratio (%)
Cellulose nitrate	Vortexing	15 s	18.42 ± 2.00 b	25.28 ± 3.22
		30 s	24.85 ± 0.91 b	26.21 ± 9.16
		60 s	41.96 ± 8.15 a	18.59 ± 8.16
	Sonication	15 s	70.72 ± 3.17	13.94 ± 8.36
		30 s	70.39 ± 2.17	28.02 ± 7.98
		60 s	85.09 ± 8.74	28.16 ± 5.66
	Stomaching	20 reps	48.83 ± 7.04 c	29.88 ± 4.25
		50 reps	71.05 ± 7.22 bc	35.97 ± 4.83
		100 reps	87.87 ± 13.66 ab	35.40 ± 3.55
		200 reps	96.16 ± 6.89 a	42.36 ± 0.80

Different lowercase letters indicate significant differences ($P < 0.05$) between recovery ratio in each recovery method

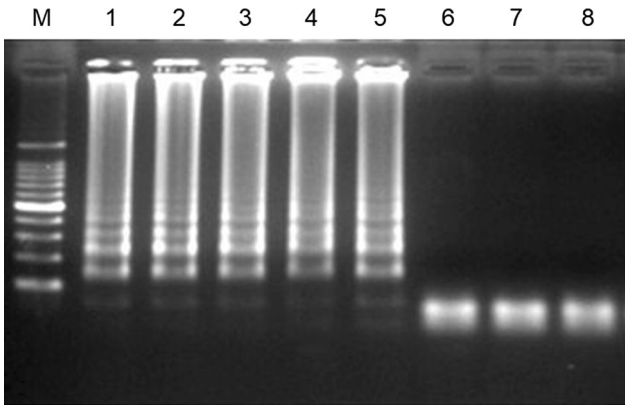

Fig. 1 LAMP product profiles at different reaction temperatures. Lane M: DNA marker (DL100); Lanes 1–8: LAMP reactions performed for 60 min at 58 °C, 58.9 °C, 60.8 °C, 63.5 °C, 66.9 °C, 69.7 °C, 71.2 °C and 72 °C, respectively

Table 4 Quantitation of *E. coli* O157:H7 in artificially contaminated beef by filtration and real-time PCR

Bacterial counts (CFU/g)	Mean Ct ± SD	
	Beef	
	Unfiltered	Filtered
10^5	29.00 ± 0.63	28.51 ± 0.26
10^4	32.40 ± 0.25	31.50 ± 1.10
10^3	35.28 ± 0.21	35.61 ± 0.57
10^2	–	37.04 ± 0.42
10^1	–	–
10^0	–	–

10-fold (Table 4). In LAMP, the sensitivity of unfiltered beef was 10^3 CFU/g and that of filtered beef was 10^1 CFU/g (Fig. 2). Filtering improves the sensitivity of real-time PCR and LAMP. LAMP–LFA showed 10 times higher sensitivity than real-time PCR.

Discussion

In this study, we successfully established a new filtration-based LAMP–LFA method targeting the *stx*2 gene of *E. coli* O157:H7. The new filtration-based assay could process 225-mL buffer containing 25-g sample and could detect a least 10^1-CFU/g (14–15 CFU/g) bacteria in 2.25-mL aliquot of the 225-mL sample within 3 h.

In artificially inoculated beef, the sensitivity of filtration-based LAMP–LFA was 10 CFU/g (0.8 CFU per reaction), which was 10 times more than that of the filtration-real-time-PCR assay (10^2 CFU/g; 8 CFU per reaction). In a previous study, the detection limit of a filtration-based LAMP–LFA assay for *E. coli* O157:H7 was more sensitive than that of an unfiltered LAMP assay using spiked food samples (> 10^4 CFU mL). Wang et al. (2012) and Zhao et al. (2010) reported that the detection limit of *E. coli* O157:H7 using LAMP was 10 pg (10^4 CFU/mL) without using enrichment. The sensitivity of their methods was low to detect foodborne pathogens present in very small amounts (< 10^3 CFU/mL). In addition, no previous study has been conducted on DNA detection with LAMP using LFA after concentrating the sample by filtration.

The enhanced sensitivity of the methods reported here is believed to be because of the following three reasons. First, LAMP has higher specificity than conventional PCR and rapidly amplifies DNA. LAMP holds great potential as a rapid and sensitive molecular method for detecting DNA from various materials, including foods. Although PCR is inhibited by various food ingredients, such inhibitors do not affect the LAMP assay (Plutzer et al. 2010), which improves the sensitivity of detection. This has already been demonstrated in previous studies, in which the effectiveness of PCR and LAMP was compared using oyster and shrimp samples (Surasilp et al. 2011; Prompamorn et al. 2011). Second, the membrane filtration method physically concentrates *E. coli* O157:H7. In this study, homogenized, filtered *E. coli* O157:H7 cells could be efficiently recovered from 2.25-mL aliquot of the 225-mL sample, theoretically resulting in a 100-fold increase in concentration. Several studies have reported the use of a filtration method for similar reasons (Wolffs et al. 2006; Kaevska and Slana 2015). Third, filtration enhances detection sensitivity by removing inhibitors, such as protein and lipid components (Rossen et al. 1992).

LFA can be easily performed by passing on assay buffer through the membrane and can decrease the total time of LAMP assays by eliminating electrophoresis. Furthermore, the LAMP–LFA assay provides more specificity because it involves hybridization with a specific probe to the LAMP amplicons (Prompamorn et al. 2011).

The use of filtration in combination with LAMP–LFA does not require specialized expertise, and the enrichment step increases the assay's sensitivity. The entire process for detecting *E. coli* O157:H7 in beef comprises sequential steps of filtration, LAMP, and LFA; the total time for detection can be decreased to ≤ 3 h.

Conclusion

In this study, a new filtration-based LAMP–LFA method was developed to detect the presence of *E. coli* O157:H7 in beef. The filtration-based pretreatment step effectively reduced the overall analysis time and improved sensitivity by concentrating bacteria up to the detection limit to

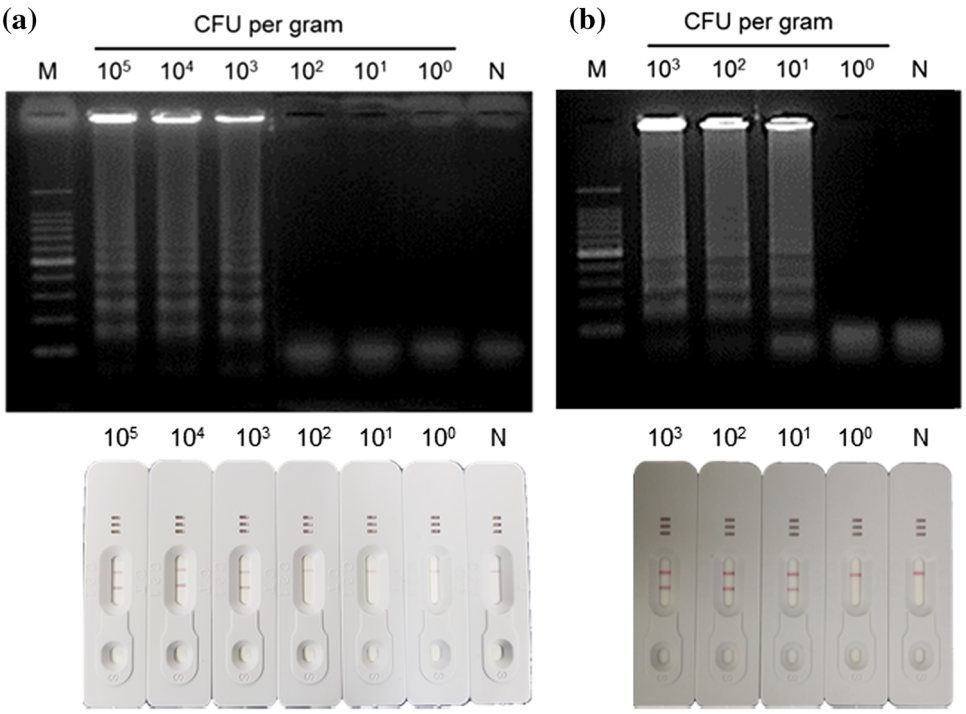

Fig. 2 LAMP sensitivity increased after filtration for detecting *E. coli* O157:H7 in inoculated beef by electrophoresis (top panel) and LFA (bottom panel). **a** Unfiltered samples and **b** filtered samples. A volume of 4 μL of LAMP amplicons from 25 μL reactions was loaded per lane. Lane M: molecular marker; lane N: negative control

overcome the need for a microbial enrichment step. The sensitivity of real-time PCR and LAMP of filtered treatment was improved 10–100 fold compared to unfiltered controls. The concentration of 10^1 CFU/g of *E. coli* O157:H7 could be detected within 3 h without microbial enrichment. LFA was used to confirm the detection of *E. coli* O157:H7 with improved accuracy and simplicity. This new filtration-based LAMP–LFA method is potential for POCT because it allows for the detection of *E. coli* O157:H7 in $\leq$ 3 h at an isothermal temperature and does not require complex equipment or trained technical personnel. Our study may enable an approach that the food industry can detect before delivering contaminated food.

Acknowledgements This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2017R1D1A1B03030859).

References

Avery SM, Hudson JA, Penney N (1994) Inhibition of *Listeria monocytogenes* on normal ultimate pH beef (pH 5.3–5.5) at abusive storage temperatures by saturated carbon dioxide controlled atmosphere packaging. J Food Prot 57:331–333

Chapman P, Ellin M, Ashton R (2001) A comparison of immunomagnetic separation and culture, RevealTM and VIPTM for the detection of *E. coli* O157 in enrichment cultures of naturally-contaminated raw beef, lamb and mixed meat products. Lett Appl Microbiol 32:171–175

Dong H-J, Cho A-R, Hahn T-W, Cho S (2014) Development of a multiplex loop-mediated isothermal amplification assay to detect shiga toxin-producing *Escherichia coli* in cattle. J Vet Sci 15:317–325

D'Urso OF, Poltronieri P, Marsigliante S, Storelli C, Hernández M, Rodríguez-Lázaro D (2009) A filtration-based real-time PCR method for the quantitative detection of viable *Salmonella enterica* and *Listeria monocytogenes* in food samples. Food Microbiol 26:311–316

Kaevska M, Slana I (2015) Comparison of filtering methods, filter processing and DNA extraction kits for detection of mycobacteria in water. Ann Agric Environ Med 22:429–432

Kim SY, Kang DH, Kim JK, Ha YG, Hwang JY, Kim T, Lee SH (2011) Antimicrobial activity of plant extracts against *Salmonella* Typhimurium, *Escherichia coli* O157: H7, and *Listeria monocytogenes* on fresh lettuce. J Food Sci 76:M41–M46

Ibekwe AM, Watt PM, Grieve CM, Sharma VK, Lyons SR (2002) Multiplex fluorogenic real-time PCR for detection and quantification of *Escherichia coli* O157:H7 in dairy wastewater wetlands. Appl Environ Microbiol 68:4853–4862

Lee D, Kim YT, Lee JW, Seo TS (2016) An integrated direct loop-mediated isothermal amplification microdevice incorporated with an immunochromatographic strip for bacteria detection in human whole blood and milk without a sample preparation step. Biosens Bioelectron 79:273–279

Maheux AF, Bissonnette L, Boissinot M, Bernier J-LT, Huppé V, Picard FJ, Bérubé È, Bergeron MG (2011) Rapid concentration and molecular enrichment approach for sensitive detection of *Escherichia coli*/*Shigella* in potable water samples. Appl Environ Microbiol 77:6199–6207

Plutzer J, Törökné A, Karanis P (2010) Combination of ARAD microfibre filtration and LAMP methodology for simple, rapid and cost-effective detection of human pathogenic *Giardia duodenalis* and *Cryptosporidium* spp. in drinking water. Lett Appl Microbiol 50:82–88

Prompamorn P, Sithigorngul P, Rukpratanporn S, Longyant S, Sridulyakul P, Chaivisuthangkura P (2011) The development of loop-mediated isothermal amplification combined with lateral

flow dipstick for detection of *Vibrio parahaemolyticus*. Lett Appl Microbiol 52:344–351

Rossen L, Nørskov P, Holmstrøm K, Rasmussen OF (1992) Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. Int J Food Microbiol 17:37–45

Sagong H-G, Lee S-Y, Chang P-S, Heu S, Ryu S, Choi Y-J, Kang D-H (2011) Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. Int J Food Microbiol 145:287–292

Shan S, Lai W, Xiong Y, Wei H, Xu H (2015) Novel strategies to enhance lateral flow immunoassay sensitivity for detecting foodborne pathogens. J Agric Food Chem 63:745–753

Singh J, Sharma S, Nara S (2015) Evaluation of gold nanoparticle based lateral flow assays for diagnosis of enterobacteriaceae members in food and water. Food Chem 170:470–483

Surasilp T, Longyant S, Rukpratanporn S, Sridulyakul P, Sithigorngul P, Chaivisuthangkura P (2011) Rapid and sensitive detection of *Vibrio vulnificus* by loop-mediated isothermal amplification combined with lateral flow dipstick targeted to *rpoS* gene. Mol Cell Probes 25:158–163

Techathuvanan C, Draughon FA, D'Souza DH (2010) Loop-mediated isothermal amplification (LAMP) for the rapid and sensitive detection of *Salmonella* Typhimurium from pork. J Food Sci 75:M165–M172

Wang F, Jiang L, Ge B (2012) Loop-mediated isothermal amplification assays for detecting Shiga toxin-producing *Escherichia coli* in ground beef and human stools. J Clin Microbiol 50:91–97

Wang F, Yang Q, Qu Y, Meng J, Ge B (2014) Evaluation of a loop-mediated isothermal amplification suite for the rapid, reliable, and robust detection of Shiga toxin-producing *Escherichia coli* in produce. Appl Environ Microbiol 80:2516–2525

Wolffs PF, Glencross K, Thibaudeau R, Griffiths MW (2006) Direct quantitation and detection of salmonellae in biological samples without enrichment, using two-step filtration and real-time PCR. Appl Environ Microbiol 72:3896–3900

Yamazaki W, Kumeda Y, Uemura R, Misawa N (2011) Evaluation of a loop-mediated isothermal amplification assay for rapid and simple detection of *Vibrio parahaemolyticus* in naturally contaminated seafood samples. Food Microbiol 28:1238–1241

Yongkiettrakul S, Jaroenram W, Arunrut N, Chareanchim W, Pannengpetch S, Suebsing R, Kiatpathomchai W, Pornthanakasem W, Yuthavong Y, Kongkasuriyachai D (2014) Application of loop-mediated isothermal amplification assay combined with lateral flow dipstick for detection of *Plasmodium falciparum* and *Plasmodium vivax*. Parasitol Int 63:777–784

Zhao X, Li Y, Wang L, You L, Xu Z, Li L, He X, Liu Y, Wang J, Yang L (2010) Development and application of a loop-mediated isothermal amplification method on rapid detection *Escherichia coli* O157 strains from food samples. Mol Biol Rep 37:2183–2188