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Comparison of Loop-Mediated Isothermal Amplification (LAMP) and Conventional PCR for Detection of Shiga Toxin-Producing *Escherichia Coli* (STEC) in Various Food Products

Hyejin Jang, Yong sun Cho*

Food Analysis Research Center, Korea Food Research Institute

***Corresponding Author:** Yong Sun Cho, Food Analysis Center, Korea Food Research Institute 516 Baekhyeon-dong Bundang-gu, Seongnam-si Gyeonggi-do, Korea. Tel: +82-31-780-9242, +82-31-780-9280 E-mail: yscho@kfri.re.kr

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Abstract

Polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) were compared as molecular detection methods for food-borne pathogens. Green salad, ground beef, sausage, and seasoned meat were inoculated with Shiga toxin-producing *Escherichia coli* (STEC) to evaluate the limits of detection (LOD) and the limits of quantification (LOQ) of PCR and LAMP. Relative PCR and LAMP sensitivity, specificity and efficiency of STEC detection were evaluated. The LODs for diluted bacterial DNA were ≤ 10^4 CFU/mL and ≤ 10^2-10^3 CFU/mL according to PCR and LAMP, respectively. The LOQs for enrichment process it was confirmed that there was no difference between the PCR and LAMP. However, in the four food types, the detection sensitivity differed maximum by 11.1%(PCR, 28.9%LAMP, 40.0%for seasoned meat and minimum by 8.1% (PCR, 38.6%; LAMP, 46.7%) for ground beef. Only PCR returned false positives for ground beef and green salad. Their efficiency differed by 9.3% for seasoned meat (PCR, 40.7%LAMP, 50.0%)and green salad (PCR, 42.6%; LAMP, 51.9%) and by 7.4% for sausage (PCR, 64.8%; LAMP, 72.2%). LAMP had high sensitivity and 100% specificity for all four food types. Therefore, LAMP is a reliable molecular detection method for STEC as its detection rate is comparable and its specificity and sensitivity may be superior to those of PCR depending on the food type.

Keywords: Escherichia coli, Polymerase chain reaction

Introduction

The detection of pathogenic bacteria is vital to food safety. Pathogenic bacteria are monitored by traditional culture-based methods (Ministry of Food and Drug Safety, 2019). However, several molecular methods are also used to detect food-borne pathogenic bacteria. These include conventional PCR (polymerase chain reaction), real-time PCR, and loop-mediated isothermal DNA amplification (LAMP) [1]. Conventional PCR is the gold standard and is commonly used to detect bacterial genes. Real-time PCR is a widely used rapid detection method [2]. Unlike the amplification results of conventional PCR, those of real-time PCR can be verified without electrophoresis. Hence, analytical results can be quickly obtained [3,4]. Loop-mediated isothermal amplification (LAMP) requires no thermal cycle as it can amplify DNA at a single temperature [5,6,2].

The molecular method has a low limit of detection (LOD), which effectively identifies bacteria. Therefore, PCR is used to detect food-borne bacterial pathogens. However, PCR is costly as it requires dedicated laboratory equipment and well-trained technicians/operators [7]. Even in the presence of enrichment culture, pathogen growth may be inhibited by competition from food flora [8]. Moreover, food matrices interfere with DNA detection and PCR amplification. Therefore, it is necessary to apply a molecular detection method that has high selectivity and can increase the detection rate without interfering with food substrates or flora [9-11].

Enterohemorrhagic *E. coli* (EHEC) is a causative agent of food-borne illness and may be accompanied by hemolytic uremic syndrome (HUS). EHEC is a type of Shiga toxin-producing *E. coli* (STEC) and classified as a highly hazardous pathogen [12]. STECs have been divided into more than 400 serotypes, differing in physiological characteristics and pathogenic potential to humans, and it is currently impassible to fully define human pathogenic STECs [13,14]. In general, the serogroups focused on detecting STEC are the serogroups most frequently associated with human disease and pathogenesis, with O26, O45, O91, O103, O111, O121, O145, and O157 serogroups likely to be toxic to humans[14-16]. In addition, serogroups that are less frequently associated with human infection but can cause HUS throught food contamination include O113:H21, O174:H21, and O104:H4 [17]. The most widely used assays are aimed solely at the detection of *E.coli* O157[18], and relatively few investigations aiming at the detection of other STEC serogroups. Therefore, there is a need for a universal detection method that can detect not only O157 but other serotypes with high detection rates in recent years.

In this study, food was artificially inoculated with serogroups O157, O26, and O111, which were found most frequently in human diseases, and O104, which induces HUS. By comparing the sensitivity, specificity, and efficiency of PCR and LAMP in artificially inoculated food, an effective molecular method applicable to the detection of pathogenic bacteria in food was identified.

Materials and Methods

Bacterial Strains and Culture Conditions

Five Shiga toxin-producing *E. coli* strains (STEC) [*E. coli* O157 (NCTC 12079), *E. coli* O111 (NCCP 13518), *E. coli* O26 (NCCP 13667), *E. coli* O104 (NCCP 13721), and *E. coli* O104 (NCCP 15648)] and one nonpathogenic *E. coli* strain (ATCC 25922) were used in the present study. They were purchased from the National Culture Collection for Pathogens (NCCP; Cheongku, South Korea). All strains were pre-cultured in tryptic soy broth (Difco, Franklin Lakes, NJ, USA) at 35 °C for 9 h and cultured again in fresh medium at 35 °C for 18 h.

Food Samples

Seasoned meat, sausage, ground meat, and fresh green salad were purchased at various markets around Jeonju City. Each sample was checked by the Korea Food Code (KFC) method for the presence or absence of STEC. Only STEC-negative samples were used

in the subsequent analyses.

Limit Of Detection (LOD)

The enrichment culture was pelleted by centrifugation at 14,000 x *g* for 10 min, 15 °C. The bacterial pellet was washed and re-suspended in 0.85% (w/v) saline. The bacteria were enumerated 4.8 x 10^7 CFU/mL and the re-suspended bacteria were serially diluted (10^6–10^{-2}). The LOD of each dilution was confirmed by LAMP and PCR. Bacteria were counted after incubation 35 °C for 48 h on a plate count agar. (Difco, Franklin Lakes, NJ, USA)

Limit Of Quantification (LOQ)

In the first assay, the STEC-negative samples were thoroughly homogenized. Then 25 g of each was taken and inoculated at low (< 10^2 CFU/g), medium (10^2~10^3 CFU/g), and high (> 10^3 CFU/g) levels and subjected to enrichment culture. The inoculated strain was diluted to 0.5 McFarland with saline and the bacteria were enumerated. For the enrichment culture, 225 mL mTSB enrichment medium (Difco, Franklin Lakes, NJ, USA) was added and the suspension was incubated at 35 °C for 24 h. The DNA was extracted and toxin confirmation tests were performed by PCR and LAMP.

In the second assay, the STEC-negative samples were thoroughly homogenized. Then 25 g of each was taken and inoculated at low (< 10^2 CFU/g), medium (10^2~10^3 CFU/g), and high (> 10^3 CFU/g) levels. The DNA was extracted, toxin confirmation tests were performed by PCR and LAMP, and the bacteria were enumerated.

PCR Based on The Korea Food Code (KFC) Method

For this research, we used the PCR method in the Korea Food Code (KFC) method.

Total DNA extraction was performed with a Quick DNA Extraction Kit (Kogene Biotech, Seoul, Korea). The extracted DNA was used to identify the Shiga toxin genes *stx*1 and *stx*2 according to the method published in the KFC. The PCR primers were provided by KFC and are listed in (Table 1)

Table 1: Primers for conventional PCR based on KFC

Target gene	Sequence (5'→3')	Annealing temperature (C)	No. base pairs (bp)
***stx*1**	(F) ATA AAT CGC CAT TCG TTG ACT AC	Reaction condition of KFC	180
	(R) AGA ACG CCC ACT GAG ATC ATC		
***stx*2**	(F) GGC ACT GTC TGA AAC TGC TCC		255
	(R) TCG CCA GTT ATC TGA CAT TCT G		

For this research, we used the Molecular Detection System (MDS; 3M Co., Two Harbors, MN, USA) designed according to the LAMP method. The assay was conducted according to the manufacturer's (3M Co.) protocol. Twenty microliters culture medium was transferred to a lysis (LS) tube, heated to 100°C for 15 min with a heating block, and cooled in a chilling block for 5 min. Then 20 µL supernatant was taken from the cooled LS, transferred to a reagent tube, and measured in the MDS apparatus. The amplification result was confirmed using the instrument. The primers for STEC detection in MDS were provided with target *stx* and *eae* genes. The MDS was deemed positive when both primers were detected. However, the MDS was judged to be positive even when the *stx* gene alone was detected in this study.

The MDS designed by combining LAMP and bioluminescence detection technologies. In MDS, six different primers with high

specificity lead to stable amplification by *Bst* DNA polymerase. In addition, in bioluminescence detection, the DNA products pyrophosphate ions (*ppi*) and adenosine-5-O-persulfate (APS) enzymatically react with adenosine triphosphate (ATP) and light is emitted in the presence of luciferase. The combination of these two technologies enables real-time confirmation of the amplified product as a fluorescence value.

Data Analysis

True positives, false positives, false negatives, and true negatives were judged based on the strain. Sensitivity, specificity, and efficiency were calculated using the following formulae [19].

$$\%\ Sensitivity = \frac{True\ positive}{True\ positive\ +\ False\ negative} \times 100$$

$$\%\ Specificity = \frac{True\ negative}{True\ negative\ +\ False\ positive} \times 100$$

$$\%\ Efficiency = \frac{True\ positive\ +\ True\ negative}{Total} \times 100$$

Results

Toxin Gene Confirmation

According to LAMP, *E. coli* ATCC 25922 was confirmed negative while NCTC 12079 and NCCP 13581 were confirmed positive for KFC *stx*1/*stx*2. The *eae* was confirmed positive by LAMP. NCCP 13667 was confirmed positive for *stx*1 while NCCP 13721 and NCCP 15648 were confirmed positive for *stx*2. The foregoing strains were *stx*-positive according to LAMP (Table 2). Therefore, LAMP and PCR detected the same *stx* gene. Additionally, LAMP also detected the *eae* gene that can identify STEC. However, LAMP cannot distinguish between *stx 1* and *stx 2*. The *eae* gene is a factor that attaches *stx* toxin to intestinal epithelial cells, and if *eae* is further analyzed, STEC can be detected more accurately.

LOD (Limit Of Detection) Test Results

The assay detection limit was determined before any other effects on the detection rate were identified. PCR established a 10^4 LOD for all strains. In contrast, LAMP determined different detection limits depending on the gene. The strains with *stx*1/*stx*2 showed LOD = 10^2 while those with either *stx1* or *stx2* (but not both) or the other gene showed LOD = 10^3.

Consequently, the LOD for the diluted DNA can be confirmed up to 10^4 CFU/mL by PCR and up to 10^2 or 10^3 CFU/mL by LAMP (Table 2.)

Table 2: Limit of detection (LOD) according to PCR and LAMP of type strain target gene

		PCR		LAMP		LOD (CFU/mL)	
		*stx*1	*stx*2	*stx*	*eae*	PCR	LAMP
E. coli	ATCC					-	-
(Negative Control)	25922						
***E. coli* O157**	NCTC	o	o	o	o	104	102
	12079						
***E. coli* O111**	NCCP	o	o	o	o	104	102
	13581						
***E. coli* O26**	NCCP	o		o	o	104	103
	13667						
***E. coli* O104**	NCCP		o	o		104	103
	13721						
***E. coli* O104**	NCCP		o	o		104	103
	15648						

LOQ (Limit Of Quantification) Test Results

STEC were directly inoculated into the sample to confirm detection in the presence of competitive food flora growth. After enrichment culture, detection was confirmed by PCR and LAMP. Seasoned meat, sausage, ground meat, and green salad did not significantly differ in terms of their detection levels (Table 3).

Table 3: Limit of quantification (LOQ) by PCR and LAMP and according to food type

***E. coli* inoculum**	**Food category**	**Inoculation level**	**PCR**		**LAMP**		
			*stx*1	*stx*2	*stx*	*eae*	No. detected
E. coli* O157 *(stx1, stx2)	seasoned meat	Low	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
		Medium	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
		High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
	sausage	Low	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
		Medium	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
		High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
	ground beef	Low	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
		Medium	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
		High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
	green salad	Low	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
		Medium	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
		High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar

E. coli (Negative Control)+G7:N33	seasoned meat	Low	0/3	0/3	0/3	0/3	0/3
		Medium	0/3	0/3	0/3	0/3	0/3
		High	0/3	0/3	0/3	0/3	0/3
	sausage	Low	0/3	0/3	0/3	0/3	0/3
		Medium	0/3	0/3	0/3	0/3	0/3
		High	0/3	0/3	0/3	0/3	0/3
	ground beef	Low	0/3	0/3	0/3	0/3	0/3
		Medium	0/3	0/3	0/3	0/3	0/3
		High	0/3	0/3	0/3	0/3	0/3
	green salad	Low	0/3	0/3	0/3	0/3	0/3
		Medium	0/3	0/3	0/3	0/3	0/3
		High	0/3	0/3	0/3	0/3	0/3

In addition, all artificially inoculated strains were detected regardless of the inoculation level. Up to a detectable range, competitive growth was possible even if food flora and a few pathogens were present. The same results were obtained by PCR and LAMP for the enrichment culture even in the presence of a few pathogens [4]. There were no significant differences between PCR and LAMP in terms of sensitivity or specificity according to the enrichment process.

Detection Rate According to Food Types of Artificially Contaminated Samples

The detection rates of PCR and LAMP were confirmed in foods artificially inoculated in three stages of low, medium, and high level (Table 4).

Table 4: Relative PCR and LAMP performance at detecting STEC inoculated at various levels in food products (n = 3)

Food category	**Inoculate *E. coli***		**Inoculation level**	**PCR**		**LAMP**		
				*stx*1	*stx*2	*stx*	*eae*	Detect
seasoned meat	*E. coli*	ATCC 25922	Low	0/3	0/3	0/3	0/3	0/3
	(Negative Control)		Medium	0/3	0/3	0/3	0/3	0/3
			High	0/3	0/3	0/3	0/3	0/3
	E. coli O157	NCTC	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*1, *stx*2)	12079	Medium	0/3	0/3	02-Mar	01-Mar	0/3
			High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
	E. coli O111	NCCP 13581	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*1, *stx*2, *eae*)		Medium	0/3	0/3	0/3	0/3	0/3
			High	03-Mar	03-Mar	02-Mar	0/3	0/3
	E. coli O26	NCCP 13667	Low	0/3	0/3	0/3	0/3	0/3

	(*stx*1)		Medium	0/3	0/3	01-Mar	0/3	0/3
			High	01-Mar	0/3	03-Mar	01-Mar	01-Mar
	E. coli O104	NCCP 13721	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*2)		Medium	0/3	0/3	01-Mar	0/3	0/3
			High	0/3	03-Mar	03-Mar	0/3	0/3
	E. coli O104	NCCP 15648	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*2)		Medium	0/3	0/3	0/3	0/3	0/3
			High	0/3	03-Mar	03-Mar	0/3	0/3
sausage	*E. coli*	ATCC 25922	Low	0/3	0/3	0/3	0/3	0/3
	(Negative Control)		Medium	0/3	0/3	0/3	0/3	0/3
			High	0/3	0/3	0/3	0/3	0/3
	E. coli O157	NCTC	Low	0/3	02-Mar	01-Mar	0/3	0/3
	(*stx*1, *stx*2)	12079	Medium	01-Mar	01-Mar	03-Mar	03-Mar	03-Mar
			High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
	E. coli O111	NCCP	Low	03-Mar	01-Mar	01-Mar	0/3	0/3
	(*stx*1, *stx*2, *eae*)	13581	Medium	02-Mar	0/3	03-Mar	03-Mar	03-Mar
			High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
	E. coli O26	NCCP	Low	01-Mar	0/3	0/3	01-Mar	0/3
	(*stx*1)	13667	Medium	0/3	0/3	02-Mar	02-Mar	02-Mar
			High	03-Mar	0/3	03-Mar	03-Mar	03-Mar
	E. coli O104	NCCP	Low	0/3	01-Mar	0/3	0/3	0/3
	(*stx*2)	13721	Medium	0/3	01-Mar	02-Mar	0/3	0/3
			High	0/3	02-Mar	03-Mar	0/3	0/3
	E. coli O104	NCCP	Low	0/3	01-Mar	0/3	0/3	0/3
	(*stx*2)	5648	Medium	0/3	0/3	03-Mar	0/3	0/3
			High	0/3	03-Mar	03-Mar	0/3	0/3
ground beef	*E. coli*	ATCC	Low	0/3	0/3	0/3	0/3	0/3
	(Negative Control)	25922	Medium	0/3	0/3	0/3	0/3	0/3
			High	0/3	0/3	0/3	0/3	0/3
	E. coli O157	NCTC	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*1, *stx*2)	12079	Medium	02-Mar	02-Mar	03-Mar	01-Mar	01-Mar
			High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar

	E. coli O111	NCCP	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*1, *stx*2, *eae*)	13581	Medium	0/3	0/3	01-Mar	01-Mar	01-Mar
			High	03-Mar	03-Mar	03-Mar	02-Mar	03-Mar
	E. coli O26	NCCP	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*1)	13667	Medium	0/3	0/3	01-Mar	0/3	0/3
			High	03-Mar	0/3	03-Mar	03-Mar	03-Mar
	E. coli O104	NCCP	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*2)	13721	Medium	0/3	0/3	0/3	0/3	0/3
			High	0/3	03-Mar	03-Mar	0/3	0/3
	E. coli O104	NCCP	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*2)	15648	Medium	01-Mar	0/3	01-Mar	0/3	0/3
			High	0/3	03-Mar	03-Mar	0/3	0/3
green salad	*E. coli*	ATCC	Low	0/3	0/3	0/3	0/3	0/3
	(Negative Control)	25922	Medium	0/3	0/3	0/3	0/3	0/3
			High	0/3	0/3	0/3	0/3	0/3
	E. coli O157	NCTC	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*1, *stx*2)	12079	Medium	01-Mar	01-Mar	02-Mar	01-Mar	0/3
			High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
	E. coli O111	NCCP	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*1, *stx*2, *eae*)	13581	Medium	0/3	0/3	02-Mar	0/3	0/3
			High	03-Mar	03-Mar	03-Mar	03-Mar	03-Mar
	E. coli O26	NCCP	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*1)	13667	Medium	01-Mar	01-Mar	0/3	01-Mar	0/3
			High	01-Mar	0/3	03-Mar	03-Mar	03-Mar
	E. coli O104	NCCP	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*2)	13721	Medium	0/3	0/3	0/3	0/3	0/3
			High	0/3	03-Mar	03-Mar	0/3	0/3
	E. coli O104	NCCP	Low	0/3	0/3	0/3	0/3	0/3
	(*stx*2)	15648	Medium	0/3	0/3	0/3	0/3	0/3
			High	01-Mar	02-Mar	03-Mar	0/3	0/3

For seasoned meat, PCR detected only at high level whereas LAMP detected at medium level. For sausage, both PCR and LAMP detected at low level. The highest detection rate was confirmed for sausage. For ground beef and green salad, both PCR and LAMP detectable at medium level. PCR detected once or twice out of three repetitions whereas LAMP detected all three times and had a higher detection rate than PCR.

Statistical Analyses

Statistical processing of the number of inoculated bacteria per food type confirmed that the sensitivity, specificity, and efficiency (Table 5.)

Table 5: Statistical analyses of PCR and LAMP detection of STEC in various food products

Food category	Detection method	False positive	True positive	False negative	True negative	Sensitivity (%)	Specificity (%)	Efficiency (%)
seasoned meat	PCR	0	13	32	9	28.9	100	40.7
	LAMP	0	18	27	9	40	100	50
sausage	PCR	0	26	19	9	57.8	100	64.8
	LAMP	0	30	15	9	66.7	100	72.2
ground beef	PCR	1	17	27	9	38.6	90	48.1
	LAMP	0	21	24	9	46.7	100	55.6
green salad	PCR	2	14	29	9	32.6	81.8	42.6
	LAMP	0	19	26	9	42.2	100	51.9

They differed in sensitivity by 11.1%for seasoned meat (PCR, 28.9%; LAMP, 40.0%) and by 8.1% for ground beef (PCR, 38.6%; LAMP, 46.7%). Hence, LAMP had high sensitivity for all four food types. While LAMP had 100% specificity for all four food groups, the specificity of PCR was 90% for ground beef and 81.8% for green salad. Only the PCR method returned false positives for ground beef and green salad. LAMP and PCR differed in efficiency by 9.3% for seasoned meat (PCR, 40.7%; LAMP, 50.0%) and green salad (PCR, 42.6%; LAMP, 51.9%) and by 7.4% for sausage (PCR, 64.8%; LAMP, 72.2%). Thus, LAMP sensitivity was high for all four food types. [20-24] reported that LAMP had higher detection probability than PCR in the presence of complex food matrices. Both the food and medium components interfere with primer binding and polymer synthesis. The probability of returning false positives with LAMP was low as this method is less susceptible to interference from food flora and matrices than PCR [21,22].

Discussion

This study compared the effectiveness of LAMP (we used the Molecular Detection System: MDS designed according to the LAMP method) and conventional PCR. In MDS, six different primers with high specificity lead to stable amplification by *Bst* DNA polymerase. In addition, in bioluminescence detection, the DNA products pyrophosphate ions (*ppi*) and adenosine-5-O-persulfate (APS) enzymatically react with adenosine triphosphate (ATP) and light is emitted in the presence of luciferase. The combination of these two technologies enables real-time confirmation of the amplified product as a fluorescence value. First, we checked the LOD of PCR and LAMP. PCR showed a LOD value of 104, but LAMP was able to confirm a lower LOD value of 102 or 103. By checking the LOD, it can be seen that the sensitivity of LAMP is superior to that of PCR. To determine the LOQ, food samples were directly inoculated with STEC and enrichment broth. Both PCR and LAMP detected artificially inoculated STEC, as there was no competitive growth between food flora and STEC regardless of food type. LAMP is reliable because its detection rate is similar to that of PCR (Table 3). The LOQ for the food types were determined by artificially inoculating them with different STEC concentrations. The detection limits slightly varied with food type and LAMP was generally more effective than PCR in this

capacity (Table 4). Overall, LAMP had relatively higher detection rate, efficiency, sensitivity, and specificity than PCR for this particular application. Except for enrichment, the detection sensitivity of food is inferior to both PCR and LAMP. Because the test sensitivity for food is not high, additional methods such as pretreatment analysis that can increase the sensitivity should be considered. The results of this study indicate that LAMP is reliable with similar detection rate, sensitivity, specificity, and efficiency compared to PCR. In addition, LAMP is not significantly affected by food flora and matrix compared to PCR. Therefore, it is a useful screening tool for foodborne bacterial pathogens.

Conclusion

According to the Korea Food Code (KFC), first, food is cultured with MTSB (Modified Tryptone Soy Broth) enriched medium. In the second step, the Shiga toxin gene is detected using PCR in the enriched culture medium. If Shiga toxin is confirmed, it must be purified and reconfirmed whether the isolated bacteria possesses the Shiga toxin gene. For isolation culture, SMAC medium and BCIG medium are used. When a typical colony is formed, the Shiga toxin gene is confirmed by PCR and biochemical testing is performed to determine STEC. In the process of confirming Shiga toxin from the enrichment broth, interference by the food matrix may occur, resulting in false-negative and false-positive results. Therefore, a more sensitive genetic-based detection method is needed. Our results demonstrate when two genetic-based detection methods were applied for each type of food, it was confirmed that the sensitivity and efficiency between the two methods were significantly different depending on the type of food. LAMP showed a higher value in all four food groups. In particular, it can be seen that the specificity is 100% because LAMP does not show false positives. Therefore, in this study, when LAMP is used as a detection method for various samples, it can be used as a more accurate detection method because it shows high sensitivity.

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Declarations

Conflict of interest The authors declare that they have no confict of interest.

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