pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 38, No. 5, pp. 347~355 (2023)
https://doi.org/10.13103/JFHS.2023.38.5.347

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Comparative Evaluation 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*

Department of Food Analysis Research Center, Korea Food Research Institute, Wanju, Korea

(Received May 18, 2023/Revised August 21, 2023/Accepted September 19, 2023)

ABSTRACT - In this study, polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) were compared in terms of their ability to detect shiga-toxin-producing *Escherichia coli* (STEC). Various foods were artificially inoculated with STEC to evaluate the limit of detection (LOD), limit of quantification (LOQ), sensitivity, specificity, and efficiency of PCR and LAMP. The LODs were $\leq 10^4$ and $\leq 10^3$ CFU/mL for PCR and LAMP, respectively. The LOQs did not differ between PCR and LAMP. However, of the four considered food types, the sensitivities differed by a maximum of 11.1% for seasoned meat and by a minimum of 8.1% for ground beef. LAMP had higher sensitivity than that of PCR and 100% specificity for all four food types. Therefore, LAMP is a reliable molecular method for detecting STEC as comparable to PCR assay, and its specificity and sensitivity are superior to those of PCR, depending on the food type.

Key words: LAMP, PCR, STEC, Food, Detection

The detection of pathogenic bacteria is vital to food safety. Pathogenic bacteria are monitored by traditional culture-based methods. However, several molecular methods are also used to detect food-borne pathogenic bacteria[1]. These include conventional polymerase chain reaction (PCR), 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[3]. Hence, analytical results can be quickly obtained[3,4]. LAMP requires no thermal cycle as it can amplify DNA at a single temperature[2,5,6].

The molecular method has a low limit of detection (LOD), which effectively identifies bacteria. Therefore, PCR is used to detect food-borne bacterial pathogens[1,4]. 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 microbes[8]. Moreover, the Korea Food Code first steps a Shiga toxin gene identification test with an enrichment medium and determines non-detection or detection according to the result[4]. 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 microbes[3,9].

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[10]. 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[11,12]. 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[13-16]. Addition, serogroups that are less frequently associated with human infection but can cause HUS into food contamination include O113:H21, O174:H21, and O104:H4[17]. The most widely used assays are aimed solely at the detection of

*Correspondence to: YongSun Cho, Department of Food Analysis Research Center, Korea Food Research Institute, Wanju 55365, Korea
Tel: +82-63-219-9242, Fax: +82-63-219-9280
E-mail: yscho@kfri.re.k

Table 1. Primers for conventional PCR based on the Korea food code (KFC)

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

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. Consequently, 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* (STEC) strains [*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, Cheongju, Korea). All strains were pre-cultured in tryptic soy broth (DIFCO, Tucker, GA, 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[4]. Only STEC-negative samples were used in the subsequent analyses.

DNA extraction

For this research, we used a Quick DNA Extraction Kit (Kogene Biotech, Seoul, Korea). The 1mL of enrichment medium was centrifuged 12,000×*g* for 5 min. The supernatant removed, and the pellet was suspended in Quick DNA extraction solution 700 μL. The suspension was heated 95-100°C for 10 min. After heating is completed, centrifuge at 12,000×*g* for 5 min, and use the supernatant as template DNA for PCR and LAMP. The extracted DNA was diluted 90-100 ng/μL before using PCR and LAMP.

Table 2. PCR (KFC) conditions used in this study

Step	Process	Temperature (°C)	Time (sec)	No. of cycle
1	Denaturation	95	60	10
	Annealing	65	120	
	Extension	72	90	
2	Denaturation	95	60	1
	Annealing	64	120	
	Extension	72	90	
3	Denaturation	95	60	1
	Annealing	63	120	
	Extension	72	90	
4	Denaturation	95	60	1
	Annealing	62	120	
	Extension	72	90	
5	Denaturation	95	60	1
	Annealing	61	120	
	Extension	72	90	
6	Denaturation	95	60	11
	Annealing	60	120	
	Extension	72	90	
7	Denaturation	95	60	10
	Annealing	60	120	
	Extension	72	150	
8	Store	4	-	-

PCR based on the Korea food code (KFC)

For this research, we used the PCR method in the Korea food code (KFC)[4].

Total DNA extraction was performed with a Quick DNA Extraction Kit (Kogene Biotech). The extracted DNA was used to identify the shiga toxin genes *stx*1 and *stx*2 according to the method published in the KFC[4]. The PCR primers were provided by KFC and are listed in Table 1 and Table 2.

LAMP assay by MDS

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.

Limit of detection (LOD)

The enrichment culture was pelleted by centrifugation at 14,000×*g* for 10 min, 15°C. The bacterial pellet was washed and re-suspended in 0.85% (w/v) saline. Bacteria counted from 1.0×10^{6}-1.0×10^{1} CFU/mL and detection was confirmed by repeating the experiment according to each test method of LAMP and PCR.

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, Tucker, GA, 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.

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[18].

% Sensitivity = True positive/(True positive + False negative) × 100

% Specificity = True negative/(True negative + False positive) × 100

% Efficiency = True positive + True negative/Total test × 100

Results

Toxin gene confirmation

As a result of comparing the LAMP and PCR (KFC) test methods, *E. coli* ATCC 25922 was analyzed as negative for both *stx* and *eae* genes in both tests. In the case of

Table 3. LOD according to PCR and LAMP of type strain target gene

		PCR (KFC)		LAMP		LOD (CFU/mL)	
		*stx*1	*stx*2	stx	eae	PCR	LAMP
E. coli (Negative control)	ATCC25922					-	-
E. coli O157	NCTC 12079	Detected	Detected	Detected	Detected	10^4	10^2
E. coli O111	NCCP 13581	Detected	Detected	Detected	Detected	10^4	10^2
E. coli O26	NCCP 13667	Detected		Detected	Detected	10^4	10^3
E. coli O104	NCCP 13721		Detected	Detected		10^4	10^3
E. coli O104	NCCP 15648		Detected	Detected		10^4	10^3

NCTC 12079 and NCCP 13581, both *stx1* and *stx2* genes were analyzed positively by PCR method, and *stx* and *eae* genes were detected by LAMP method. NCCP 13667 was confirmed *stx1* positive by PCR, and *stx* and *eae* genes were analyzed by LAMP method. NCCP 13721 and NCCP 15648 were positive for *stx2*, and the *stx* gene was detected by LAMP assay (Table 3). Both test methods were able to detect the *stx* gene of the standard strain. Although the LAMP test method does not discriminate stx1 and stx2 genes, both *stx1* and *stx2* genes can be detected, so STEC *E. coli* analysis is expected to be possible in the same way as the test method in KFC. In addition to Shiga toxin, STEC attaches to the cytoplasmic membrane of intestinal epithelial cells, destroys intestinal microvilli, and causes attaching and effacing lesions. At this time, the *eae* gene is known to be essential[10].

Limit of detection (LOD) test results

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

Limit of quantification (LOQ) test results

STEC were directly inoculated into the sample to confirm detection in the presence of competitive food microorganisms. 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 4). In addition, all artificially inoculated strains were detected regardless of the inoculation level. Up to a

Table 4. Limit of quantification (LOQ) by PCR and LAMP according to various food type

E. coli inoculum	Food category	Inoculation level	PCR (KFC)		LAMP		
			*stx*1	*stx*2	stx	eae	Detect
E. coli O157 (*stx*1, *stx*2)	Seasoned meat	Low	3/3	3/3	3/3	3/3	3/3
		Medium	3/3	3/3	3/3	3/3	3/3
		High	3/3	3/3	3/3	3/3	3/3
	Sausage	Low	3/3	3/3	3/3	3/3	3/3
		Medium	3/3	3/3	3/3	3/3	3/3
		High	3/3	3/3	3/3	3/3	3/3
	Ground beef	Low	3/3	3/3	3/3	3/3	3/3
		Medium	3/3	3/3	3/3	3/3	3/3
		High	3/3	3/3	3/3	3/3	3/3
	Green salad	Low	3/3	3/3	3/3	3/3	3/3
		Medium	3/3	3/3	3/3	3/3	3/3
		High	3/3	3/3	3/3	3/3	3/3
E. coli (Negative control)	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

detectable range, competitive growth was possible even if food microbes 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 5). 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.

Table 5. Relative PCR (KFC) and LAMP performance at detecting STEC inoculated at various levels in food products (n=3)

Food category	Inoculate *E. coli*		Inoculation level	PCR (KFC)		LAMP		
				*stx*1	*stx*2	stx	eae	Detect
Seasoned meat	*E. coli* (Negative control)	ATCC 25922	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
	E. coli O157 (*stx*1, *stx*2)	NCTC 12079	Low	0/3	0/3	0/3	0/3	0/3
			Medium	0/3	0/3	2/3	1/3	0/3
			High	3/3	3/3	3/3	3/3	3/3
	E. coli O111 (*stx*1, *stx*2, *eae*)	NCCP 13581	Low	0/3	0/3	0/3	0/3	0/3
			Medium	0/3	0/3	0/3	0/3	0/3
			High	3/3	3/3	2/3	0/3	0/3
	E. coli O26 (*stx*1)	NCCP 13667	Low	0/3	0/3	0/3	0/3	0/3
			Medium	0/3	0/3	1/3	0/3	0/3
			High	1/3	0/3	3/3	1/3	1/3
	E. coli O104 (*stx*2)	NCCP 13721	Low	0/3	0/3	0/3	0/3	0/3
			Medium	0/3	0/3	1/3	0/3	0/3
			High	0/3	3/3	3/3	0/3	0/3
	E. coli O104 (*stx*2)	NCCP 15648	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	3/3	3/3	0/3	0/3
Sausage	*E. coli* (Negative control)	ATCC 25922	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
	E. coli O157 (*stx*1, *stx*2)	NCTC 12079	Low	0/3	2/3	1/3	0/3	0/3
			Medium	1/3	1/3	3/3	3/3	3/3
			High	3/3	3/3	3/3	3/3	3/3
	E. coli O111 (*stx*1, *stx*2, *eae*)	NCCP 13581	Low	3/3	1/3	1/3	0/3	0/3
			Medium	2/3	0/3	3/3	3/3	3/3
			High	3/3	3/3	3/3	3/3	3/3
	E. coli O26 (*stx*1)	NCCP 13667	Low	1/3	0/3	0/3	1/3	0/3
			Medium	0/3	0/3	2/3	2/3	2/3
			High	3/3	0/3	3/3	3/3	3/3
	E. coli O104 (*stx*2)	NCCP 13721	Low	0/3	1/3	0/3	0/3	0/3
			Medium	0/3	1/3	2/3	0/3	0/3
			High	0/3	2/3	3/3	0/3	0/3
	E. coli O104 (*stx*2)	NCCP 5648	Low	0/3	1/3	0/3	0/3	0/3
			Medium	0/3	0/3	3/3	0/3	0/3
			High	0/3	3/3	3/3	0/3	0/3

Table 5. (Continued) Relative PCR (KFC) and LAMP performance at detecting STEC inoculated at various levels in food products (n=3)

Food category	Inoculate *E. coli*		Inoculation level	PCR (KFC)		LAMP		
				*stx*1	*stx*2	stx	eae	Detect
Ground beef	*E. coli* (Negative control)	ATCC 25922	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
	E. coli O157 (*stx*1, *stx*2)	NCTC 12079	Low	0/3	0/3	0/3	0/3	0/3
			Medium	2/3	2/3	3/3	1/3	1/3
			High	3/3	3/3	3/3	3/3	3/3
	E. coli O111 (*stx*1, *stx*2, *eae*)	NCCP 13581	Low	0/3	0/3	0/3	0/3	0/3
			Medium	0/3	0/3	1/3	1/3	1/3
			High	3/3	3/3	3/3	2/3	3/3
	E. coli O26 (*stx*1)	NCCP 13667	Low	0/3	0/3	0/3	0/3	0/3
			Medium	0/3	0/3	1/3	0/3	0/3
			High	3/3	0/3	3/3	3/3	3/3
	E. coli O104 (*stx*2)	NCCP 13721	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	3/3	3/3	0/3	0/3
	E. coli O104 (*stx*2)	NCCP 15648	Low	0/3	0/3	0/3	0/3	0/3
			Medium	1/3	0/3	1/3	0/3	0/3
			High	0/3	3/3	3/3	0/3	0/3
Green salad	*E. coli* (Negative control)	ATCC 25922	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
	E. coli O157 (*stx*1, *stx*2)	NCTC 12079	Low	0/3	0/3	0/3	0/3	0/3
			Medium	1/3	1/3	2/3	1/3	0/3
			High	3/3	3/3	3/3	3/3	3/3
	E. coli O111 (*stx*1, *stx*2, *eae*)	NCCP 13581	Low	0/3	0/3	0/3	0/3	0/3
			Medium	0/3	0/3	2/3	0/3	0/3
			High	3/3	3/3	3/3	3/3	3/3
	E. coli O26 (*stx*1)	NCCP 13667	Low	0/3	0/3	0/3	0/3	0/3
			Medium	1/3	1/3	0/3	1/3	0/3
			High	1/3	0/3	3/3	3/3	3/3
	E. coli O104 (*stx*2)	NCCP 13721	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	3/3	3/3	0/3	0/3
	E. coli O104 (*stx*2)	NCCP 15648	Low	0/3	0/3	0/3	0/3	0/3
			Medium	0/3	0/3	0/3	0/3	0/3
			High	1/3	2/3	3/3	0/3	0/3

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 6). They differed in sensitivity by 11.1% for seasoned meat (PCR, 28.9%; LAMP, 40.0%) and by

Table 6. Statistical analyses of PCR (KFC) 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	100.0	40.7
	LAMP	0	18	27	9	40.0	100.0	50.0
Sausage	PCR	0	26	19	9	57.8	100.0	64.8
	LAMP	0	30	15	9	66.7	100.0	72.2
Ground beef	PCR	1	17	27	9	38.6	90.0	48.1
	LAMP	0	21	24	9	46.7	100.0	55.6
Green salad	PCR	2	14	29	9	32.6	81.8	42.6
	LAMP	0	19	26	9	42.2	100.0	51.9

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 [19, 20] 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 microbes and matrices than PCR[19,21].

Discussion

This study compared the effectiveness of PCR and LAMP at detecting food-borne bacterial pathogens. First, we checked the LOD of PCR and LAMP. PCR showed a LOD value of 10^4, but LAMP was able to confirm a lower LOD value of 10^2 or 10^3. 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. Both PCR and LAMP detected artificially inoculated STEC, as there was no competitive growth between food microbes and STEC regardless of food type. LAMP is reliable because its detection rate is similar to that of PCR (Table 4).

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 5). 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.

PCR is a universally used genetic method, but it has the disadvantage of low detection rate when it is interfered with by food matrix, so it is thought that a genetic method to compensate for this is necessary. Through this study, it was confirmed that LAMP has a higher detection rate than PCR because it has less interference with the food matrix. The more complex the food matrix, the better the detection by LAMP rather than PCR. In addition, PCR selectively detected Shiga toxin primers according to serotypes, but on the other hand, LAMP were detected regardless of O157, O111, O26, O104, so it was confirmed that various serotypes could be confirmed in one experiment (Table 3). Therefore, in order to use conventional PCR, additional studies on the nucleotide sequence of primers with broad selectivity according to serotype are required.

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 microbes and matrix compared to PCR. Therefore, it is a useful screening tool for foodborne bacterial pathogens.

국문 요약

본 연구에서는 시가독소 생성 대장균(STEC)을 검출하기 위해 식품공전의 polymerase chain reaction (PCR)검사법과 loop-mediated isothermal amplification (LAMP)를 비교하였다. PCR 및 LAMP의 검출 한계(LOD) 및 정량화 한계(LOQ), 민감도, 특이성 및 효율성을 평가하기 위해 다양한 식품에 STEC를 접종하였다. LOD는 PCR의 경우 10^4

CFU/mL 이하, LAMP의 경우 10^3 CFU/mL 이하로 측정되었다. LOQ 값은 PCR과 LAMP 간에 차이가 없었다. 그러나 4가지 식품군에서 민감도는 양념육이 최대 11.1%, 간소고기가 최소 8.1% 차이가 났다. LAMP는 네 가지 음식 유형 모두에 대해 높은 민감도와 100% 특이도를 보였다. 따라서 LAMP는 식품 유형에 따라 검출률이 비슷하고 특이도와 민감도가 식품공전 PCR보다 우수하기 때문에 STEC에 대한 신뢰할 수 있는 분자 검출 방법이다.

Acknowledgement

This research was supported by Main Research Program of the Korea Food Research Institute (KFRI) funded by the Ministry of Science, ICT & Future Planning and NEOGEN Korea

Conflict of interests

The authors declare no potential conflict of interest.

ORCID

Hyejin Jang https://orcid.org/0000-0002-1485-9196
Yong Sun Cho https://orcid.org/0000-0001-9972-1454

References

1. Hein, I., Flekna, G., Krassnig, M., Wagner, M. Real-time PCR for the detection of Salmonella spp. in food: an alternative approach to a conventional PCR system suggested by the FOOD-PCR project. *J. Microbiol. Methods*, **66**, 538-547 (2006).
2. Bird, P., Flannery, J., Crowley, E., Agin, J.R., Goins, D., Monteroso, L., Evaluation of the 3M™ Molecular Detection Assay (MDA) 2 - Salmonella for the Detection of Salmonella spp. in Select Foods and Environmental Surfaces: Collaborative Study, First Action 2016.01. *J. AOAC Int.,* **99**, 980-997 (2016).
3. Bird, P., Flannery, J., Crowley, E., Agin, J.R., Goins, D., Monteroso, L., Evaluation of the 3M™ Molecular Detection Assay (MDA) 2 - Listeria monocytogenes for the Detection of Listeria monocytogenes in a Variety of Foods and Select Environmental Surfaces: Collaborative Study, First Action 2016.08. *J. AOAC Int.,* **100**, 454-469 (2017).
4. Gwak, S.H., Lee, S.Y., Kim, J.H., Oh, S.W. Comparison of Loop-mediated Isothermal Amplification and Korea Standard Food Code (KFSC) Method for Detection of Salmonella Typhimurium, Listeria monocytogenes Artificially Inoculated in Yuk-hwe and Yuk-Sashimi.. *J. Food Hyg. Saf.*, **34**, 277-282 (2019).
5. Bird, P., Fisher, K., Boyle, M., Huffman, T., Benzinger, M.J.Jr., Bedinghaus, P., Flannery, J., Crowley, E., Agin, J., Goins, D., Benesh, D., David, J., Evaluation of 3M™ molecular detection assay (MDA) Salmonella for the detection of Salmonella in selected foods: collaborative study. *J. AOAC Int.,* **96**, 1325-1335 (2013).
6. Kiddle, G., Hardinge, P., Buttigieg, N., Gandelman, O., Pereira, C., McElgunn, C.J., Rizzoli, M., Jackson, R., Appleton, N., Moore, C., Tisi, L.C., Murray, J. A., GMO detection using a bioluminescent real time reporter (BART) of loop mediated isothermal amplification (LAMP) suitable for field use. *BMC Biotechnol.*, **12**, 1-13 (2012).
7. Shan, L., Abdul Haseeb, H., Zhang, J., Zhang, D., Jeffers, D. P., Dai, X., Guo, W., A loop-mediated isothermal amplification (LAMP) assay for the rapid detection of toxigenic Fusarium temperatum in maize stalks and kernels. *Int. J. Food Microbiol.*, **291**, 72-78 (2019).
8. Fortes, E.D., David, J., Koeritzer, B., Wiedmann, M., Validation of the 3M molecular detection system for the detection of listeria in meat, seafood, dairy, and retail environments. *J. Food Prot.*, **76**, 874-878 (2013).
9. Hu, L., Ma, L.M., Zheng, S., He, X., Wang, H., Brown, E.W., Hammack, T.S., Zhang, G., Evaluation of 3M Molecular Detection System and ANSR Pathogen Detection System for rapid detection of Salmonella from egg products. *Poult. Sci.*, **96**, 1410-1418 (2017).
10. Paton, J.C., Paton, A.W., Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. *Clin. Microbiol. Rev.*, **11**, 450-479 (1998).
11. Bettelheim, K.A., The non-O157 shiga-toxigenic (verocytotoxigenic) Escherichia coli; under-rated pathogens. *Crit. Rev. Microbiol.*, **33**, 67-87 (2007).
12. European Food Safety Authority (EFSA), Scientific opinion of the panel on biological hazards on a request from EFSA on monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types. *EFSA J.,* **579**, 1-61 (2007).
13. Auvray, F., Lecureuil, C., Taché, J., Leclerc, V., Deperrois, V., Lombard, B., Detection, isolation and characterization of Shiga toxin-producing Escherichia coli in retail minced beef using PCR based techniques, immunoassays and colony hybridization. *Lett. Appl. Microbiol.*, **45**, 646-651 (2007).
14. Fratamico, P.M., Bagi, L.K., Cray, W.C.Jr., Narang, N., Yan, X., Medina, M., Liu, Y., Detection by multiplex real-time polymerase chain reaction assays and isolation of Shiga toxin-producing Escherichia coli serogroups O26, O45, O103, O111, O121, and O145 in ground beef. *Foodborne Pathog. Dis.*, **8**, 601-607 (2011).
15. Madic, J., Vingadassalon, N., de Garam, C.P., Marault, M., Scheutz, F., Brugère, H,, Jamet, E., Auvray, F., Detection of Shiga toxin-producing Escherichia coli serotypes O26:H11, O103:H2, O111:H8, O145:H28, and O157:H7 in raw-milk cheeses by using multiplex real-time PCR. *Appl. Environ. Mcrobiol.*, **77**, 2035-2041 (2011).
16. Scheutz, F., Nielsen, E.M., Frimodt-Møller, J., Boisen, N., Morabito, S., Tozzoli, R., Nataro, J.P., Caprioli, A., Characteristics of the enteroaggregative Shiga toxin/verotoxin-pro-

ducing Escherichia coli O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. *Euro Surveill.,* **16**, 19889 (2011).
17. Rani, A., Ravindran, V.B., Surapaneni, A., Mantri, N., Ball, A.S., Review: Trends in point-of-care diagnosis for Escherichia coli O157:H7 in food and water. *Int. J. Food Microbiol.*, **349**, 1-12 (2021).
18. Abatcha, M.G., Tan, P.L., Chuah, L.O., Rusul, G., Chandraprasad, S.R., Effarizah, M.E., Evaluation of 3M™ loop-mediated isothermal amplification-based kit and 3M ready-to-use plating system for detection of Listeria in naturally contaminated leafy vegetables, chicken, and their related processing environments. *Food Sci. Biotechnol.*, **29**, 1141-1148 (2020).
19. Kim, J.H., Oh, S.W., Comparison of Loop-Mediated Isothermal Amplification and Real-Time PCR for the Rapid Detection of Salmonella Typhimurium, *Listeria monocytogenes* and Cronobacter sakazakii Artificially Inoculated in Foods. *J. Food Hyg. Saf.*, **34**, 135-139 (2019).
20. Wang, X., Seo, D.J., Lee, M.H., Choi, C., Comparison of conventional PCR, multiplex PCR, and loop-mediated isothermal amplification assays for rapid detection of Arcobacter species. *J. Clin. Microbiol.*, **52**, 557-563 (2014).
21. Lee, S.Y., Gwak, S.H., Kim, J.H., Oh, S.W., Comparison of Isolation Agar Method, Real-Time PCR and Loop-Mediated Isothermal Amplification-Bioluminescence for the Detection of Salmonella Typhimurium in Mousse Cake and Tiramisu. *J. Food Hyg. Saf.*, **34**, 290-295 (2019).