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Development of a LAMP Assay Targeting the rfbE Gene for Rapid Detection of Escherichia coli O157:H7

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ABSTRACT

Infections with *Escherichia coli (E. coli)* O157:H7 can lead to severe health complications. This pathogen is commonly found in contaminated meat and fresh produce, posing significant public health risks. The Loop-Mediated Isothermal Amplification (LAMP) method offers a rapid and accessible alternative to conventional nucleic acid amplification techniques, making it particularly suitable for on-site diagnostic systems. This study aimed to design a quick method using LAMP to detect the rfbE gene of *E. coli* O157:H7. *E. coli* ATCC 43888 was used as the positive control, while *Candida albicans* ATCC 10231, *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 served as negative controls. Positive and negative controls were tested 10 times each for both analyses. Following DNA isolation, real-time PCR and LAMP were performed and compared with culture methods. For the positive control strain (*E. coli* ATCC 43888) at 10 CFU/mL, positivity was detected in 8 out of 10 samples by real-time PCR and in 7 out of 10 samples by LAMP. The sensitivity, specificity, negative predictive value, and positive predictive value of LAMP and real-time PCR were 95.00%–96.67%, 100%–100%, 94.34%–96.15%, and 100%–100%, respectively. In conclusion, our study successfully developed a rfbE gene-specific LAMP kit for *E. coli* O157, demonstrating comparable sensitivity and specificity to real-time PCR and culture methods. This kit can be effectively utilized in resource-limited settings.

INTRODUCTION

Escherichia coli (E. coli) is a ubiquitous microorganism that resides in the gastrointestinal tracts of all living organisms. It plays a significant role in global morbidity and mortality in both humans and animals, with animal hosts being crucial in the epidemiology of infections. The adaptive and versatile characteristics of *E. coli* highlight the need for ongoing research within the One Health approach, which integrates human, animal, and environmental health (García and Fox, 2021).

E. coli is a highly adaptable pathogen capable of causing a wide range of diseases and is responsible for at least 2 million human fatalities annually. Its involvement in both intestinal and extraintestinal diseases was recognized shortly after its discovery (Foster-Nyarko and Pallen, 2022). Enteropathogenic *E. coli* strains are categorized based on serogroups, pathogenicity, and clinical symptoms. Enterohemorrhagic strains, in particular, spread through contaminated food and water, producing Shiga toxins. The most common strain, O157, has been associated with numerous foodborne outbreaks worldwide (Yinur et al., 2023).

E. coli O157:H7 is a significant public health concern, as it can cause severe illnesses such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). Even a small number of bacteria can result in serious infections. Given its high risk, the development of rapid and accurate diagnostic methods for *E. coli* O157:H7 is a global priority.

Traditional methods for detecting *E. coli* O157:H7 are often inefficient and time-consuming. Although newer immunological and genetic methods have been developed, they frequently face limitations such as low specificity or sensitivity. Molecular methods like PCR are more

promising but require specialized equipment and expertise. Thus, there remains a need for a more efficient and accessible diagnostic method for detecting *E. coli* O157:H7 (Ranjbar et al., 2016). In 2000, a new nucleic acid amplification technique called Loop-Mediated Isothermal Amplification (LAMP) was introduced. LAMP provides a faster and more accessible alternative to conventional nucleic acid amplification techniques. Its simplicity and ease of use make it particularly suitable for on-site diagnostic systems, and it can be adapted for detecting various pathogens (Kirkoyun et al., 2024).

Notably, all *E. coli* O157 strains have tested positive for the rfbE gene, which is specific to the O157 antigen, using PCR analysis (Tóth et al., 2009). The aim of this study was to develop a quick and simple LAMP-based method for detecting the rfbE gene of *E. coli* O157:H7.

MATERIALS AND METHODS

Escherichia coli American Type Culture Collection (ATCC) 43888 was performed as positive control for *E. coli* O157:H7. Candida albicans ATCC 10231, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 13883, Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 29213 were served as negative control. Positive and negative control used for 10 times for both real-time PCR and LAMP PCR analysis. A fresh bacterial culture was prepared by inoculating 10 ml of TSB (Oxoid, USA) with 1 µl of each strain and incubating overnight at 37°C (Liao et al., 2022).

DNA isolation was revealed using the boiling method, and dilutions of the DNA were prepared for standardization in optimization (Kirkoyun et al., 2024).

To design the LAMP assay for *E. coli* O157, LAMP Primer Sets were designed using PrimerExplorer Ver.5 (http://primerexplorer.jp/lampv5e/index.html) (accessed on 24/10/2024) with FASTA sequence file of the rfbE gene (GenBank ID: AF163332.1) as the target. B3, F3 (outer) and BIP, FIP (inner) primers were identified, and LF and LB primers were created to target single-stranded loop regions, utilizing database of NCBI's primer. The rfbE gene (GenBank ID: AF163332.1), a conserved sequence in *E. coli* O157:H7, was chosen as the target and highly conserved regions within this gene were identified using the BLAST program. Table 1 showed these primers and sequences.

Table 1. Oligonucleotid primers and sequence informations for *E. coli* O157

Label	5′pos	3′pos	len	Sequence
F3	63	87	25	GGAAATAAAACTATTACTACAGGTG
B3	248	271	24	CGTGATATAAAATGATCAGCTTGT
FIP			44	TTGGCCTTTAAAATGTAAACAACGGAAGGTGGAATGGTTGTCAC
BIP			45	AGCTGTACATAGGCAATATTGGCATCTGGGCTAATCCTATAGCAG
F2	88	106	19	AAGGTGGAATGGTTGTCAC
F1c	128	152	25	TTGGCCTTTAAAATGTAAACAACGG
B2	223	242	20	CTGGGCTAATCCTATAGCAG
B1c	158	182	25	AGCTGTACATAGGCAATATTGGCAT
LF	109	127	18	TGACAAAACACTTTATGA
LB	202	220	18	ATAGGATGACAAATATCT

Protocols of LAMP analysis were applied to each tube using the T1 System (BioRad) at 65°C for 30 minutes. Amplified products were analyzed on the LightCycler 480 (Roche Diagnostics) using a melting curve protocol, and melting analysis was used to consider the LAMP reactions. Real-Time PCR was conducted in a 96-well microplate using the LightCycler FastStart SYBR Green kit (Roche Diagnostics) on the LightCycler 480 instrument. Each run included a negative control without template and a positive control. The optimized reaction mixture for the stx1 gene primers (Jothikumar and Griffiths, 2002) with SYBR Green assays and 2µl of template DNA in a 20-µl volume. The cycling profile involved an initial 10-minute denaturation at 95°C, followed by 45 cycles of 95°C for 15 seconds, 55°C for 10 seconds, and 72°C for 15 seconds, with fluorescence measured at the end of each extension step. After PCR melting curve protocol was used (Jothikumar and Griffiths, 2002).

Test performance indicators for LAMP and Real-Time PCR results were calculated using the classical culture method as a reference. Sensitivity was measured using the formula true positive / (true positive + false negative), specificity with true negative / (true negative + false positive), positive predictive value with true positive / (true positive + false positive), and negative predictive value with true negative / (true negative + false negative) (Kirkoyun et al., 2024).

RESULTS

Table 2 summarizes the results obtained for all samples analyzed in this study. Using the positive control strain *Escherichia coli* ATCC 43888, positivity was observed in 8 out of 10 samples when tested with real-time PCR at a detection limit of 10 CFU/mL. Similarly, the LAMP PCR method successfully detected positivity in 7 out of 10 samples under the same conditions. These results demonstrate that both methods exhibit high sensitivity and are effective in detecting *E. coli* at low concentrations.

Table 3 provides a comparative analysis of the LAMP PCR and real-time PCR methods in relation to the results obtained through classical culture techniques, which are considered the gold standard in bacterial identification. This comparison highlights the practical applicability and performance metrics of each molecular technique when validated against traditional culture methods.

Table 4 presents the calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the LAMP PCR and real-time PCR methods. While both methods demonstrated high accuracy, it is noteworthy that the LAMP PCR method

exhibited a slightly lower sensitivity compared to real-time PCR. Despite this slight difference, the specificity of the LAMP PCR method was found to be comparable to that of real-time PCR, indicating its reliability in accurately identifying *E. coli* O157:H7.

These findings collectively suggest that LAMP PCR, despite minor differences in sensitivity, is a robust and reliable alternative to real-time PCR for detecting *E. coli* O157:H7, especially in resource-limited settings where advanced equipment may not be readily available.

Table 2. Distribution of the number of positives obtained after culture, Real-time PCR and LAMP PCR

		Culture	Real-Time PCR	LAMP PCR
*Escherichia coli ATCC 43888 - 10*6*	*Positive Control*	10	10	10
*Escherichia coli ATCC 43888 - 10*5*	*Positive Control*	10	10	10
*Escherichia coli ATCC 43888 - 10*4*	*Positive Control*	10	10	10
*Escherichia coli ATCC 43888 - 10*3*	*Positive Control*	10	10	10
*Escherichia coli ATCC 43888 - 10*2*	*Positive Control*	10	10	10
*Escherichia coli ATCC 43888 - 10*1*	*Positive Control*	10	8	7
Escherichia coli ATCC 25922	*Negative Control*	10	0	0
Staphylococcus aureus ATCC 29213	*Negative Control*	10	0	0
Pseudomonas aeruginosa ATCC 27853	*Negative Control*	10	0	0
Klebsiella pneumoniae ATCC 13883	*Negative Control*	10	0	0
Candida albicans ATCC 10231	*Negative Control*	10	0	0

Table 3. Comparison of LAMP PCR and Real-time PCR assay with culture results

		Culture		
		Positive	Negative	
LAMP PCR	Positive	57	0	57
	Negative	3	50	53
		60	50	
		Culture		
		Positive	Negative	
Real-Time PCR	Positive	58	0	58
	Negative	2	50	52
		60	50	

Table 4. Comparison of diagnostic results for LAMP PCR and Real-Time PCR tests

	LAMP PCR	Real-Time PCR
Sensitivity	95.00%	96.67%
Specificity	100.00%	100.00%
Positive predictive value	100.00%	100.00%
Negative predictive value	94.34%	96.15%

DISCUSSION AND CONCLUSION

E.coli O157 is a major foodborne pathogen with significant implications for food safety and public health. Its rapid and precise detection is crucial to minimizing the risk of foodborne illnesses and preventing outbreaks (Yin et al., 2024). In this study, we utilized the LAMP PCR technique as a potential tool for rapid and reliable detection of EHEC O157:H7.

Previous studies have emphasized the advantages of the LAMP technique in detecting EHEC O157:H7. Yinur et al., (2023) demonstrated that LAMP has higher sensitivity and specificity compared to traditional PCR. Their results also showed a high correlation between LAMP and PCR outcomes, suggesting that LAMP is particularly useful in resource-limited settings where cost-effectiveness and simplicity are essential. Consistent with their findings, our study observed that the LAMP assay displayed strong diagnostic accuracy. However, real-time PCR outperformed LAMP PCR in terms of precision under laboratory conditions, likely due to the controlled environment that enhanced its performance. Despite this, the LAMP technique remains a viable option in settings where advanced laboratory infrastructure is unavailable.

Ranjbar et al., (2016) highlighted the utility of targeting the rfbE gene for EHEC O157:H7 detection using the LAMP assay. Their assay achieved remarkable sensitivity, detecting DNA concentrations as low as 78 pg per reaction, and exhibited high specificity, with no cross-reactivity with non-EHEC strains. Our findings align with these results, as our rfbE-targeted LAMP assay also demonstrated high sensitivity and specificity. In addition, Wang et al., (2023) developed two LAMP-based methods: a real-time fluorescent LAMP (RT-LAMP) and a visual LAMP assay using calcein as an indicator. These assays targeted the Ecs_2840 gene and demonstrated excellent performance in detecting EHEC O157:H7 from pure bacterial cultures and milk samples. The RT-LAMP method detected as few as 8.8×10^0 CFU/mL, while the visual LAMP assay achieved a detection limit of 2.35×10^0 CFU/mL. These findings underline the adaptability of LAMP for use in various sample types, supporting our assertion that LAMP assays are promising tools for rapid pathogen detection in food safety applications.

Cui et al., (2024) evaluated two complementary methods, colorimetric LAMP and immunochromatographic test strips (ICTs), for detecting EHEC O157:H7. Both methods showed high specificity, with detection limits of 5.7 CFU/mL. Similarly, our study found that the LAMP assay we developed had a detection limit of 10 CFU/mL, closely aligning with these results. This level of sensitivity confirms the capability of LAMP as a competitive alternative for pathogen detection.

Qin et al., (2018) further expanded on the potential of LAMP by combining it with immunomagnetic separation (IMS), targeting the rfbE gene. Their IMS-LAMP method demonstrated both high sensitivity and specificity, detecting 3×10^1 CFU/mL in meat samples. Our findings, with a detection limit of 10 CFU/mL, are consistent with their results, demonstrating that LAMP can provide reliable performance across different applications and sample types. Despite these promising results, our study had certain limitations. We did not incorporate colorimetric analysis, which could have broadened the applicability of the LAMP assay in non-laboratory settings. Additionally, we did not test the assay with a wide range of routine biological or environmental samples. Future studies should address these limitations to further validate and optimize the utility of the LAMP assay in diverse conditions.

In conclusion, our study successfully developed an rfbE-specific LAMP kit for detecting *E. coli* O157. The assay demonstrated sensitivity and specificity comparable to real-time PCR, and its simplicity and cost-effectiveness make it an excellent option for resource-limited settings. These findings contribute to the growing evidence that LAMP is a valuable tool for improving food safety and public health diagnostics, offering a robust alternative to traditional methods in various settings.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authorship contributions

Concept: M.D., S.E., Design: M.D., S.E., Data Collection or Processing: M.D., S.E., Analysis or Interpretation: M.D., S.E., Literature M.D., S.E., Writing: M.D., S.E.

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