Chen *et al. Zoonoses* (2023) 3:37
DOI 10.15212/ZOONOSES-2023-0022

ORIGINAL ARTICLE

Visual and Rapid Detection of *Escherichia coli* O157:H7 in Stool Samples by FTA Card-based Loop-mediated Isothermal Amplification

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Abstract

Objective: *Escherichia coli* O157:H7 (*E. coli* O157:H7) can induce severe diseases in animals and humans that result in significant public health problems. Therefore, the development of rapid and visual detection methods to diagnose *E. coli* O157:H7 infections and monitor its prevalence is critical for the prevention and control purposes.

Methods: A colorimetric loop-mediated isothermal amplification (LAMP) assay was utilized to detect *E. coli* O157:H7. A DNA extraction kit and Flinders Technology Associates (FTA) cards were used to extract nucleic acid in conjunction with colorimetric LAMP detection.

Results: The method developed effectively distinguished *E. coli* O157:H7 from other pathogens with a detection limit of 25 CFU/mL in spiked stool samples. In addition, the nucleic acid of these samples was easily extracted and transported with an FTA card at room temperature. The entire detection process was completed within 35 min using simple constant-temperature equipment.

Conclusion: The colorimetric LAMP method with FTA card-based nucleic acid purification was shown to rapidly detect *E. coli* O157:H7 with sensitivity and specificity. This visual method is expected to be widely used to control *E. coli* O157:H7 infections, particularly in resource-limited settings.

Key words: *Escherichia coli* O157:H7, loop-mediated isothermal amplification (LAMP), Flinders Technology Associates (FTA) card, molecular diagnosis

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Received: June 8 2023
Revised: July 24 2023
Accepted: August 24 2023
Published Online: September 6 2023

INTRODUCTION

Escherichia coli O157:H7 (*E. coli* O157:H7) poses a significant threat to the health of poultry, such as ducks and chickens, causing a range of diseases, including egg peritonitis, perihepatitis, pericarditis, and salpingitis [1]. Additionally, consumption of foods or water contaminated by *E. coli* O157:H7 induces severe diseases and symptoms in human beings, such as abdominal pain, inflammation, diarrhea, and hemorrhagic colitis, particularly in vulnerable populations [2,3]. Therefore, it is critical to detect *E. coli* O157:H7 and monitor its prevalence in susceptible animals and human beings to minimize the adverse effects, which will ensure food

safety, protect human and animal health, and avoid financial losses [4].

Various technologies have been established for the detection of *E. coli* O157:H7, including molecular methods (e.g., polymerase chain reaction [PCR]), cultivation and plant counting, and serologic testing. The PCR method provides exceptional precision and accuracy, but typically requires long temperature cycles, specialized equipment, and well-trained personnel [5,6]. Serologic tests can produce negative results in the acute phase of the disease because antibodies are typically generated 7-14 days after infection. Therefore, these methods are not ideal for the on-site detection of *E. coli* O157:H7, particularly in resource-limited settings.

Recently, isothermal amplification techniques, such as recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), and nucleic acid sequence-based amplification (NASBA), have been developed with advantages of rapid detection at constant reaction temperature [7,8]. Among the techniques, LAMP is user-friendly and has shown great potential for the detection of *E. coli* O157:H7 in resource-limited areas [9]. Although several techniques for LAMP results readout have been reported, the most popular readout, gel electrophoresis, is time-consuming and easily induces false-positive results from aerosol contamination of amplicons [7]. To overcome this challenge, fluorescence-based methods have been developed. Specifically, Huang et al. [10] developed a fluorescence-based LAMP assay with high sensitivity to detect *E. coli* O157:H7 (10 copies/mL) and reported good performance in 150 food samples. Fluorescence methods have good sensitivity, sophisticated and bulky equipment with optical filters to collect detection results are typically required, which is unattainable in resource-limited areas.

Sample preparation, including nucleic acid extraction, is a key point for on-site detection of *E. coli* O157:H7; however, there are still some challenges in detecting bacterial DNA in complex stool samples [11,12]. For example, stool samples contain complex microbial mixtures, including viruses, bacteria, fungi, and parasites, and the existence of different microbial communities makes it difficult to specifically distinguish target bacteria [12]. In addition, the organic components in stool samples, including complex polysaccharides, urates, bilirubin, and byproducts of bile salt decomposition, interfere with the function of DNA polymerase required for nucleic acid amplification. Many nucleases contained in stool samples can also degrade nucleic acid targets. When transporting samples to the laboratory for storage and analysis in the absence of a stable, available, and reliable power supply, the cold chain may be difficult to maintain, which may lead to a destructive freeze-thaw cycle [11]. To address these issues, the Flinders Technology Associates (FTA) card offers a potential solution [13]. The FTA card is a functional filter paper modified with denatured and chelating agents that not only lyse captured bacteria, but also immobilize the genomic DNA. The FTA card contains chemicals that lysate cells and denature proteins, and protects nucleic acids from oxidative, nuclease, and UV damage. The FTA card is widely used for nucleic acid extraction from bacterial samples and is known for the rapid purification process, ease of use, and versatility [14]. The suitability of the FTA card for the colorimetric LAMP assay of stool samples, however, is not well-established [15]. Consequently, this study aimed to develop straightforward, swift, and rapid detection of *E. coli* O157:H7 from stool samples using the FTA card-based colorimetric LAMP method.

MATERIALS AND METHODS

Materials and reagents

Luria-Bertani (LB) broth medium, LB agar medium, brain heart infusion (BHI) broth medium, the Whatman FTA card, an FTA purification reagent, 1×TE buffer (10 mM Tris-HCl and 1 mM EDTA; pH=8.0) were purchased from Sigma-Aldrich (Shanghai, China). The WarmStart® Colorimetric LAMP 2X Master Mix was purchased from New England BioLabs (Ipswich, MA, USA). Rnase/Dnase-free water was purchased from Spark Jade (Shangdong, China). A 2-mm punch was purchased from Dongguan Allwin Stationery Co., Ltd. (Dongguan, China). A *Trelief*® Bacteria Genomic DNA kit for bacterial DNA extraction was purchased from Tsingke (Beijing, China). LAMP primers were purchased from Tsingke. *E. coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Salmonella enteritis* were purchased from CICC (Beijing, China). A dry bath was purchased from Coyote Bioscience Yixing Co., Ltd. (Yixing, China). All chemicals used were of analytical grade or better.

Bacterial cultivation and DNA extraction

Single colonies of *E. coli* O157:H7, *L. monocytogenes*, *P. aeruginosa*, and *S. enteritis* from LB agar plates were incubated in 5 mL of LB broth medium at 37°C with shaking at 200r/min until the OD 600 reached 0.8-1.0. Genomic DNA of *E. coli* O157:H7, *L. monocytogenes, P. aeruginosa*, and *S. enteritis* were extracted from *Trelief*® Bacteria Genomic DNA kits (Tsingke, Beijing, China) [16] or the Whatman FTA card, according to the manufacturer's instructions. The bacterial DNA extracted from the Whatman FTA card was constructed using the following protocols: 1) a 2-mm punch was used to create a circular 2-mm FTA membrane, and the samples were added onto the membrane and dried at room temperature [17] (considering the efficiency of nucleic acid extraction by an FTA card and the suitability with PCR tubes, the most suitable diameter of FTA is 2 mm); 2) the FTA membrane was placed into a 1.5-mL tube and washed with 200 μL of the FTA purification reagent; and 3) the FTA membrane was further washed with 200 μL of 1×TE buffer (10 mM Tris-HCl and 1 mM EDTA; pH = 8.0), then dried at 56°C for 20 min before detection.

LAMP and PCR primers synthesis

The LAMP and PCR primers for the detection of *E. coli* O157:H7 were synthesized by Tsingke (Nanjing) and are listed in Table 1.

Colorimetric LAMP reaction

The colorimetric LAMP reaction was carried out at 65°C for 35 min with specific LAMP primers in WarmStart® Colorimetric LAMP 2X Master Mix (New England BioLabs). The images of results from colorimetric LAMP reactions were captured using a cellphone camera and the hue value change of each tube was extracted by applications of hue analysis (e.g., Adobe Photoshop 2021 or our custom Android app-dubbed "Hue Analyzer"), and analyzed by GraphPad Prism 8 [19]. Three sets of parallel repeated LAMP reactions were performed. A one-way ANOVA test of GraphPad Prism 8 was used for statistical analysis. Significance thresholds were as follows: *p < 0.05; **p < 0.01; ***p < 0.001; and ****p < 0.0001.

Sensitivity and specificity

To evaluate the sensitivity of the method developed, *E. coli* O157:H7 DNA was extracted from FTA membranes at different bacterial concentrations (2.5-2.5×10^5 CFU/mL), which was quantified by bacterial culture and counting, and detected using the colorimetric LAMP method. To

TABLE 1 | LAMP and PCR primers of *uidA*.

Target pathogen	Target gene	Primer	Sequence (5′-3′)	Ref.
E. coli O157:H7	*uidA*	F3(LAMP)	CAGTAGAAACCCCAACCCG	[18]
		B3(LAMP)	ATACGCAGCACGATACGC	
		FIP(LAMP)	TAACGCGCTTTCCCACCAACGGCCTGTGGGCATTCAGTC	
		BIP(LAMP)	TAACGATCAGTTCGCCGATGCACTGCCCAACCTTTCGGTAT	
		LF(LAMP)	TCCACAGTTTTCGCGATCCA	
		LB(LAMP)	ACGTCTGGTATCAGCGCGAAGT	
		F(PCR)	CAGTAGAAACCCCAACCCG	
		R(PCR)	ATACGCAGCACGATACGC	

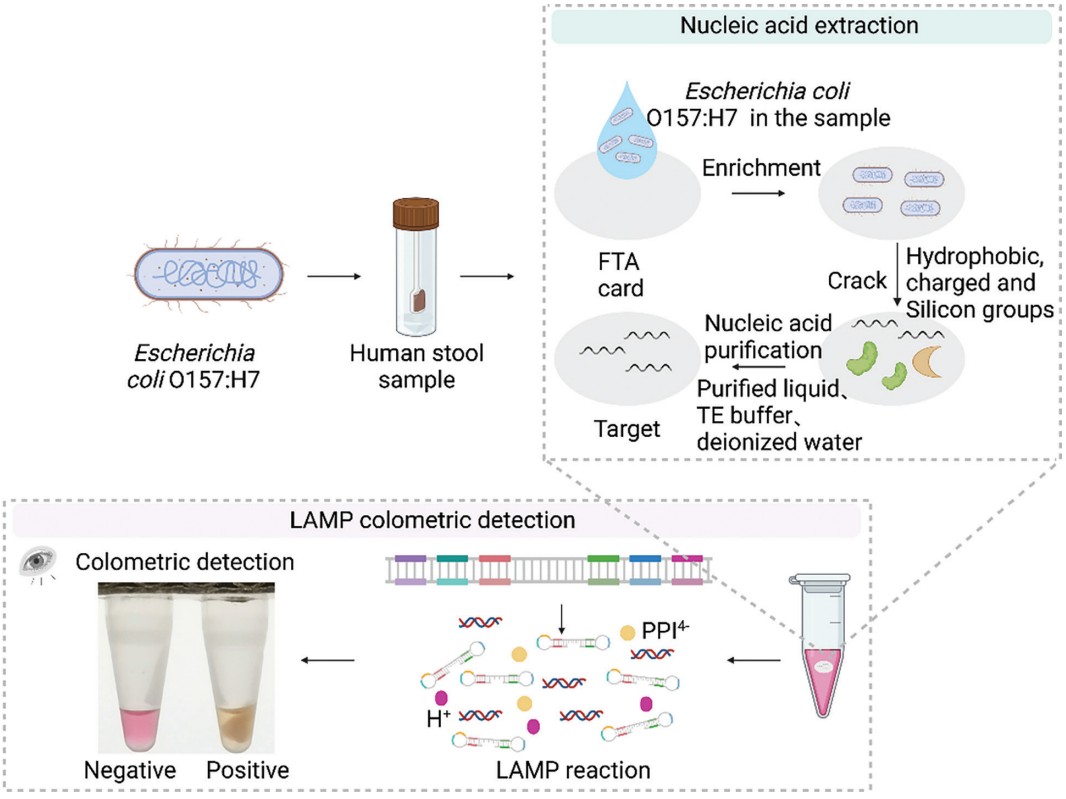

FIGURE 1 | Schematic illustration of FTA card-based colorimetric LAMP detection.

assess the specificity of the method, the DNA from *E. coli* O157:H7, *L. monocytogenes*, *P. aeruginosa*, and *S. enteritis* extracted from the FTA membrane was detected by the colorimetric LAMP assay with specific LAMP primers to *E. coli* O157:H7. Rnase/Dnase-free water was used as a negative control.

Pathogen detection using spiked human stool samples

The clinical samples were collected from Ruijin Hospital North (Shanghai Jiao Tong University School of Medicine, Shanghai, China) with Ethics Committee approval (No. 2017-02-01). The stool samples were spiked with different concentrations of *E. coli* O157:H7 bacteria (2.5-2.5×10^5 CFU/mL). Then, the stool samples were smeared onto a 2-mm FTA membrane and dried at room temperature before nucleic acid extraction and colorimetric LAMP detection.

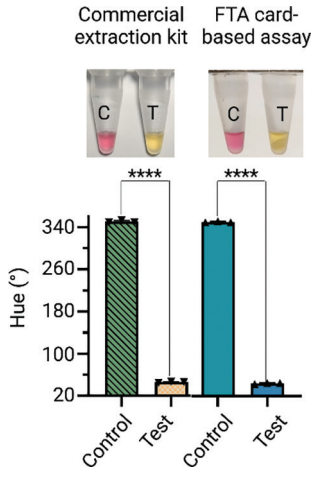

FIGURE 2 | Colorimetric LAMP detection of *E. coli* O157:H7 using the DNA from a commercial extraction kit and FTA card-based assay. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. All the experiments were repeated three times.

RESULTS

Establishment of FTA card-based colorimetric LAMP detection

In this study we utilized the FTA card for pathogen enrichment, cell lysis, collection, and purification of nucleic acids from human stool samples to develop a simple visual method (Fig 1) for the detection of *E. coli* O157:H7 from human stool samples by combining FTA-based nucleic acid sample preparation with colorimetric LAMP detection.

E. coli O157:H7 DNA extracted from a commercial DNA extraction kit was first used to test the performance of colorimetric LAMP detection. As shown in Fig 2, the positive group exhibited a color change from pink-to-yellow, while the negative group remained pink. Our previous study demonstrated that hue value-based colorimetric detection outperforms traditional red, green, and blue (RGB)-based colorimetric assays [19]. Therefore, the images after the reaction were first captured by a cell-phone camera, and the hue value change of each tube was extracted for analysis.

Next, the performance of the established FTA-based colorimetric LAMP detection was determined by comparison with the method using a DNA extraction kit. As shown in Fig 2, the detection result was consistent with the LAMP assay based on a commercial DNA extraction kit.

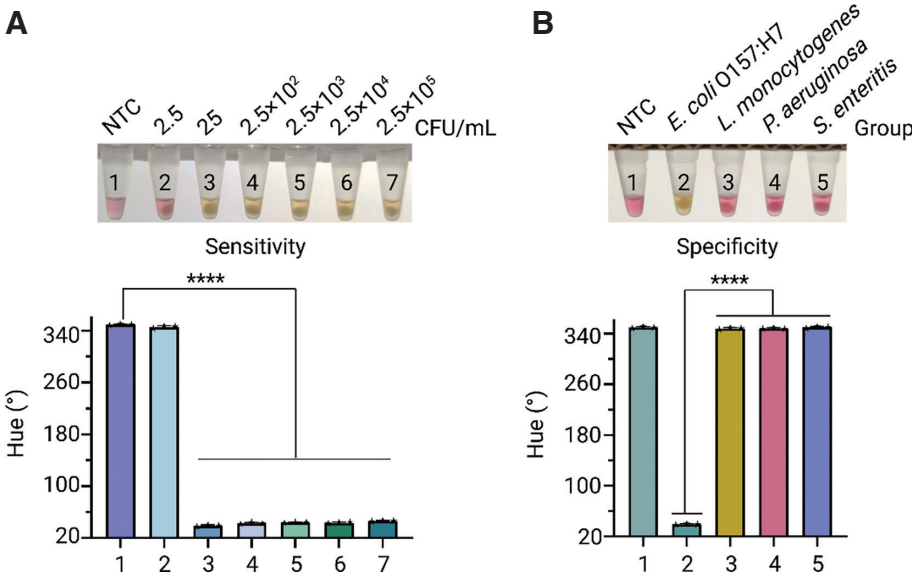

FIGURE 3 | Sensitivity and specificity of the FTA card-based colorimetric LAMP detection. A. Sensitivity of *E. coli* O157:H7 detection at different concentrations. B. Selectivity of *E. coli* O157:H7 detection. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001; NTC, no template control. All the experiments were repeated three times.

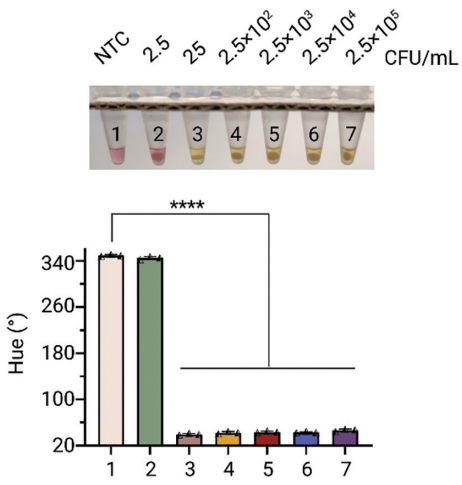

FIGURE 4 | FTA card-based colorimetric LAMP detection of *E. coli* O157:H7 in spiked human stool samples. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001; NTC, no template control. All the experiments were repeated three times.

Sensitivity and specificity

The sensitivity of the colorimetric LAMP detection method with FTA card-based DNA extraction was first evaluated by detecting different concentrations of *E. coli* O157:H7 (2.5-2.5×10^5 CFU/mL). As shown in Fig 3A, the established FTA card-based colorimetric LAMP detection achieved a sensitivity of 25 CFU/mL within 35 min.

To determine the specificity of the FTA card-based colorimetric LAMP method, *E. coli* O157:H7, *L. monocytogenes*, *P. aeruginosa*, and *S. enteritis* were detected. As shown in Fig 3B, only *E. coli* O157:H7 was detected with the specific LAMP primers of *E. coli* O157:H7.

Detection of *E. coli* O157:H7 in stool samples

The ability of the developed FTA card-based colorimetric LAMP method to detect *E. coli* O157:H7 in stool samples was determined. The clinical human stool samples collected from Ruijin Hospital North were directly detected or spiked with concentrations of *E. coli* O157:H7 (2.5-2.5×10^5 CFU/mL) and detected by the established method. As shown in Fig 4, the simple, rapid, and visual detection of *E. coli*. O157:H7 was achieved within 35 min, with excellent sensitivity at a limit of detection (LOD) of 25 CFU/mL. In addition, the detection results were in good agreement with the PCR assay results (Table 2), which verified the reliability of the developed method.

TABLE 2 | Comparison of detection *E. coli* O157:H7 results in stool samples by PCR and FTA card-based colorimetric LAMP detection.

Stool samples		PCR	LAMP colorimetric assay (this study)
Clinical stool samples	1	-	-
	2	-	-
	3	-	-
	4	-	-
	5	-	-
	6	+	+
	7	+	+
	8	-	-
	9	-	-
	10	-	-
	11	-	-
	12	-	-
	13	-	-
	14	-	-
	15	-	-
	16	+	+
	17	-	-
Spiked stool samples	0 CFU/mL	-	-
	2.5 CFU/mL	-	-
	25 CFU/mL	+	+
	2.5×10^2 CFU/mL	+	+
	2.5×10^3 CFU/mL	+	+
	2.5×10^4 CFU/mL	+	+
	2.5×10^5 CFU/mL	+	+

+: detected; -: not detected.

DISCUSSION

E. coli O157:H7 is an important zoonotic pathogen that is usually detected by laboratory tests, such as DNA sequencing, enzyme-linked immunosorbent assay (ELISA), and PCR, which typically require well-trained professionals, complicated procedures, and expensive equipment. In addition, sample preparation, such as nucleic acid extraction, is usually constructed by commercial kits with a centrifuge, which is also a major challenge for the rapid, visual, and on-site detection of *E. coli* O157:H7, especially in resource-limited areas [17]. Of note, the FTA card has recently emerged as a solution for collecting, transporting, and purifying nucleic acids [17,20]. Herein a visual and rapid detection of *E. coli* O157:H7 method was developed by combining FTA card-based nucleic acid extraction and the LAMP colorimetric assay.

Compared to the gold standard method (PCR) for the detection of *E. coli* O157:H7 from stool samples, the assay we developed not only achieved higher sensitivity (25 CFU/mL) in a shorter time (35 min) without complex sample preparation, operation, reaction, and analysis steps, but was also tolerant to PCR inhibitors and reduced interference to the reaction [18,21]. Remarkably, our approach

only requires a compact, portable, and lightweight dry bath, eliminating the requirement for cumbersome and complicated equipment. Furthermore, the FTA card-based bacteria concentration and DNA extraction effectively lysed and deactivated cells, denatured proteins, and stabilized nucleic acids, allowing for long-term storage at room temperature [15]. Therefore, the FTA card-based colorimetric detection shows great potential for clinical screening and monitoring of *E. coli* O157:H7, particularly in recourse-limited settings.

CONCLUSIONS

In conclusion, an FTA card-based colorimetric LAMP assay has been successfully developed for the simple and rapid detection of *E. coli* O157:H7 with satisfactory sensitivity and selectivity. This method offers several advantages, including the ability to perform nucleic acid extraction and transport without complex equipment, as well as the ability to read the results visually. Moreover, this method achieved high sensitivity (LOD=25 CFU/mL) and excellent specificity within 35 min; in contrast, the conventional PCR requires 1.5 h. In addition, the detection results of FTA card-based colorimetric LAMP detection agreed well with the results from the PCR assay. With the design of specific primers, the method can also be applied for the detection of other pathogens in the stool. In the future this study is expected to align with the recent advances in the detection of *E. coli* O157:H7, including digital [22], integrated [23], and portable analysis systems [24]. This method shows promise as a valuable diagnostic tool for the clinical detection and monitoring of zoonotic infections, which also offers significant potential for on-site detection, particularly in resource-limited settings.

ACKNOWLEDGEMENTS

This work was supported by the Shanghai Agriculture Applied Technology Development Program, China (X2020-02-08-00-12-F01110), the Natural Science Foundation of Shanghai (22ZR1436200), and the National Natural Science Foundation of China (22104090). All images in the figures were adapted with permission from Biorender.com with a paid academic subscription unless otherwise stated in the captions.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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