A Colorimetric LAMP-Based Method for Detecting *E. coli* and *Coliform* in Domestic Water

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Tóm tắt

Household water contaminated with *Escherichia coli* and Coliforms poses a serious risk to public health, particularly in areas lacking access to regular monitoring. Conventional detection methods are often slow, technically demanding, and reliant on laboratory infrastructure, limiting their usefulness in real-time or field-based applications. This study introduces a rapid detection method based on Loop-mediated Isothermal Amplification (LAMP) for the identification of *E. coli* and Coliforms in household water. The assay delivers results in under 30 minutes and produces a visible color change that can be interpreted without the need for sophisticated analytical equipment. This simplified, equipment-light and more accessible approach offers an effective solution for on-site water quality assessment, supporting early intervention and improved water safety at the community level.

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Từ khóa
LAMP; household water; Coliforms; *E.coli*

1. Introduction

Ensuring the microbiological safety of domestic water is essential for protecting public health. Contamination of household water with pathogenic microorganisms poses a significant risk, particularly in rural and peri-urban areas where centralized water treatment is limited[1]. *Escherichia coli* and total Coliform are widely used as indicator organisms for assessing water quality, reflecting the potential presence of fecal contamination and waterborne pathogens[2-4]. Traditional detection methods, including membrane filtration, multiple-tube fermentation, and chromogenic media, are accurate but require laboratory infrastructure, trained personnel, and considerable processing time[5-7]. These constraints limit their application in routine monitoring of domestic water, especially in settings with limited resources[8].

Loop-mediated isothermal amplification (LAMP) offers a rapid, sensitive, and field-deployable alternative for microbial detection[9]. This technique amplifies target DNA under isothermal conditions and, when combined with colorimetric indicators, allows visual detection without specialized equipment[10]. Such advantages make LAMP particularly suitable for onsite water quality testing[11]. In recent years, LAMP has been successfully applied for detecting various waterborne pathogens beyond *E. coli* and Coliform, including *Salmonella*, *Vibrio*, and other bacteria in aquaculture water, wastewater, and groundwater[12-13]. Some studies have developed multiplex LAMP platforms capable of simultaneously detecting multiple pathogens, thereby enhancing monitoring efficiency in environmental and rural water systems. Others have integrated LAMP with portable devices, smartphone-based readers, or microfluidic systems to improve usability in field conditions and enable real-time data interpretation[14-15]. These advancements demonstrate the versatility of LAMP technology in water quality surveillance, particularly for decentralized testing in low-resource settings.

This study aims to develop and evaluate a simple, colorimetric LAMP assay for the rapid detection of *E. coli* and Coliform in domestic water. The method is designed to provide a practical solution for routine water monitoring, contributing to improved water safety management in household and community settings.

2. Materials and methods

2.1. Sample collection and DNA extraction

A total of 100 domestic water samples (approximately 20 per province) were collected from household taps or storage containers used for drinking and cooking in five provinces of the Mekong Delta, Vietnam: Can Tho, Hau Giang, Soc Trang, Bac Lieu, and Kien Giang. A 100 mL volume of each sample was filtered through a 0.45 µm membrane (Sartorius, Germany). The membrane was

placed in 500 µL of nuclease-free water and vortexed to release bacterial cells. DNA extraction was performed using the Monarch® Genomic DNA Purification Kit (NEB, UK) following the manufacturer's protocol. DNA was stored at –20°C until it was used.

2.2. LAMP primer design

LAMP primers targeting the *uidA* gene for *E. coli* and the *malB* gene for total Coliform were designed based on conserved regions retrieved from the NCBI GenBank database. Each set consisted of four primers: Forward Outer (F3), Backward Outer (B3), Forward Inner Primer (FIP), Backward Inner Primer (BIP). Primer sequences are listed in Table 1.

Table 1. Designed LAMP primers used in this study.

Target gene	Primer	Sequence 5' – 3'
uidA	F3	GCCCACGTCTTTAATGTCGT
	B3	CCCGATCGCTGTTGAAGC
	FIP	GAGCGCGCTGATGTTCAACC T-CTTGAACGCATAACCCCCG
	BIP	TCAGTTCTTTATCCAGCGCCG G-AAAGATCTGCTGCCGAACC
malB	F3	GAAAAGCCGCCGACTTCG
	B3	CCGACATGTGGAGTGAAGAG
	FIP	TTTTGCGACCTCGCAAGGCAT -GTCGCGAGTGAAGATCCCT
	BIP	CCATACCTGTTCACCGACGAC G-GGCTGGATATGTATCACCGC

2.3. Colorimetric LAMP assay

LAMP reactions were performed in a 15 µL mixture containing 7.5 µL of WarmStart® Colorimetric LAMP 2X Master Mix (NEB, UK), 1.6 µM each of FIP and BIP, 0.2 µM each of F3 and B3, and 5 µL of DNA template. Nuclease-free water was added to adjust the final volume. The reaction was incubated at 65°C for 30 minutes using a dry block heater (Benchmark, USA). A color change from pink to yellow indicated a positive result, while a negative result remained pink.

2.4. Optimization of LAMP reaction conditions

The LAMP assay was optimized by evaluating temperatures from 55°C to 68°C and incubation times from 5 to 55 minutes. Reactions were performed in 15 µL containing WarmStart® Colorimetric LAMP 2X Master Mix (NEB, UK), specific primers, and 5 µL of DNA template. Amplifications were conducted using a dry block heater and visualized based on color change.

2.5. Limit of detection evaluation

LoD95 was determined using intact cells of ATCC reference strains without DNA extraction. Bacterial concentrations were standardized using OD_{600} and CFU counts before serial dilution.. The bacterial suspensions were adjusted to a known concentration and serially diluted to achieve final concentrations ranging from 1 to 50 CFU per reaction. Each dilution was tested in triplicate (n = 3) to assess the minimum detectable concentration. The limit of detection with 95% probability (LoD95) was calculated using the PODLOD ver12 software, based on the presence or absence of amplification signals across replicates at each dilution level.

2.6. LAMP detection on domestic water samples

The performance of the LAMP assay was tested on domestic water samples. Each sample was analyzed in parallel using the standard membrane filtration method for *E. coli* and Coliform detection to compare the results with those obtained from LAMP.

3. Results

3.1. Primer Performance for LAMP Assay

The primer sets designed for *E. coli* and Coliform successfully amplified the target DNA. Positive reactions exhibited a clear color change from pink to yellow, while negative controls remained pink, indicating no non-specific amplification. This confirmed that the primers were highly specific and efficient for detecting both *E. coli* and Coliforms. These primers were subsequently used in all further experiments.

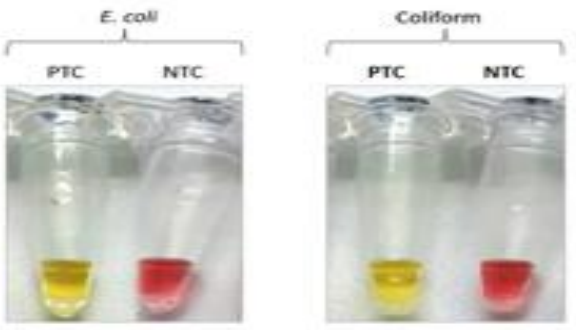

Figure 1. Performance evaluation of LAMP primer sets for E. coli and Coliform detection.

LAMP reactions were conducted at 65°C for 30 minutes with target DNA at a concentration of 10^3 CFU/reaction.

3.2. Optimization of LAMP Reaction Conditions

Optimization experiments were conducted to determine the most suitable temperature and incubation time for the LAMP assay. Amplification was successfully observed at temperatures ranging from 58°C to 68°C, with the most stable and distinct color change at 61°C. In terms of incubation time, amplification began to appear from 20 minutes, but stable and reliable results were achieved at 30 minutes. No amplification was

detected at temperatures below 57°C or with incubation times shorter than 15 minutes. Therefore, the optimal conditions for the LAMP assay were set at 61°C for 30 minutes.

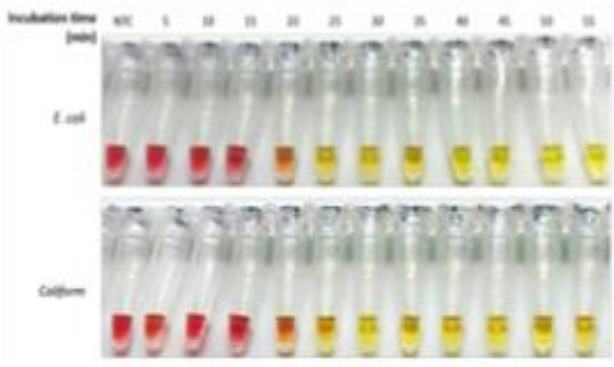

Figure 2. Time optimization for the LAMP assay detecting *E. coli* and Coliform.

The LAMP reactions were conducted at 65°C, with incubation times ranging from 5 to 55 minutes to determine the optimal reaction duration.

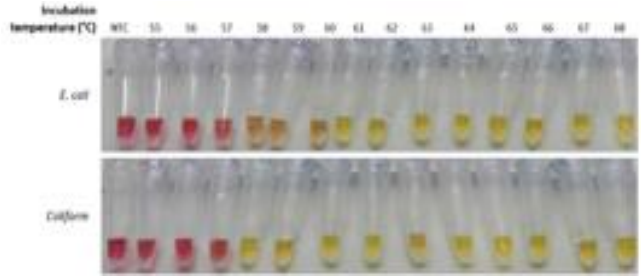

Figure 3. Temperature optimization for the LAMP assay detecting *E. coli* and Coliform.

The LAMP reactions were conducted at temperatures ranging from 55°C to 68°C to determine the optimal amplification temperature for the assay.

3.3. Detection Limit (LoD95)

The detection limit of the assay was determined using serial dilutions of purified DNA from *E. coli* and Coliform, ranging from 1 to 50 CFU per reaction. For *E. coli*, positive amplification was observed in one out of three replicates at 1 CFU/reaction and increased with higher concentrations. For Coliform, positive detection started from 1 CFU/reaction, with two or more replicates showing amplification from 5 CFU/reaction upwards. The calculated LoD95 was 9.675 CFU/reaction (3.701–25.291) for *E. coli* and 4.907 CFU/reaction (1.537–15.670) for Coliform, indicating that the LAMP assay has high sensitivity and can detect low bacterial concentrations in domestic water samples.

Table 2. LoD95 of the LAMP assay for *E. coli* and Coliform.

CFU/reaction	Number of Positive Results (*E. coli*)	Number of Positive Results (Coliform)
1	1	1
5	2	3
10	3	3
20	3	3
50	3	3
LoD95 result	**9.675 CFU (3.701–25.291)**	**4.907 CFU (1.537–15.670)**

3.4. Application of LAMP for Domestic Water Samples

The LAMP assay was applied to 100 domestic water samples collected from various sources in the Mekong Delta region. The assay detected *E. coli* in 8 samples, Coliform in 9 samples, and 3 samples showed co-contamination with both *E. coli* and Coliform. The remaining 83 samples were negative for both targets. All LAMP results were confirmed by conventional PCR, with 100% consistency. The results also strongly agreed with those from the membrane filtration method..

Table 3. Field validation of the LAMP assay for detecting *E. coli* and Coliform in domestic water samples (N = 100)

Contamination Type	Number of Samples
E. coli	8
Coliform	9
E. coli & Coliform	3
Total contaminated	17
Negative samples	83

4. Discussion

This study developed a simple, rapid LAMP assay for detecting *E. coli* and Coliform in domestic water. The assay demonstrated high specificity without cross-reactivity to non-target bacteria, confirming the reliability of the primer sets targeting the *malB* and *uidA* genes. Optimization showed that the assay performs best at 61°C for 30 minutes, supporting its suitability for rapid detection. The detection limits were 9.675 CFU/reaction for *E. coli* and 4.907 CFU/reaction for Coliform, demonstrating high sensitivity comparable to previous LAMP assays, which reported detection limits ranging from 10 to 100 CFU/reaction for *E. coli*[9, 11].Field validation showed strong agreement with the standard membrane filtration method, detecting 17 contaminated samples out of 100, including single and

co-contaminations. The visual colorimetric readout and simple workflow make the assay highly applicable for onsite water monitoring, especially in resource-limited settings. This LAMP method provides a practical alternative to conventional methods, offering faster results with minimal equipment, supporting improved water safety management at the community level.

Acknowledgments

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Phát hiện nhanh *E. coli* và *Coliform* trong nước sinh hoạt bằng phương pháp LAMP theo chỉ thị màu

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Tóm tắt Ô nhiễm nước sinh hoạt bởi vi khuẩn *Escherichia coli* và Coliform là mối đe dọa nghiêm trọng đối với sức khỏe cộng đồng, đặc biệt ở những khu vực thiếu điều kiện giám sát thường xuyên. Các phương pháp phát hiện truyền thống thường chậm, phức tạp và phụ thuộc vào cơ sở hạ tầng phòng thí nghiệm, hạn chế khả năng ứng dụng trong giám sát tại chỗ hoặc thời gian thực. Nghiên cứu này giới thiệu một phương pháp phát hiện nhanh dựa trên kỹ thuật Loop-mediated Isothermal Amplification (LAMP) để nhận diện *E. coli* và Coliform trong nước sinh hoạt. Phản ứng cho kết quả trong vòng 30 phút với sự thay đổi màu sắc rõ ràng, có thể quan sát trực tiếp mà không cần sử dụng các thiết bị phân tích phức tạp. Phương pháp đơn giản và chi phí thấp này mang đến giải pháp hiệu quả cho đánh giá chất lượng nước tại chỗ, góp phần hỗ trợ can thiệp sớm và nâng cao an toàn nguồn nước cho cộng đồng.
Từ khóa LAMP; nước sinh hoạt; *E. coli*; Coliforms