 DOI: 10.35382/TVUJS.16.6.2025.226

RAPID DETECTION OF *Vibrio parahaemolyticus* USING POLYDOPAMINE MEMBRANES IN COMBINATION WITH LAMP

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Abstract – *This study was conducted from September 2025 to April 2026 at Tra Vinh University, Vietnam, with the aim of developing a simple detection strategy for screening Vibrio parahaemolyticus using polydopamine membranes and loop-mediated isothermal amplification. Polydopamine membranes were synthesized by coating Whatman filter paper with dopamine and used for nucleic acid extraction. The extracted DNA was then amplified using loop-mediated isothermal amplification at 65^{o}C for 25 minutes. Results showed that an incubation time of 20 minutes with a suitable A260/A280 ratio was selected for downstream analysis, including amplification and detection. The loop-mediated isothermal amplification result was directly observed due to the color change from red (negative) to yellow (positive), indicating samples containing the target Vibrio parahaemolyticus. This detection method opens potential applications for rapid bacterial detection in low-resource areas.*

Keywords: colorimetric detection, LAMP, on-site detection, polydopamine, V. parahaemolyticus.

I. INTRODUCTION

Vibrio parahaemolyticus (V. parahaemolyticus) is a Gram-negative and halophilic bacterium found in coastal environments. It typically uses its flagellum to move through water or attach to living organisms such as shellfish [1]. Since its discovery in 1950, *V. parahaemolyticus* has become one of the leading causes of seafood-derived pathogenic bacteria throughout the world [2]. The food poisoning caused by *V. parahaemolyticus* usually occurs in summer (from June to October) and is primarily associated with contaminated water and seafood such as shrimps, crabs, prawns, clams, fish, and oysters [3]. Consumption of seafood contaminated with *V. parahaemolyticus*, particularly when inadequately cooked, is a major risk factor for gastrointestinal infection [4]. Typical symptoms of *V. parahaemolyticus* infection include diarrhea, abdominal pain, nausea, and fever [5]. Severe symptoms include unconsciousness, seizures, pale or cyanotic skin, and even death [6]. Therefore, rapid and accurate detection of this pathogen is crucial for food safety control and human health.

II. LITERATURE REVIEW

Currently, different methods are used to detect *V. parahaemolyticus*, including bacterial culture, immunology, and molecular diagnostics [7]. However, culture methods are time-consuming and labor-intensive, often taking up to a week to obtain results [8]. Immunological diagnostic techniques have faster analysis times but are limited in sensitivity and selectivity [7]. With the advances of molecular biology, the polymerase chain reaction (PCR) is considered the gold standard for diagnostics due to its high stability and sensitivity. PCR has been widely applied for the rapid detection of *V. parahaemolyticus*. Despite these advantages, PCR requires a complex thermal cycler, and the reaction takes several hours, thereby limiting its use to laboratory settings [9–11]. In 2000, Notomi et al. [12] developed a DNA amplification method called loop-mediated isothermal amplification (LAMP). The method employs the *Bst* DNA polymerase enzyme, which

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Received date: 28 February 2026; Revised date: 9 June 2026; Accepted date: 19 August 2026

is capable of both strand separation and new strand synthesis for amplification. LAMP uses four or six primers to amplify the target gene at a constant temperature of 60^{o}C–65^{o}C for 15–60 minutes. The results of the LAMP reaction can be observed directly with the naked eye via turbidity or color change, making it suitable for use outside of a laboratory as well as in laboratories with limited equipment.

Polydopamine (PDA) membranes have been considered a promising material in recent years. PDA is a biopolymer inspired by the adhesion mechanism of marine mollusks [13]. PDA materials have high hydrophilicity and biocompatibility because their chemical structure contains many functional groups, including catechol hydroxyl, amino, and quinone, that can react with many molecules [14]. Specifically, during the extraction process, the quinone groups in PDA can react with free amine groups present in biological molecules through Schiff-base reactions [15].

Gao et al. [16] developed a rapid method for detecting *Staphylococcus aureus (S. aureus)* using magnetic nanoparticles coated with PDA to aid in DNA extraction and integrate with PCR techniques. The study showed that the PDA was capable of capturing and purifying DNA for amplification reactions. In addition, Trinh et al. [17] published a study using a microcentrifuge device integrating the LAMP technique combined with fluorescence detection using the fisetin biological dye. In the study, PDA-coated paper was used as a filter layer for direct sample collection from milk containing *Salmonella* spp. At this stage, amplification inhibitors (such as cell debris, milk proteins, and calcium ions) are retained on the PDA membrane via Schiff-base interactions. Subsequently, the DNA is transferred into the reaction chambers for the LAMP reaction. The device was suitable for rapid pathogen detection in an environmentally friendly and easy-to-operate manner.

Therefore, this study was conducted to develop a rapid, simple, and effective method for detecting *V. parahaemolyticus* based on the use of PDA material in combination with LAMP for DNA analysis. The detection process was completed within 50 minutes. This method opens a new avenue in the analysis of pathogenic bacteria, allowing for rapid on-site diagnosis without the need for complex equipment.

III. MATERIALS AND METHODS

A. Materials

All experiments were conducted from September 2025 to April 2026 at the Biotechnology Institute, Tra Vinh University, Vietnam. *V. parahaemolyticus* (ATCC 33844) was provided by the Biotechnology Institute, Tra Vinh University. Luria–Bertani (LB) broth and Mueller–Hinton agar (MHA) were purchased from Himedia (India). Dopamine hydrochloride was obtained from Sigma, and Whatman No. 1 filter paper was used. WarmStart® Colorimetric LAMP 2X Master Mix (New England Biolabs) was used for the amplification reactions. LAMP primer sets were designed based on specific gene regions of the target bacterial strains using Primer Explorer V5 software (Table 1).

B. Methods

Preparation of polydopamine membrane

The polydopamine membrane was prepared following a previous method by Chae et al. [18]. Dopamine hydrochloride was dissolved in Tris–HCl buffer solution (10 mM, pH 8.0) to obtain a final concentration of 2 mg/mL. Filter paper was directly immersed in the solution and incubated for 20 hours at room temperature. The membrane was then thoroughly rinsed with distilled water and dried under room conditions.

Bacterial culture

V. parahaemolyticus (ATCC 33844) was cultured and counted using LB broth and MHA at 37^{o}C for 16 hours with shaking.

DNA extraction using polydopamine-coated membranes

PDA-coated membranes were cut into strips (0.25 $\times$ 2 cm) and dipped in 100 μL of *V. parahaemolyticus* suspension at a concentration of 10^5 CFU/mL. The membranes were incubated

at room temperature for four different periods (15, 20, 25, and 30 minutes) to determine the optimal incubation time for bacteria binding. Subsequently, the membranes were washed twice with 200 μL of water. The membranes were then heated at 90^{o}C for five minutes and stored at 4^{o}C for subsequent experiments. Each treatment was replicated three times. To evaluate the quality of extracted DNA, the treated membrane was eluted using nuclease-free water. The DNA concentration and purity were determined using a NanoDrop^{TM} spectrophotometer.

Loop-mediated isothermal amplification reaction

LAMP primer set targeting the *toxR* gene of *V. parahaemolyticus* was designed using PrimerExplorer V5 software. The primer sequences are listed in Table 1. Each 12.5 μL of LAMP reaction mixture contained 1.6 μM each of the inner primers (FIP and BIP), 0.2 μM each of the outer primers (F3 and B3), 0.4 μM of the loop primer (LF), 6.5 μL of WarmStart® Colorimetric LAMP 2X Master Mix, and 5 μL of deionized water. Then, 12.5 μL of LAMP mixture was dropped into the PDA membrane obtained from the previous experiment. The amplification reaction was carried out at 65^{o}C for 25 minutes. Amplification results were evaluated by color change. A negative control without template DNA was included to validate the reliability of the experiments. Figure 1 illustrates the workflow of *V. parahaemolyticus* detection using PDA-based DNA extraction and colorimetric LAMP reaction.

Specificity of loop-mediated isothermal amplification assay

To evaluate the specificity of the method, *Enterococcus faecalis (E. faecalis)* was used in the reaction as a non-target bacterium. DNA of *E. faecalis* was prepared using the same procedure as for *V. parahaemolyticus*. The LAMP reaction was performed under the same conditions as described above.

Performance with spiked sample

In this experiment, tap water was selected to test the performance of this approach on a real sample. Sterile tap water was separately spiked with different types of pathogenic bacteria, including *V. parahaemolyticus*, *E. faecalis*, *S. aureus*, and *Salmonella*. The samples were then analysed using the proposed approach to evaluate its ability to detect *V. parahaemolyticus*.

Data analysis

Experimental data were processed using Microsoft Excel software. Colorimetric analysis of amplification results was performed in ImageJ using red, green, and blue (RGB) color intensity values. Color images of the reaction tubes were captured. The images were then analyzed using ImageJ through the RGB channel analysis function. The color intensity of each treatment was measured. The bar charts are drawn to compare the relationship between color intensity and treatment.

IV. RESULTS AND DISCUSSION

A. The polymerization of polydopamine onto the filter membrane

Visual observations revealed clear differences in color between the PDA-coated membrane and pristine Whatman filter paper (Figure 2). The PDA-coated membrane exhibited a stable brown color, which was due to the oxidation and polymerization of dopamine during the coating process. In contrast, the pristine Whatman filter paper appeared white. The distinct color change between the two membranes proved the successful coating of PDA, resulting in material properties distinct from those of the pristine substrate.

B. Effect of incubation time on DNA extraction efficiency using the polydopamine membrane

The results presented in Table 2 demonstrate that the incubation time remarkably affects the efficiency of DNA extraction from the PDA membrane, as reflected by variations in both DNA concentration and purity. At an incubation time of 15 minutes, the DNA purity was acceptable with an A_{260}/A_{280} ratio of 1.80. When the incubation time was increased to 20 minutes, the DNA concentration reached 44.53 ng/μL, while

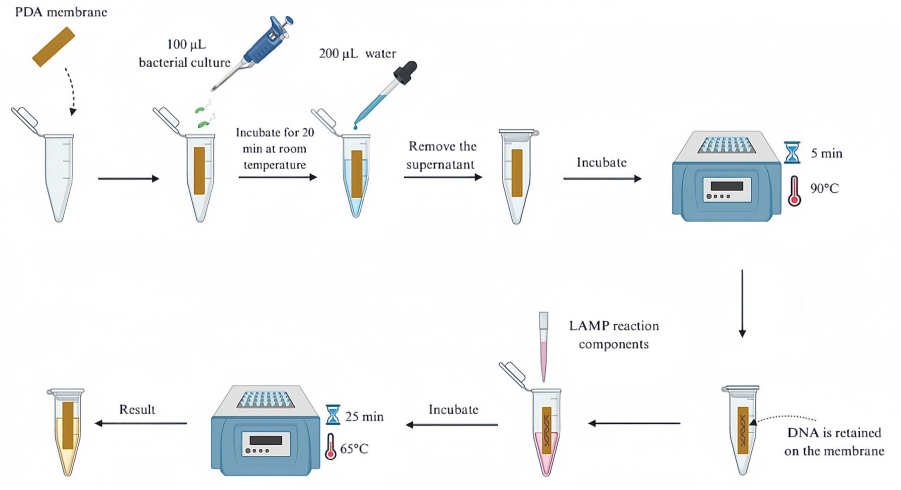


Fig. 1: Workflow of *V. parahaemolyticus* detection using LAMP and PDA-based DNA extraction

Table 1: Primer sequences used for the detection of *V. parahaemolyticus*

Primer	Sequence (5'–3')	5' pos	Length
F3	ACGCCTCTGCTAATGAGGTA	183	20
B3	GAGATTCCGCAGGGTTTGT	368	19
FIP	GGCGCTTCTGGTTCAACGATTG-GAAACGATCGTAGAGCCGTC		42
BIP	TGTGGCTTCTGCTGTGAATCCT-GCAGTACGCAAATCGGTAGT		42
LF	CCACGCGTTATTTTATTTTGGCAC		25



Fig. 2: Images of PDA-coated membrane (A) and pristine membrane (B)

the A_{260}/A_{280} ratio was 1.85, indicating good DNA purity with no or minimal contamination of protein and other reagents used in the extraction process. At the incubation times of 25 and 30 minutes, the concentration of DNA was not statistically different from the incubation of 20 minutes. From those results, an incubation time of 20 minutes was selected as the optimal condition to carry out subsequent experiments. PDA-coated membrane offered a promising platform for simple and centrifugal-free DNA extraction because the membrane successfully captured the bacteria via a Schiff-base reaction, which resulted in simple separation of the bacteria from other components in samples.

Table 2: Effect of incubation time on the concentration and purity of DNA extracted from the proposed approach

Incubation time (minutes)	Concentration (ng/µL)
15	$25.60^{a} \pm 0.87$
20	$44.53^{b} \pm 3.44$
25	$44.30^{b} \pm 1.73$
30	$45.63^{b} \pm 1.38$

Note: Values are presented as mean ± standard deviation. Means followed by the same superscript letter are not significantly different at the 5% significance level ($p < 0.05$).

C. Performance of loop-mediated isothermal amplification assay

The performance of the LAMP reaction was evaluated by observing the color change of the reaction mixture. The successful amplification of target DNA was monitored using a common pH indicator – phenol red. As described in a previous

study [19], in an amplification reaction, there are various by-products, including pyrophosphate and hydrogen ions. The production of protons can be used as an indicator for a successful amplification reaction. By lowering the buffer concentration in the reaction mixture, the proton production results in a dramatic drop in pH. Therefore, using pH indicators can indirectly validate the performance of the LAMP reaction. In this study, phenol red was used for colorimetric detection of LAMP. When there is target DNA, the primer set recognizes specific sequences and triggers amplification catalyzed by *Bst* polymerase. The reaction results in a drop in initial pH from 8.8 to below 7.0, which leads to the change of phenol red from red to yellow. Meanwhile, the absence of target DNA keeps the pH of the mixture unchanged, and the color of phenol red stays red. Figure 3 shows the results of the LAMP reaction after 25 minutes at 65^{o}C using DNA extracted from previous experiments. A clear color change from red to yellow was observed in tubes containing target DNA in the reaction mixture. In contrast, no color change was observed in tubes without target DNA template. Therefore, extracted DNA using the PDA membrane resulted in the most pronounced color change, demonstrating the potential of PDA membranes for DNA extraction. To confirm the reliability of the results, image analysis was performed using ImageJ software based on RGB values (Figure 4). The images revealed two distinct color groups: red (samples 1, 2, and 3) and yellow (samples 4, 5, and 6). ImageJ is a powerful tool for scientific analysis, especially in analytical chemistry. In ImageJ, to qualify color intensity, there can be various methods, including grayscale conversion, RGB channel decomposition, color thresholding, and optical density calibration. Among them, in colorimetric analysis, the RGB channel decomposition is commonly used to quantify color differences among samples, in which the R, G, and B values represent red, green, and blue intensities, respectively. Although visual observation allows color differences to be perceived, it does not provide a quantitative measure of color intensity. RGB channel decomposition in ImageJ can solve the problem. To measure color intensity, the raw image was input into the program. The color of the image was then processed to split into three distinct 8-bit grayscale images (R, G, and B). The intensity of the image could be measured. Following the previous publication, the G value was selected as the most representative parameter to distinguish between the red and yellow groups in this study [20]. The results indicated that samples 1, 2, and 3 exhibited color intensity values ranging from 64.33 to 81.33, whereas samples 4, 5, and 6 showed higher color intensities of 137.67, 157.33, and 160.67, respectively. This difference demonstrates the potential of using ImageJ to create digital data for colorimetric LAMP analysis, further confirming the potential application of PDA membranes for DNA extraction.



Fig. 3: Performance of the LAMP reaction using DNA extracted with a PDA membrane

Note: Samples 1–3 show negative reactions (red); samples 4–6 show positive reactions (yellow).

D. Specificity of loop-mediated isothermal amplification assay

To evaluate the specificity of the proposed method for targeting *V. parahaemolyticus*, *E. faecalis*, a common opportunistic pathogen that is also present in food, was selected as a non-target bacterium. The results presented in Figure 5 demonstrated that samples containing *V. parahaemolyticus* exhibited a distinct color change from red to yellow, indicating the presence of

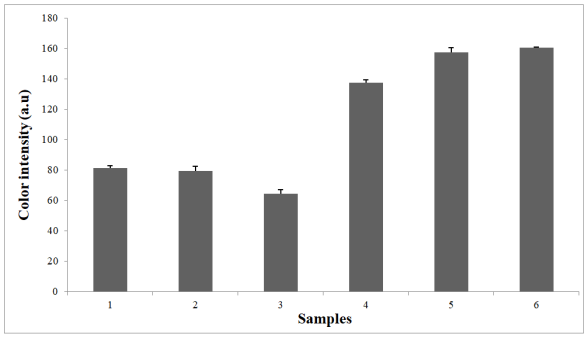


Fig. 4: The graph shows the color intensities of samples analyzed by ImageJ

Note: Samples 1–3 represent negative controls; samples 4–6 contain V. parahaemolyticus.

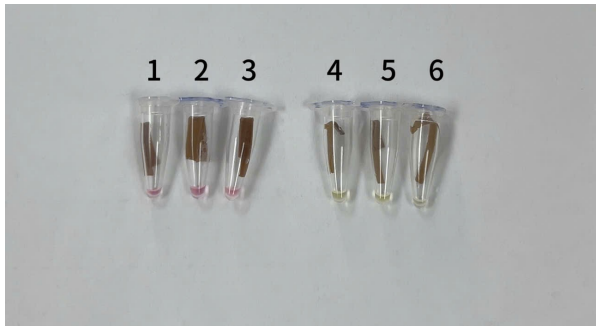


Fig. 5: Specificity of the LAMP assay for the detection of *V. parahaemolyticus*

Note: Samples 1–3 show negative reactions (red); samples 4–6 show positive reactions (yellow).

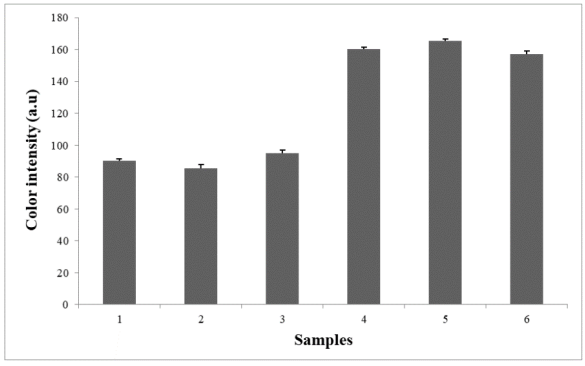


Fig. 6: Color intensity comparison and error analysis for LAMP assay specificity

Note: Samples 1–3 contain E. faecalis; samples 4–6 contain V. parahaemolyticus.

the target toxR gene and the success of LAMP amplification. In contrast, samples containing *E. faecalis* showed no color change, demonstrating that no amplification occurred. These results confirmed that the LAMP primer set exhibits high specificity for *V. parahaemolyticus* and does not generate cross-reactivity with non-target bacteria. Quantitative analysis of color intensity based on the RGB model (Figure 6) revealed a clear distinction between positive and negative samples. Specifically, the positive samples (samples 4, 5, and 6) showed high color intensity values. The consistency in color intensity among the positive samples indicates high stability of the LAMP reaction. Meanwhile, Figure 6 shows that the negative samples (samples 1, 2, and 3) exhibited low color intensity. These findings further confirmed the specificity of the designed LAMP primers toward *V. parahaemolyticus* and demonstrated the accuracy and ease of use of the LAMP-based detection method.

E. Performance with spiked sample

The performance of the method on real samples was evaluated using tap water samples spiked with different common pathogens, including *V. parahaemolyticus*, *E. faecalis*, *S. aureus*, and *Salmonella*. The results are shown in Figure 7. Only the sample containing *V. parahaemolyticus* (sample 4) exhibited a distinct color change from red to yellow after the LAMP reaction, indicating a positive result. In contrast, the samples containing other bacterial species (samples 1, 2, and 3) showed no color change, indicating negative results. These results demonstrate that the method has a good ability to differentiate between target and non-target bacteria under the experimental conditions. Furthermore, the successful detection of *V. parahaemolyticus* in tap water samples also indicates the potential application of this method in real sample analysis.

This study was also compared with previous studies to highlight its strengths and weaknesses,



Fig. 7: Performance of the LAMP assay for the detection of *V. parahaemolyticus* in spiked samples

Note: Samples 1–3 show negative reactions (red); sample 4 shows a positive reaction (yellow).

as shown in Table 3. In a previous study focused on detecting pathogenic *V. parahaemolyticus* in shrimps, Mai-Hoang et al. [21] developed a PCR-based strategy to recognize mutated bacteria in the sample. Although the method was sensitive and suitable for screening both wild-type and mutated targets, it was still limited in a laboratory setting due to the use of phenol-chloroform extraction and PCR. Trinh et al. [20] and Li et al. [22] proposed a rapid approach for bacteria detection using isothermal amplification methods. Another study by Oh et al. [23] integrated isothermal amplification into a lab-on-a-disc platform to quickly and accurately detect *V. parahaemolyticus* in food samples. These approaches, which combine isothermal amplification methods with rapid extraction methods such as Flinders Technology Associates cards or microfluidics, have demonstrated the promising potential of isothermal amplification for detecting *V. parahaemolyticus* in low-resource areas. Benefiting from isothermal amplification methods, the proposed method offered a simple and comparably rapid strategy for detecting a common foodborne bacterium – *V. parahaemolyticus*. The approach employed a simple PDA membrane for DNA extraction without the need for a centrifugal process and toxic solvents. Moreover, isothermal amplification – LAMP was also selected as an ideal on-site genetic amplification. The strategy successfully detected *V. parahaemolyticus* within 50 minutes without the need for any sophisticated equipment.

Table 3: Comparison of extraction methods, amplification method, and assay times from previous studies for the detection of *V. parahaemolyticus*

Study	Extraction method	Amplification method	Time
Mai-Hoang et al. [21]	Phenol-chloroform extraction method	PCR	NA
Trinh et al. [20]	Flinders Technology Associates (FTA) card-embedded tube	LAMP	75 minutes
Li et al. [22]	Nucleic acid extraction kit (TaKaRa, China)	Recombinase Polymerase Amplification (RPA) - CRISPR/Cas12a	~ 2 hours
Oh et al. [23]	Lysis buffer integration in lab-on-a-disc format	LAMP	65 minutes
This study	PDA membrane-based extraction	LAMP	50 minutes

V. CONCLUSION

This study proposed a novel and simple approach for the rapid detection of *V. parahaemolyticus* through the integration of the PDA – coated membrane for nucleic acid extraction with LAMP for amplification. The PDA – coated membrane demonstrated efficient and stable DNA extraction capability. In combination with the LAMP reaction, the strategy offered high specificity with no cross-reactivity with non-target bacteria. LAMP reaction produced a clear and fast colorimetric signal, which is suitable for naked-eye observation without the need for an extra instrument. The results highlighted the practical significance of the proposed approach for rapid bacterial analysis and provided a foundation for the development of on-site detection systems. However, this research only provided simple discrimination of the presence of *V. parahaemolyticus*, which is a qualitative analysis. Moreover,

further study should be carried out to expand the specificity and sensitivity of the approach. Future studies should focus on validating the method for quantitative purposes as well as using real samples and integrating multiplex detection of multiple pathogens to further expand its practical applicability. The possibility of multiplexing with quantitative analysis can be extended by the integration of our strategy, which uses PDA in combination with colorimetric LAMP into a microfluidic platform.

ACKNOWLEDGEMENTS

This research was fully funded by Tra Vinh University (TVU) under grant contract number 62/2025/HĐ.HĐKH&ĐT-ĐHTV.

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