Development of *Vibrio parahaemolyticus* sensitive and specific loop-mediated isothermal amplification combined with lateral flow device

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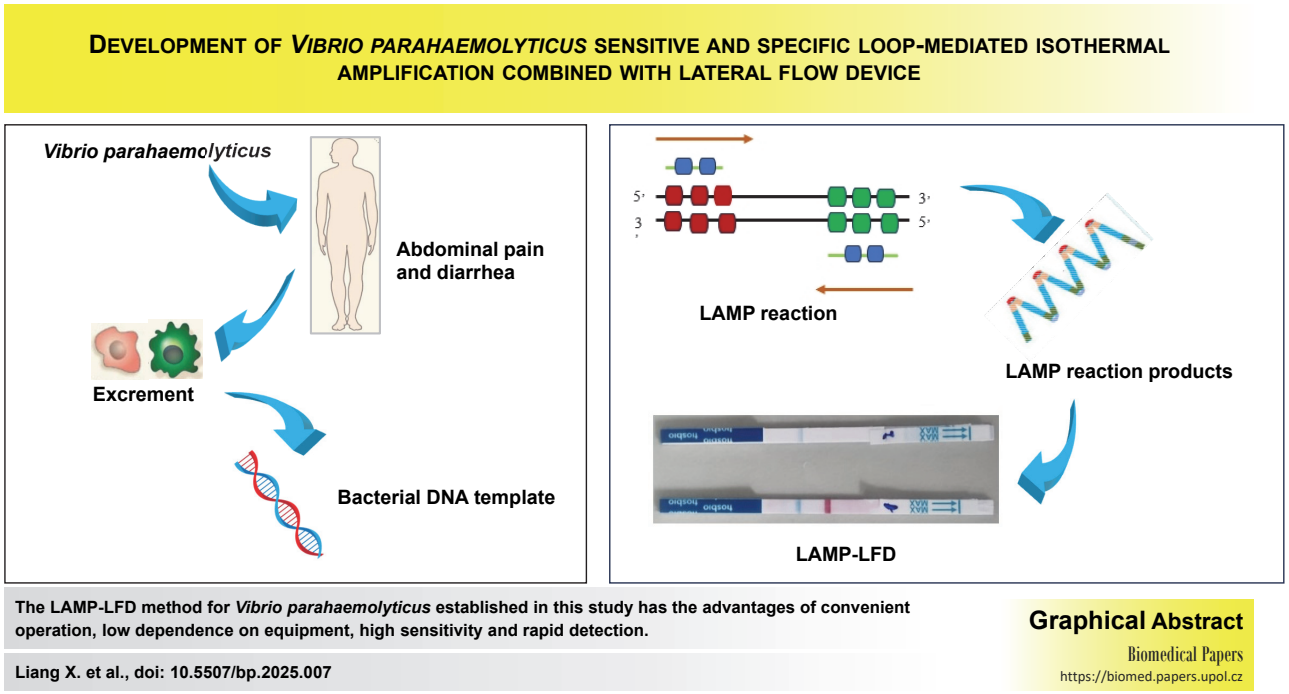
Background and Aim. *Vibrio parahaemolyticus* a gram-negative, rod-shaped bacterium with salinophilic properties is found mainly in rivers, oceans, and coastal environments. With the expanding scale of aquaculture in coastal regions of China, the contamination of seafoods with *Vibrio parahaemolyticus* is becoming a significant cause of food poisoning with symptoms including gastroenteritis, wound infection and sepsis. Current methods for detecting this microorganism are unsuitable in the present context. We developed a rapid LAMP-LFD method-by combining the loop-mediated isothermal amplification technique (LAMP) and lateral flow device (LFD).
Methods. The thermolabile hemolysin *tlh* gene of *Vibrio parahaemolyticus* was used as the target, and we designed five specific primers in its conserved region. The primers were used to carry LAMP reaction with biotin labelling, the products completed hybridisation with the FAM-labelled primers, and the hybridisation products were tested for results on LFD.
Results. The results showed that the LAMP-LFD method specifically detected *Vibrio parahaemolyticus* and was negative for proximate strains such as *Vibrio vulnificus* and other *Vibrio* pathogens as well as common pathogens such as *Escherichia coli*. The optimised reaction conditions for LAMP were 40 min at 60 °C, plus 5 min of probe hybridisation and 3–5 min of LFD color development. The lowest concentration of *Vibrio parahaemolyticus* pure culture bacterial fluid of 1.5×10^2 cfu/mL could be detected, and the pathogen could be detected from tissue samples with a contamination concentration of 0.75×10^3 cfu/mL. The method has higher specificity and sensitivity, and the pathogen can be detected within 1.5 h.
Conclusion. The LAMP-LFD method for *Vibrio parahaemolyticus* established in this study has the advantages of convenient operation, low dependence on equipment, high sensitivity and rapid detection, all of make it ideally suited to the detection of *Vibrio parahaemolyticus* at the grass-roots level.



Key words: *Vibrio parahaemolyticus*, loop-mediated isothermal amplification, *tlh* gene, LFD

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INTRODUCTION

Vibrio parahaemolyticus is a Gram-negative, curved, rod-shaped bacterium with salinophilic properties that spreads mainly in rivers, oceans, and coastal environments, can also be found in salted and processed seafood products[1,2], and it is the most common foodborne pathogen in aquatic products[3]. The earliest recorded food poisoning caused by *Vibrio parahaemolyticus* occurred in Osaka, Japan, in the 1950s, when more than 200 people became ill and 20 died as a result of consuming sardine products[4]. Since then, an increasing number of *Vibrio parahaemolyticus* food poisoning events have been reported around the world, and *Vibrio parahaemolyticus* has become one of the foodborne pathogens[5,6]. In recent years, with the expanding scale of aquaculture in coastal areas of China, the contamination of aquatic products and their products with pathogenic microorganisms has attracted much attention, among which *Vibrio parahaemolyticus* has the highest detection rate, and *Vibrio parahaemolyticus* has become the most common pathogen of food poisoning in coastal areas and islands of China[7,8]. The main symptoms of food poisoning caused by *Vibrio parahaemolyticus* are gastroenteritis, wound infection and sepsis, among which gastroenteritis symptoms can be manifested as nausea and vomiting, abdominal pain and diarrhea, fever and chills[9].

Currently, *Vibrio parahaemolyticus* can be detected using a variety of methods, mainly traditional culture method, immunological detection, nucleic acid amplification detection and others[10,11]. The most classic and standard clinical microbial isolation culture and identification[12], in China, *Vibrio parahaemolyticus* detection is mainly followed in the food safety national testing standards, the method of detection is accurate, the results are reliable, and the false-positive rate is low, but the traditional method is time-consuming and, cumbersome[13], the knowledge and background skills of the test personnel required are demanding and, far from being able to meet the market demand for rapid detection. Immunological methods have been widely used in the detection of a variety of microorganisms because there is no need for bacterial enrichment[14], but the false positive rates are high. Molecular biology techniques relying on nucleic acid detection have become important in laboratory diagnosis for various pathogens due to their advantages of high sensitivity and specificity. These techniques include, the Loop-Mediated Isothermal Amplification (LAMP) (ref.[15]). LAMP relying on *Bst* DNA polymerase can achieve the rapid amplification of nucleic acids at a constant temperature (60–65 °C), which shows a superior performance to PCR in terms of time-consumption and cost of instrumentation[16]. This technique has been widely used in the case of bacteria, viruses and parasites because of its superior performance in terms of detection time and instrumental cost[17-19]. In this study, four primers and one 6-FAM-labeled primer were designed according to the conserved region of *Vibrio parahaemolyticus tlh* gene[20], and the conditions were optimized with the aim of establishing an accurate and efficient LAMP-LFD method for *Vibrio parahaemolyticus*, which can provide a more reliable and convenient technical means for its rapid detection and immediate diagnosis in the case of poisoning.

MATERIALS AND METHODS

Experimental materials

The Vibrio strains used in this experiment, as *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio fluvialis*, *Vibrio cholerae*, and *Vibrio alginolyticus* were purchased from Qingdao Hi-Tech Industrial Park HaiBo Biotechnology Co. Ltd (with strain identification report) and the other strains, such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* were provided by Guangxi Preliminary Examination Center and kept in our laboratory for subsequent experiments. *Bst* DNA polymerase was purchased from Beijing Baozhi Biotechnology Co. Ltd; genomic DNA extraction kit was purchased from Beijing Solebaum Technology Co. LFD was purchased from Libo Technology Co, LTD.

Bacterial recovery culture and genome extraction

The freeze-drying tube was opened in a biological safety cabinet, and about 0.5 mL of sterile water was sucked into the freeze-drying tube with a sterile pipette, dripped into the freeze-drying tube, gently oscillated until it was dissolved, sucked up all the bacterial suspensions, inoculated on the medium and put into the warm box to incubate for 12–24 h, and then a single colony of the culture was selected to be analyzed for bacterial identification. A single colony of *Vibrio parahaemolyticus* was used as the target, and the bacterial solution was prepared and the concentration was adjusted to 0.5 McKnight's unit (equivalent to 1.5×10^8 cfu/mL) by a bacterial McCloud turbidimeter, and this concentration was taken as the starting concentration, and a 10-fold gradient dilution of concentration was performed to obtain the concentrations of 1.5×10^7, 1.5×10^6, 1.5×10^5, 1.5×10^4, 1.5×10^3, 1.5×10^2, 1.5×10^1 cfu/mL and other concentrations of bacterial suspension, and set aside. DNA was extracted by ordinary boiling method: 1 mL of the above bacterial suspension was pipetted into a heat-free EP tube, centrifuged at 10,000 rpm for 3 min, and the supernatant was discarded, leaving the precipitate; 300 μL of distilled water was added, and it was vigorously vortexed and mixed, and centrifuged at 10,000 rpm for 3 min, and the supernatant was discarded to obtain the precipitate; 150 μL of distilled water was added to the precipitate, vortex vigorously and well mixed, and boiled at 100 °C for 10 min; centrifuged at 10,000 rpm for about 1 min, and the supernatant is the extracted nucleic acid genome, which is used as the template for amplification. Other negative control bacteria also used this method for bacterial DNA extraction, stored in the refrigerator at −20 °C, standby.

Primers of LAMP-LFD

We checked the related literature of *Vibrio parahaemolyticus* and identified the *tlh* gene as its specific target gene, and looked up the sequence of this gene (GenBank:

Table 1. LAMP primers.

Primer name	Sequence (5'-3')	Length (bp)
tlh-F3	CGCTGACAATCGCTTCTCAT	20
tlh-B3	GTTCTTCGCTTTGGCAATGT	20
tlh-FIP	**Biotin**-TCACCGAGTGCAACCACTTTGT-AACCACACGATCTGGAGCA	41
tlh-BIP	TTAACGCGTCACAATGGCGCT-ACCGTTGGAGAAGTGACCTA	41
tlh-LB	**6-FAM**-CCCTAACCCGAACAGCTGGT	20

MH047288.1) from NCBI database, and this sequence was compared to the specific sequence of *Vibrio parahaemolyticus*, and Blast analysis was utilized to confirm its specificity. Through Blast analysis, it was found that the *tlh* gene sequence was specific and homologous to *Vibrio parahaemolyticus* and could be used as the target sequence for LAMP primer design; the selected target sequence was imported into the LAMP primer design software Primer ExplorerV5 (http://primerexplorer.jp/) through FASTA format, and the primer was selected according to the principles of LAMP primer design (Table 1). Primers F3 and B3 were used as the upstream and downstream primers for PCR amplification, and the expected fragment size of amplification was 242bp. Blast function was applied to compare the sequences of the designed primer sets with the sequences of other strains to test the specificity of the primer sets; the designed primers were synthesized by Bio-engineering Biotechnology Co.

Establishment of initial LAMP reaction system

According to the instructions of the LAMP reaction kit, the preliminary LAMP detection system for *Vibrio parahaemolyticus* was established: the primer mix was prepared: 8 μL each of FIP and BIP, 1 μL each of F3 and B3, and the distilled water was replenished to 25 μL, and at this time the ratio of the inner primer to the outer primer was 8:1. The primer mix was prepared and used now for each experiment; and the initial reaction condition was 65 °C for 60 min; after preparation, the reagents were vortexed and centrifuged to collect the reagents in the tubes for reaction.

Optimization of LAMP reaction conditions

The LAMP reaction conditions were optimized, and the reaction temperatures were adjusted to 56 °C, 58 °C, 60 °C, 62 °C and 64 °C, and the reaction was carried out in a metal bath with different temperatures set. The reaction time was adjusted to 10 min, 20 min, 30 min, 40 min, and 50 min, respectively, and reaction product was taken at the end of the reaction and analyzed by 2% agar gel electrophoresis to determine the optimal reaction temperature and time. After selecting the optimal reaction temperature and time, the external primer: the internal primer ratio in the Primer Mix was adjusted to 1:2, 1:4, 1:6, 1:8, 1:10, 1:12, and the products were analyzed by 2% agar gel electrophoresis to determine the optimal ratio of internal and external primers. The amount of *Bst* DNA polymerase was optimized and adjusted to 0.6 μL, 0.8 μL, 1 μL, 1.2 μL, and the optimal concentration of *Bst* DNA polymerase in the reaction system was determined by gel electrophoresis according to the band.

LAMP-LFD reaction system was established

The LAMP internal primer FIP was labeled with biotin and the primer LB was labeled with 6-FAM for subsequent LFD analysis and determination[21]. According to the optimized reaction system, each component was added to the reaction tube and placed in a constant temperature metal bath, and the biotin-labeled LAMP amplification product could be obtained at the end of the reaction[22], without inactivating the enzyme at the end of the reaction, the 6-FAM-labeled primer LB was added to the reaction system and warmed up in the bath for 5 min, and then 5 μL of LAMP-amplified hybridization products were taken and added to 100 μL of buffer solution mix well, immerse the LFD paper strip into the mixture, and check the results after 5 min; the LAMP-labeled product hybridizes specifically with the 6-FAM-labeled LB to form a complex, which interacts with the solid-phase biotin-labeled antibody at the detection line (T) of the LFD paper to form a red band, which leads to a positive reading; while the unhybridized 6-FAM-labeled primer without biotin crosses over the detection zone and binds to the biotin-labeled antibody, and the LAMP-labeled antibody binds to the biotin-labeled antibody. The unhybridized 6-FAM-labeled primer without biotin crosses the detection zone and binds to the QC line (C) forming a blue band.

Specificity analysis of LAMP-LFD

To evaluate the specificity of the assay, several Vibrio spp. pathogens similar to *Vibrio parahaemolyticus*, such as *Vibrio vulnificus*, *Vibrio fluvialis*, *Vibrio cholerae*, and *Vibrio alginolyticus*, and other common pathogenic strains were selected as controls, such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella Pneumoniae*, *Streptococcus pyogenes*. New culture colonies of each strain were picked, and the bacterial solution was prepared using saline and the concentration was adjusted to $1.5x10^8$ cfu/mL, and the bacterial DNA extracted according to the boiling method was placed into a metal bath for LAMP reaction with the optimized system and reaction conditions; the amplification products were synchronized to read the results by LFD and 2% agarose gel electrophoresis ass and compared with the PCR assay methods, and the external Primers B3 and F3 were the upstream and downstream primers for PCR (Table 2), and the DNA template was consistent with the LAMP reaction, and the reaction program was divided into three stages[23]. Phase I: 94 °C, 5 min. Phase II: 94 °C, 30 s; 60 °C, 30 s; 72 °C, 30 s; a total of 35 cycles. Phase III: 72 °C, 5 min. PCR reaction products were analyzed by 1% agarose gel electrophoresis.

Sensitivity analysis of LAMP-LFD

The LAMP reaction was carried out in a metal bath in accordance with the optimized reaction system and reaction conditions by taking the gene DNA extracted from *Vibrio parahaemolyticus* bacterial fluids prepared at

Table 2. Reagents for PCR.

Reagents	
Premix Es taq	10 μL
Template DNA	1 μL
Primer F3/B3	0.2 μmol/L
ddH_2O	8 μL

Table 3. Reagents for LAMP-LFD.

Reagents	
Bca BEST Buffer	12.5 μL
Bca BEST DNA Polymerase	8 U
Template DNA	1 μL
Primer FIP/BIP	2.0 μmol/L
Primer F3/B3	0.2 μmol/L
Primer LB	0.4 μmol/L
ddH_2O	8 μL

different dilution concentrations as templates and using distilled water as a negative control; the reaction products were detected synchronously by 2% agar gel electrophoresis and LFD paper, and the lowest concentration of the bacterial fluids detected was the sensitivity of the reaction system and was compared with that of the PCR detection method. To verify the sensitivity of the LAMP assay comparing with the PCR assay.

Repeatability experiment of LAMP-LFD

Freshly cultured *Vibrio parahaemolyticus* colonies were taken, and the bacterial solution was prepared using saline and the concentration was adjusted to 0.5 McNeil units (equivalent to 1.5×10^8 cfu/mL) by a McNeil turbidimeter, and successive 10-fold isobaric dilutions were made to the lowest concentration that could be detected by the LAMP-LFD (i.e., 1.5×10^2 cfu/mL), and the three groups of samples were prepared in parallel; the genetic DNA was extracted and used as a template, distilled water as a negative control, the optimized reaction system was used to add the components to the reaction tube in a metal bath; the three groups of amplification products were detected by LFD, 2% agarose gel electrophoresis, respectively, to verify the feasibility of the method.

Experiment on artificial contamination of shrimp tissue by *Vibrio parahaemolyticus*

Fresh shrimp tissue was selected 200 mg, added into the sterile water to fully homogenize, and then fixed to 2 mL. Freshly cultured *Vibrio parahaemolyticus* colonies were picked, and the bacterial solution was adjusted to a concentration of 1.5×10^8 cfu/mL, with successive 10-fold isocratic dilutions. 1 mL was taken each of the different concentrations of bacterial solution, and mix with the fresh shrimp tissue homogenate in equal volume. Then centrifuge at 12,000 rpm for 5 min to remove the supernatant and leave the sediment; add 150 μL of distilled water to the sediment, vortex to mix well, and extract the bacterial DNA according to the instructions of the DNA extraction kit to be used as a template for PCR and LAMP-LFD assay. The optimized system and conditions were put into a metal bath for the LAMP reaction; the LAMP amplification products were analyzed by PCR, LAMP-AGE, and LAMP-LFD method, respectively to verify the feasibility of the LAMP-LFD assay in practical applications.

RESULTS

Optimization of LAMP-LFD reaction conditions

2% agarose gel electrophoresis was used to evaluate the LAMP results at temperatures between 56 °C and 64 °C. At 56 °C, no amplified bands were seen; at the other four temperatures, clear trapezoidal bands were seen, indicating consistent results (Fig. 1A). Of these, the bands produced at 60 °C showed increased brightness and clarity, suggesting a greater output of amplified products within the same reaction time and making identification easier. As a result, 60 °C was chosen as the temperature for the following LAMP reactions. After 20 min of incubation, the optimization of LAMP reaction time revealed faint white bands. The reaction time reached its maximum band intensity at 40 min (Fig. 1B), but there was no improvement after that. An ideal reaction time of 40 min was established in order to maximize reaction duration and accomplish quick detection. Variations in the depths of trapezoidal strips under various primer ratios were observed in the optimization experiment involving the ratio of internal and exterior primers. Notably, the trapezoidal strip showed better strength and clarity than other ratios evaluated when using a 1:10 ratio of external primers to internal primers (Fig. 1C). For the ensuing LAMP reactions, this primer ratio was selected. Additionally, maximizing the concentration of *Bst* DNA polymerase demonstrated that adding more enzyme increased the quantity of LAMP product to a certain degree, along with a brighter and more distinct electrophoretic band. Higher enzyme concentrations were found to result in no discernible increase in brightness beyond the second or third band, indicating that the benefits of improving amplification efficiency decreased as enzyme concentration rose. To maximize cost-effectiveness without sacrificing performance quality, a final addition volume of 1.0 μL was chosen (Fig. 1D), which corresponds to an ideal concentration of 8.0 U/μL for *Bst* DNA polymerase. In negative control hole No. 1, there was no evidence of an amplification reaction. Through enhancing the response and modifying the experimental setup. The primers in the system were mixed at 1:10 ratio to form Primer Mix, and the reaction condition was 60 °C for 40 min.

Establishment of LAMP-LFD reaction system

Utilizing 1.5×10^8 cfu/mL of isolated *Vibrio parahaemolyticus* DNA as a template for the LAMP reaction (Table 3), the results were examined using LFD and 2% agarose gel electrophoresis. Agarose gel electrophoresis revealed no bands in the negative control, but well defined trapezoidal bands in hole 1 (Fig. 2A). The *Vibrio para-*

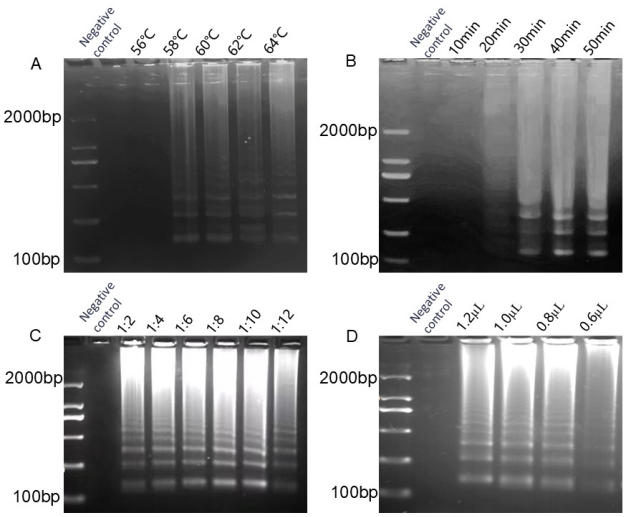

Fig. 1. LAMP reaction system condition optimization results. **A.** Optimization of temperature; **B.** Optimization of reaction time; **C.** Optimization of inner-primers with outer-primers; **D.** Optimization of *Bst* DNA.

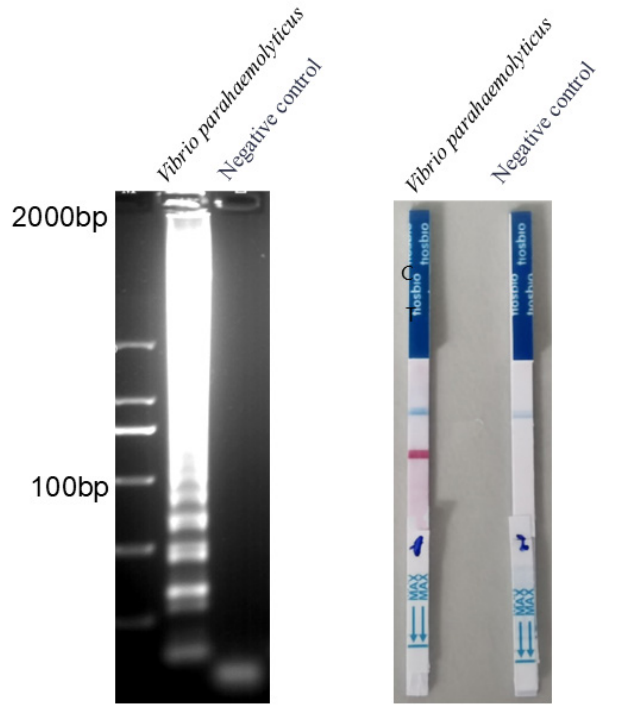

Fig. 2. The establishment of *Vibrio parahaemolyticus* LAMP-LFD assay by agarose gel electrophoresis (LAMP-AGE; A) and by lateral flow dipstick (LAMP-LFD; B).

haemolyticus DNA group had a blue strip at the quality control line (C) and a red strip at the detection line (T), according to the LAMP-LFD results, whereas the negative control group only had a blue strip at the quality control line (C) (Fig. 2B). The findings demonstrated the viability of the established LAMP-LFD detection method and the consistency of LAMP product identification using gel electrophoresis and LFD test paper.

Specificity analysis of LAMP-LFD

Using the biotin-labeled inner primer *tlh*-FIP, the LAMP reaction was performed. After optimization, the reaction condition was heated in a metal bath for 40 min at 60 °C. The expected trapezoidal bands could be formed after LAMP product when *Vibrio parahaemolyticus* DNA was used as the template. Following 2% agar gel electrophoresis the labeled LAMP product's hybridization with the 6-FAM labeled primer, the hybrid product produced a clear red strip at the LFD test strip's detection line point, signifying a successful detection. The LAMP results were all negative when the template contained the DNA of *Vibrio vulnificus*, *Vibrio fluvialis*, *Vibrio cholerae*, *Vibrio alginolyticus*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Streptococcus pyogenes*. The hybridization result with primer LB was negative and did not produce any strip at the detection line position on the LFD strip. The distilled water's No. 12 negative control hole did not exhibit any positive effect. The PCR and LAMP results agreed with each other (Fig. 3).

Sensitivity analysis of LAMP-LFD

Using concentrations ranging from 1.5×10^8 to 1.5×10^2 cfu/mL of *Vibrio parahaemolyticus* DNA as templates, LAMP reactions were conducted under optimized conditions. Subsequently, the products underwent 2% agar gel electrophoresis, resulting in the visualization of distinct trapezoidal bands. Following hybridization of the LAMP products with labeled primers, a conspicuous red strip appeared in the detection line area on the LFD strip, confirming a positive detection outcome. However, at a lower bacterial solution concentration of 1.5×10^1 cfu/mL, no amplified bands were observed post-agar gel electrophoresis, and no discernible bands appeared in the detection line area of the LFD strip after hybridization with labeled primers. This indicated that the minimum detectable DNA concentration using LAMP-LFD was 1.5×10^2 cfu/mL. The lowest template concentration detectable via agar gel electrophoresis was also 1.5×10^2 cfu/mL. In comparison, the PCR method utilizing F3 and B3 primers could detect the lowest template concentration at 1.5×10^4 cfu/mL. Notably, in the negative control group, where distilled water served as the template, no amplification reaction was observed (Fig. 4).

Repeatability experiment of LAMP-LFD

Vibrio parahaemolyticus was re-cultured, and a single colony was selected to prepare a fresh bacterial suspension. The concentration was adjusted to the lowest detectable level of 1.5×10^2 cfu/mL, followed by DNA extraction to serve as a template. The optimized reaction system was incubated in a metal bath at 60 for 40 min. The results showed positive outcomes in testing LAMP products using both 2% agar gel electrophoresis and LFD methods when the minimum concentration of *Vibrio parahaemolyticus* DNA was used as the template. Conversely, all tests resulted in negative outcomes with good repeatability when distilled water was used as the template (Fig. 5).

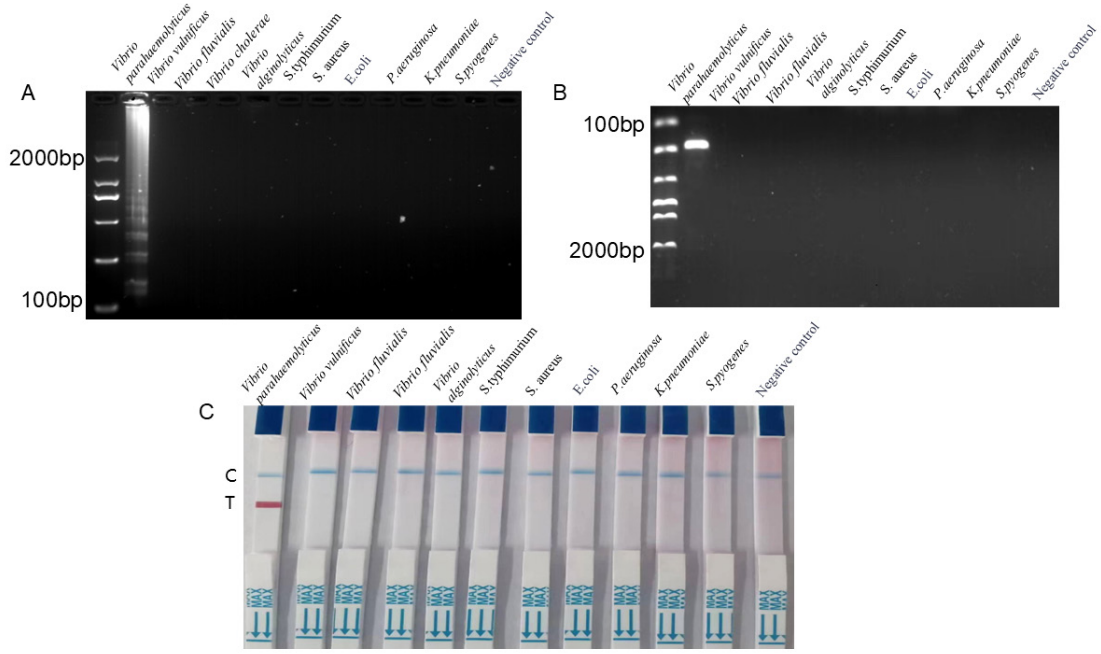

Fig. 3. Specific analysis results of LAMP-LFD. **A.** 2% gel electrophoresis of LAMP products; **B.** PCR products were detected by 1% gel electrophoresis; **C.** LAMP-LFD method.

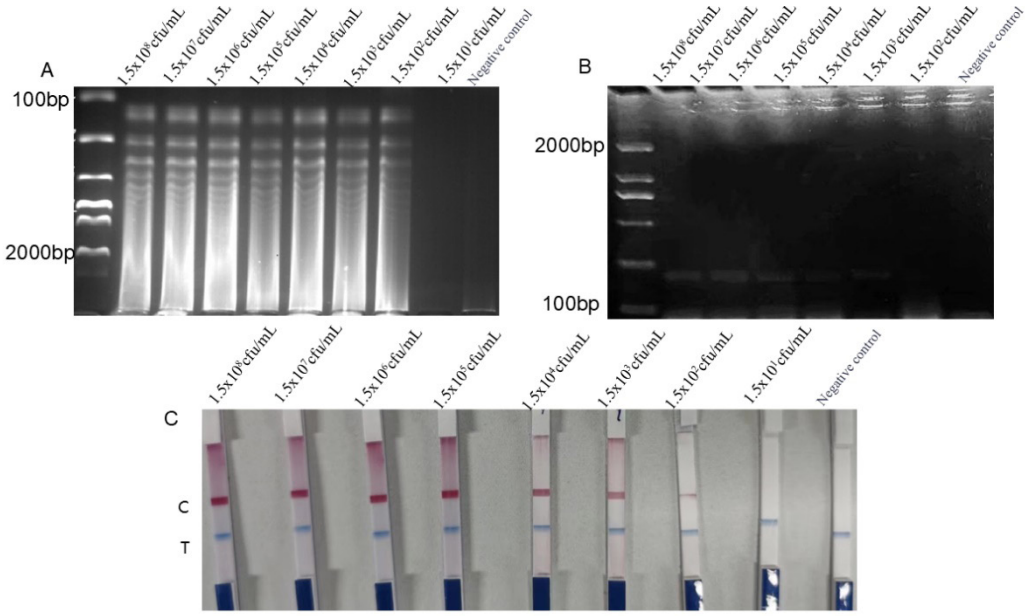

Fig. 4. Sensitivity analysis results of LAMP-LFD. **A.** 2% gel electrophoresis of LAMP products; **B.** PCR products were detected by 1% gel electrophoresis; **C.** LAMP-LFD method.

Experiment on artificial contamination of shrimp tissue by *Vibrio parahaemolyticus*

Vibrio parahaemolyticus bacterial solution of different concentrations of fresh shrimp tissue equal volume contamination, extraction of mixed homogenous plasma DNA, by LAMP-LFD, LAMP-AGE and PCR methods, respectively, it can be seen that LAMP-LFD and LAMP-AGE can be detected from the concentration of 1.5×10^3 cfu/mL bacterial solution contaminated with pathogens in fresh shrimp tissues, which is equivalent to 0.75×10^3 cfu/mL concentration of tissue homogenate, and the PCR reaction could only detect the pathogen from 0.75×10^5 cfu/mL fresh shrimp tissue homogenate (Fig. 6).

DISCUSSION

Vibrio parahaemolyticus is one of the foodborne pathogenic bacteria, and a serious pathogen of fish, shrimp, shellfish and crab culture, widely present in a variety of seafood and salty pickled food[2], mainly due to food poisoning caused by the consumption of food containing the

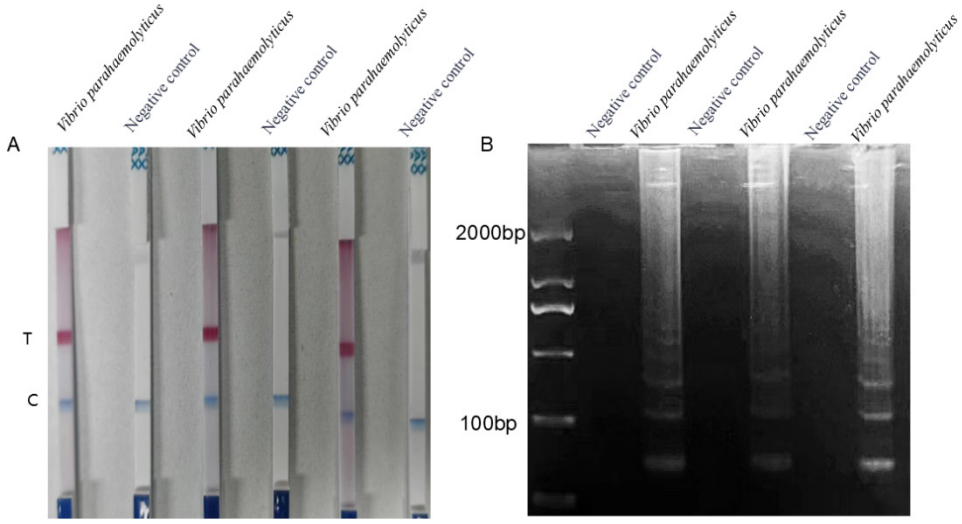

Fig. 5. Repeatability analysis results of LAMP-LFD. **A.** 2% gel electrophoresis of LAMP products; **B.** LAMP-LFD method.

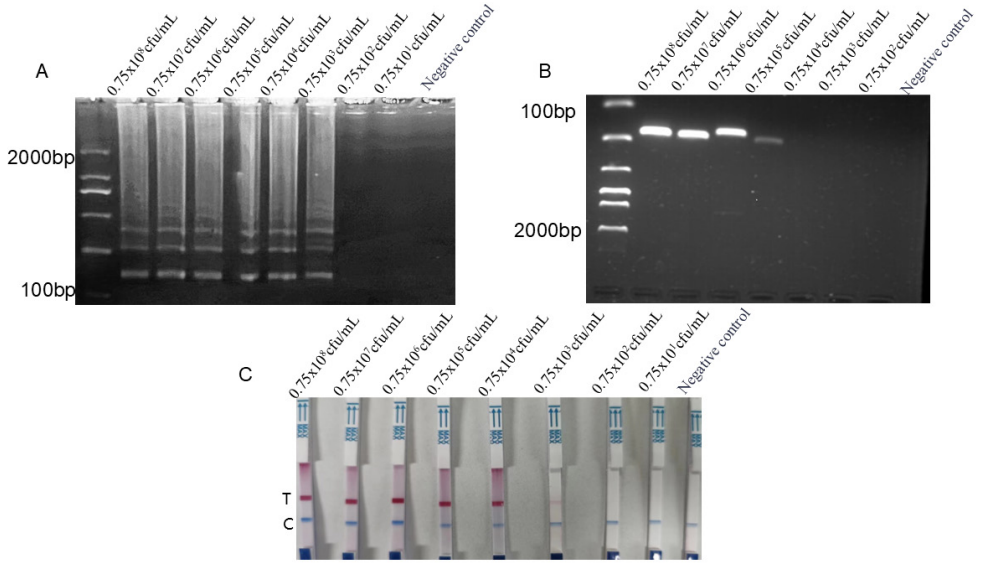

Fig. 6. LAMP-LFD analysis results of shrimp tissue samples artificially contaminated with *Vibrio parahaemolyticus*. **A.** 2% gel electrophoresis of LAMP products; **B.** PCR products were detected by 1% gel electrophoresis; **C.** LAMP-LFD method.

bacteria, with the gradual increase in the proportion of seafood products in the dietary structure, human diseases caused by *Vibrio parahaemolyticus* have also received more and more attention[24], and have become one of the potential factors that seriously threaten human health. Therefore, it is of great significance to establish a rapid, convenient and accurate detection technology to realize the early diagnosis and immediate detection of this pathogen for the effective prevention and detection of diseases. The selection of target genes and the design of LAMP primers are particularly important for the high specificity and sensitivity of the detection. *Tdh* gene, *trh* gene and *tlh* gene are the most representative of multiple virulence genes of *Vibrio parahaemolyticus*, but *tdh* and *trh* genes do not have a high specificity, and it was later found that the *tlh* gene exists in all *Vibrio parahaemolyticus*, and according to the relevant studies, the *tlh* gene has been used as the target-specific detection of *Vibrio parahaemolyticus*. Therefore, the *tlh* gene of *Vibrio parahaemolyticus* was selected as the target gene in this study.

Due to its high sensitivity and strong amplification efficiency, LAMP is prone to environmental contamination to produce false positive results, which seriously affects the accuracy of the detection method. The main reason for contamination during the nucleic acid detection process is that the amplification products form aerosols, which adhere to the clothes, gloves, pipettes, reaction tubes and other objects of the experimental personnel, thus affecting the next reaction process and causing false-positive results. As the reaction reagents need to be added in the middle of the experimental process, the opening of the lid cannot be avoided, so it is necessary to strictly use physi-

cal isolation methods, the experimental area is divided into the reagent preparation area, the sample area, the reaction amplification area and the amplification product detection area and other different areas, and at the same time, the reagents and the experimental equipment are not mixed between the various areas, and according to the standardized operation to prevent the cross-contamination of the areas. Moreover, after the operation, we use alcohol to sterilize and then irradiate with ultraviolet light for 30–60 min, which reduces the possibility of product contamination to a certain extent.

Loop-Mediated Isothermal Amplification with its high specificity and high sensitivity has gradually gained the attention of the majority of scholars, is currently one of the rapid, accurate and simple nucleic acid amplification methods, but the detection of turbidity or dye changes in the experimental results through visual observation has a greater subjectivity, and with the help of PCR amplifier or agarose gel electrophoresis technology read the results of the more complex and does not have the advantage of the way. In this study, LAMP detection method was combined with LFD technology to establish a rapid detection method for *Vibrio parahaemolyticus* LAMP-LFD, which is simple to operate, has low requirements for experimental conditions, and visualizes the detection, and reads the results directly on the LFD test paper with the naked eye. Compared with the standard culture method, it avoids the time-consuming and tedious operation steps such as bacterial enrichment and culture, and its nucleic acid amplification process does not need the repeated temperature change process similar to that of PCR, which greatly shortens the detection time, and the amplification process can be completed under constant temperature, which reduces the dependence on the reaction instrument. The use of fluorescein-labeled probe primers and LAMP product specific binding through the LFD test paper for detection, to a certain extent, to make up for the limitations of the detection of the lack of testing equipment cannot be detected, as long as the color bands on the test strips can be determined by the results, more suitable for on-site and grass-roots level of detection use.

CONCLUSION

In this study, LAMP primers were designed with *Vibrio parahaemolyticus tlh* as the target gene. After optimizing the reaction temperature, reaction time, internal and external primer ratio and enzyme addition, the final LAMP-LFD reaction system was determined as follows: 25 μL of the total reaction, of which 2.5 μL of the primer mixture (including the internal primer at a concentration of 2.0 μmol/L; the external primer at 0.2 μmol/L); 1 μL of template DNA; 12.5 μL of LAMP reaction buffer; and 8.0 U *Bst* DNA polymerase. The specificity of LAMP-LFD in detecting *Vibrio parahaemolyticus* is not significantly different from that of conventional PCR method, while the sensitivity is better than that of conventional PCR method, and it has good stability, which greatly shortens the reaction time and is more suitable for rapid on-site detection.

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