

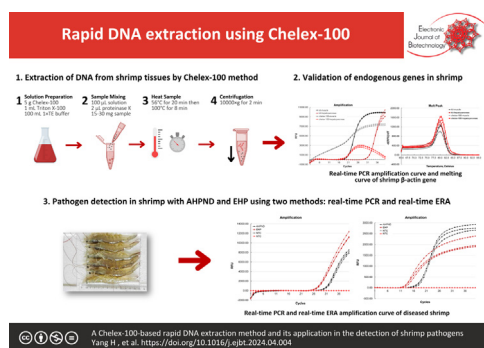


Research article

A Chelex-100-based rapid DNA extraction method and its application in the detection of shrimp pathogens ☆

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GRAPHICAL ABSTRACT



Rapid DNA extraction using Chelex-100

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ABSTRACT

Background: The Pacific white shrimp is one of the world's most economically significant aquatic species, being one of the top three species cultured globally. However, the increasing incidence of diseases such as acute hepatopancreatic necrosis disease and hepatopancreatic microsporidia has led to a serious decline in shrimp production and severe economic losses. With the increasing demand for pathogen detection in shrimp farms, rapid DNA extraction technology has become more sophisticated. In this study, a rapid and crude method of extracting genomic DNA from shrimp muscle and hepatopancreas using Chelex-100 was established.

Results: DNA was successfully extracted from muscle and hepatopancreatic tissues using both the Chelex-100 method and commercial kits. The internal reference genes of shrimp were successfully amplified via PCR and real-time PCR using the obtained DNA samples. Moreover, a field assay was successfully conducted using real-time PCR and real-time enzymatic recombinase amplification (real-time ERA), indicating that the quality of the DNA extracted using Chelex-100 is sufficient for use in conjunction with nucleic acid amplification to detect pathogens in shrimps.

Conclusions: Chelex-100 is an efficient method for extracting DNA from shrimp muscle or hepatopancreas tissues, with a short extraction time, high extraction efficiency, and simple operation, making it appropriate for use in the detection of pathogens in shrimp.

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1. Introduction

The cultivation of Pacific white shrimp currently dominates the world's shrimp farming industry; however, this species is threatened by serious diseases, which pose a significant challenge to the shrimp farming globally. Acute hepatopancreatic necrosis disease (AHPND) is a disease caused primarily by *Vibrio parahaemolyticus* infection. Since 2009, AHPND has caused a significant decline in shrimp production, resulting in severe economic losses. In areas affected by AHPND, shrimp production has decreased by approximately 60%, and the global shrimp farming industry has suffered a loss of USD 43 billion [1,2]. Shrimp affected by AHPND exhibit lethargy, anorexia, and slow growth. During the first 30 d after shrimp larvae are stocked, many epithelial cells can be observed in the hepatopancreas or digestive tract [3,4,5]. Hepatopancreatic microsporidiosis (HPM) is another shrimp disease caused by *Enterocytozoon hepatopenaei* (EHP) bacteria. EHP mainly parasitizes shrimp hepatopancreatic tissue, specifically the epithelial cells of the hepatic tubules [6]. It is often found in Pacific white shrimp, spot prawns, and other economically important shrimp species, and is highly contagious. Since its discovery in 2003, EHP infections have been reported in several countries, including China, Thailand, Australia, Malaysia, Venezuela, India, and Indonesia [7,8,9].

Rapid and effective pathogen detection technologies are crucial in controlling and mitigating shrimp disease outbreaks. Nucleic acid molecular detection methods have been successfully developed in recent years and are now widely used in the detection of shrimp pathogens, particularly real-time quantitative polymerase chain reaction (real-time PCR) testing and isothermal amplification techniques such as real-time enzymatic recombinase amplification (real-time ERA) [10]. Almost all detection methods require the rapid preparation of high-quality DNA samples as the initial step. Hence, in the realm of shrimp pathogen detection, developing an effective, fast, and straightforward method for extracting DNA from shrimp tissue is of paramount importance.

There are various techniques for extracting shrimp DNA, and the most common methods include the phenol–chloroform method, the centrifugal column adsorption method, and the magnetic bead method. However, these techniques are time-consuming, costly, and difficult and have other shortcomings when applied to shrimp pathogen detection. The phenol–chloroform method is a traditional approach. The DNA obtained through the phenol–chloroform method exhibits high purity and satisfies multiple experiment requirements [11,12,13]. However, the used solvents may differ in effectiveness and the residual phenol in the extraction method may potentially hinder PCR amplification [14]. In addition, the extraction procedure in the phenol–chloroform method is arduous, time-consuming, and prone to cross-contamination. Finally, the chemicals used in this process are potentially hazardous, posing health risks to the operator and leading to environmental pollution [15]. DNA extracted using the centrifugal adsorption column method has superior purity, thus ensuring the safety of the extracted DNA. Furthermore, the centrifugal column technique can be applied using micro-volumes, with the caveat that it requires repeated centrifugation of the extracted DNA [11]. It is therefore not conducive to high-

throughput, automated operations and is both costly and time-consuming. Magnetic beads, on the other hand, eliminate the need for organic solvents and repeated centrifugation and can be used in combination with high-throughput techniques. However, the process is more intricate, is time-consuming, and requires specialized expertise to operate [16]. Currently, when testing for shrimp pathogens, laboratories typically use commercial kits to extract DNA from shrimp tissues. However, DNA extraction using kits takes about 1–2 h to yield results [17]. In addition, commercial kits are expensive, cumbersome to use, and require a high level of skill from the laboratory technician, making them unsuitable for the field detection of pathogens in shrimp.

In recent years, several scholars have conducted in-depth research to increase the speed of existing DNA extraction methods. For example, the integration of magnetic bead adsorption of genomic DNA in the traditional cetyltrimethylammonium bromide (CTAB) method reduced the number of operating steps and shortened the processing time, thus improving the method [18]. Moreover, the utilization of sodium dodecyl sulfate (SDS) in combination with adsorbent silica gel columns not only achieved effective cell lysis but also provided a safe and non-polluting means of isolation and purification [19]. Additionally, the operation is faster and more cost-effective, effectively avoiding the use of harmful organic solvents such as chloroform. Although the above methods have improved the efficiency of DNA extraction compared to traditional methods, their processes are still time-consuming and their operational procedures somewhat inconvenient, which could hinder the subsequent shrimp pathogen detection process.

Recently, the Chelex-100-based method was shown to be an efficient and rapid DNA extraction method with low cost and high ease of use, making it an ideal method for use in shrimp pathogen detection. Chelex-100 is a chelating resin with a high affinity for polyvalent metal ions. It is composed of a copolymer of styrene and divinylbenzene paired with iminodiacetic acid ions, functioning as chelating groups. Chelex-100 ruptures the cell membranes of a limited number of cells, releasing their DNA via boiling. It also prevents DNA degradation through the chelated metal ions during boiling. Currently, Chelex-100 is predominantly utilized to extract DNA from various matrices, including bacteria, fungi, insects, and other biological sources [20]. Additionally, it facilitates the efficient amplification of DNA for sequencing, genotyping, and specific assays, among other applications [21,22,23,24,25,26].

The Chelex-100 method enables fast, simple, and cost-effective DNA extraction without the use of organic solvents or the need for extensive sample preparations or transfer [27]. In addition, the method can effectively bind other exogenous substances that may interfere with nucleic acid amplification during extraction. However, the method is not commonly used for DNA extraction from marine organism tissues, and reports on DNA extraction from shrimp tissue are scarce. Nonetheless, based on its advantages, the Chelex-100 method can be employed alongside nucleic acid amplification techniques to detect field pathogens in shrimp farms. The main objectives of this study are to optimize the DNA extraction technique to reduce the DNA extraction time and enhance the efficiency of shrimp pathogen detection by combining the Chelex-100 DNA extraction technique with real-time PCR and real-time ERA.

2. Materials and methods

2.1. Shrimp anatomy

Pacific white shrimp (average body weight 30 g, average length 10 cm) were taken from an aquaculture farm in Wenchang, Hainan, China. The shrimp were dissected using sterilized scissors and forceps. Muscle and hepatopancreatic tissues were then placed into cryopreservation tubes, labeled, and stored in a -80°C freezer.

2.2. DNA extraction

For DNA extraction, 5 g of Chelex-100 and 1 mL of TritonX-100 were added in 100 mL of $1 \times$ Tris-EDTA buffer. Next, 100 mL of the above solution was pipetted and 2 μL of 20 mg/mL fresh proteinase K was added to it to enzymatically cleave the histones bound to the nucleic acids so that the DNA was released from the solution. Then, 15–30 mg of shrimp muscle or hepatopancreas tissue was added, followed by vortexing and centrifugation for 10 s to mix. The samples were heated at 56°C for 20 min in a metal bath, denatured at 100°C for 8 min in another metal bath, then immediately cooled on an icebox for 3 min and vortexed on an oscillator for 15 s. They were subsequently centrifuged for 1–2 min at a speed of 8,000–10,000 g, and the supernatants were stored in a refrigerator at -20°C for future use (Fig. 1). Control experiments were also carried out using a Marine Biological Tissue DNA Extraction Kit (DP324-02, Tiangen, China). Finally, the DNA extracted from the shrimp muscle tissue using the two methods was uniformly mixed with $10 \times$ loading buffer and ddH₂O in a ratio of 1:1:8 and detected via 1.5% agarose gel electrophoresis, respectively.

2.3. Validation of endogenous genes in shrimp

Decapod-specific primers 143F (5'-TGCCTTATCAGCTNTCGATTG TAG-3') and 145R (5'-TTCAGNTTTCGAACCATCTCC-3') were

used to amplify an 848 bp amplicon of the 18S rRNA gene. The 25 μL PCR reaction mixture used for this purpose consisted of 12.5 μL of $2 \times$ Taq Master Mix Buffer (E005 Novoprotein China), 1 μL each of 10 μM primers, 1 μL of template DNA, and 9.5 μL of ddH₂O. PCR reaction was programmed as follows: an initial denaturation step at 94°C for 3 min, followed by 37 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 45 s, with a final extension at 72°C for 7 min. PCR amplification utilized the above-mentioned DNA extracted from shrimp muscle and hepatopancreas tissues as templates using Chelex-100 method and commercial kits, respectively. PCR products were identified via electrophoresis on 1.5% agarose gels. This PCR reaction was carried out in a Mini Amp PCR system (Thermo Fisher Scientific, USA). Real-time PCR amplification employed decapod-specific primers 178F (5'-GGTCGGTATGGGTCAGAAGGA-3') and 228R (5'-TTGCTTTGGGCTCATCAC-3') for the β -actin gene. The 20 μL real-time PCR reaction mixture consisted of 10 μL of Premix Ex Taq Buffer (RR390A Takara Japan), 0.4 μL each of 10 μM primers, 0.2 μL of probes, 0.8 μL of template DNA, and 8.2 μL of ddH₂O. The real-time PCR reaction was programmed as follows: an initial denaturation step at 95°C for 30 s, followed by 40 cycles of cyclic response at 95°C for 10 s and 60°C for 1 min. The above-mentioned DNA was isolated from the muscle and hepatopancreas tissues of the shrimp using the Chelex-100 method and commercial kits served as templates. The real-time PCR reaction was performed in a CFX96 Touch real-time PCR system (Bio-Rad, USA).

2.4. Optimization of incubation times

To optimize the reaction conditions, incubation time was set at 12, 14, 16, 18 and 20 min at 56°C for DNA extraction from muscle tissue using the Chelex-100 method. The DNA extracted from these different incubation times was used as a template to amplify the 18S rRNA endogenous gene using primers 143F and 145R and the amplification system and procedures described above. The

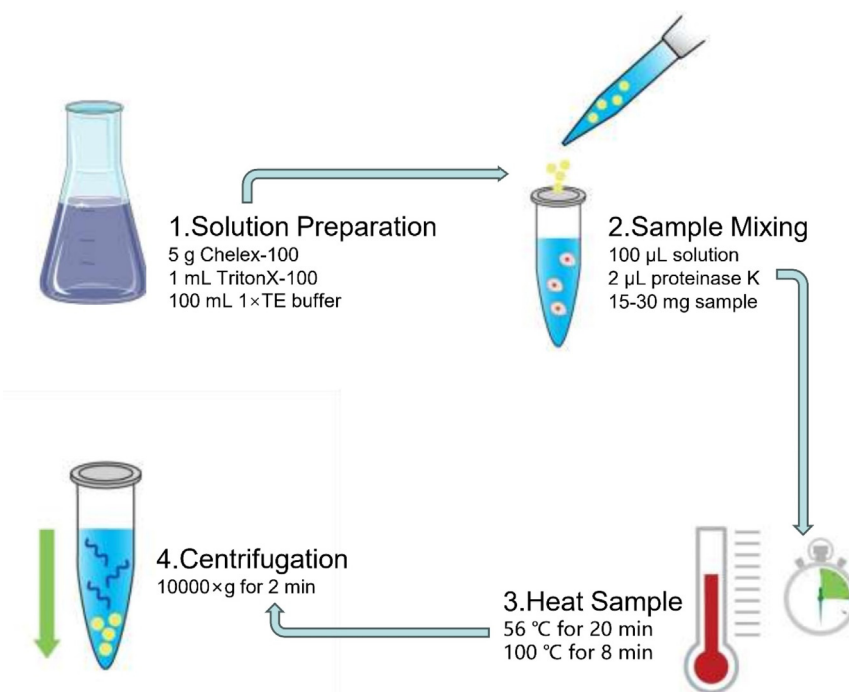


Fig. 1. Flow chart of DNA extraction via the Chelex-100 method. 1) Solution Preparation: 5 g of Chelex-100 and 1 mL of TritonX-100 were added in 100 mL of $1 \times$ TE buffer; 2) Sample Mixing: Fill with 100 μL of the above solution and 2 μL of 20 mg/mL of fresh proteinase K and 15–30 mg of sample; 3) Sample Heating: 56°C for 20 min and 100°C for 8 min; 4) Centrifugation: 10,000 g for 2 min.

resulting PCR products were analyzed using 1.5% agarose gel electrophoresis.

2.5. Practical sample testing

Twenty-four shrimp samples were collected from a farm in Fujian, China. Samples with probable EHP infection were obtained from this shrimp pond, and samples with probable AHPND infection were prepared using the *V. parahaemolyticus* stimulation assay. The pathogenic *V. parahaemolyticus* specimens were kindly provided by Prof. Lei Wang from the Institute of Oceanology, Chinese Academy of Sciences [28]. The hepatopancreatic tissues were dissected using sterile scissors and forceps. Hepatopancreatic tissues from 24 shrimp samples were used for DNA extraction using either the Chelex-100 method or the Marine Biological Tissue DNA Extraction Kit (DP324-02, Tiangen, China). Pathogen detection was conducted on DNA samples of these 24 shrimps utilizing both real-time PCR and real-time ERA. DNA extracted by both methods was used as template for real-time PCR amplification. The primers of real-time PCR for AHPND and EHP detection are listed in Table 1. Real-time PCR of AHPND was performed in a total volume of 20 µL containing 10 µL of Premix Ex Taq Buffer (RR390A, Takara, Japan), 0.6 µL each of 10 µM primers, 0.2 µL of probes, 0.8 µL of template DNA, and 7.8 µL of ddH₂O. Real-time PCR of EHP was performed in a total volume of 25 µL containing 12.5 µL of Premix Ex Taq Buffer (RR390A, Takara, Japan), 1 µL each of 10 µM primers, 0.5 µL of probes, 1 µL of template DNA, and 9 µL of ddH₂O. Real-time PCR reactions of AHPND or EHP were programmed as follows: an initial denaturation step at 95°C for 20 s, followed by 40 cycles of cyclic response at 95°C for 3 s and 60°C for 30 s. DNA extracted using both methods was used as template for real-time ERA amplification. The primers used in the real-time ERA for AHPND or EHP detection are listed in Table 1. Real-time ERA of AHPND and EHP was performed in a total volume of 50 µL containing 20 µL of Lytic agent, 2 µL of 10 µM preprimers, 2 µL of 10 µM primers, 0.6 µL of probes, 2 µL of template DNA, 2 µL of activator, and 21.4 µL of ddH₂O. Real-time ERA reactions of AHPND and EHP were programmed at 42°C for 20 min. Finally, to test the accuracy of the clinical samples, samples of diseased shrimp suffering from AHPND or EHP were selected for replication of the experiment.

3. Results

3.1. DNA agarose gel electrophoresis

The concentration of muscle tissue DNA extracted using Chelex-100 was 3174.9 ng/µL, and the concentration of hepatopancreatic DNA using Chelex-100 was 10225.9 ng/µL. The concentration of muscle DNA extracted with a commercial kit was 47.4 ng/µL, and

the concentration of hepatopancreatic DNA was 813.5 ng/µL. DNA extracted from shrimp muscle tissue was subjected to agarose gel electrophoresis using the commercial kit and Chelex-100 resin methods. The DNA extracted using the commercial kit showed a single, well-defined band in the electropherogram, while the DNA extracted using Chelex-100 resin did not show clear and visible bands (Fig. 2).

3.2. Validation of endogenous genes in shrimp

Using the DNA extracted from shrimp muscle and hepatopancreas tissues with the commercial kit and Chelex-100 as templates, the amplification of the 18S rRNA gene via PCR showed only one major band with a correct position (Fig. 3), as confirmed via sequencing. The β-actin gene was amplified via real-time PCR using DNA from shrimp hepatopancreas and muscle tissues extracted with Chelex-100 and commercial kit as templates. The intersection of the baseline (the horizontal line in the figure) and the amplification curves for kit–muscle, kit–hepatopancreas, Chelex-100–muscle, and Chelex-100–hepatopancreas represent their respective Cts (threshold cycles). The amplification curves displayed a single melting peak (Fig. 4).

3.3. Optimization of incubation times

Within the 20-min incubation period, the band exhibited the maximum brightness, indicating the highest amount of product extracted. Thus, 20 min was shown to be the optimal incubation time (Fig. 5).

3.4. Practical sample testing

In the real-time PCR assay, five samples testing positive for EHP and three samples testing positive for AHPND were detected using

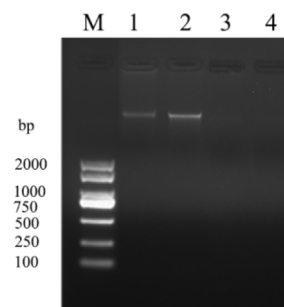


Fig. 2. DNA agarose gel electrophoresis. Lane M: DL2000 Marker (3427A, Takara, Japan); lanes 1–2: template is DNA of shrimp muscle tissue extracted using Commercial kits; lanes 3–4: template is DNA of shrimp muscle tissue extracted using Chelex-100 resin.

Table 1
Primers and probes information for AHPND and EHP.

Virus	Method	Primer	Sequences (5'-3')	length (bp)
AHPND	Real-time PCR	PirA-F	TTGGACTGTGCAACCAACG	135
		PirA-R	GCACCCATTGGTATTGAATG	
	Probe	6FAM-AGACAGCAAACATACACCTATCATCCCGGA-TAMRA		
EHP	Real-time ERA	PirA-F1	ATGGCGCTAGTCGGTITCTGTACAAT	233
		PirA-R1	ATTCCATAACGTTGATAAAAAACCCACC	
	Probe	CTCACATGACTAACGAATACGTTGTAACAA/i6FAMdT//idSp//iBHQ1dT/CATCTTTGACGGAAATTT/iSpC3/		
EHP	Real-time PCR	157F	AGTAAACTATGCCGACAA	156
		157R	AATTAAGCAGCACAAATCC	
	Probe	6FAM-TCCTGGTAGTGTCTTCCGT-TAMRA		
	Real-time ERA	EHP-F	TTGCAGAGTGTGTTAAGGGTTAAGTA	193
EHP-R		CTTCTTAAGCTGTTGTCTCCAACGTATT		
	Probe	AGAGACGATATTTACACAGACACAGCATT/i6FAMdT//idSp//iBHQ1d		

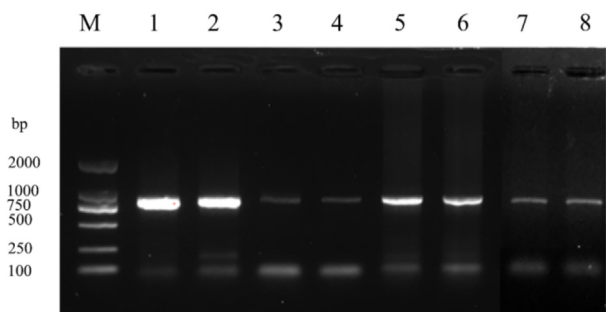


Fig. 3. PCR amplification of 18S rRNA gene in shrimp. Lane M: DL2000 Marker (3427A, Takara, Japan); lanes 1–2: template is DNA of shrimp muscle tissue extracted using kits; lanes 3–4: template is DNA of shrimp hepatopancreas tissue extracted using kits; lanes 5–6: template is DNA of shrimp muscle tissue extracted using Chelex-100 resin; lanes 7–8: template is DNA of shrimp hepatopancreas tissue extracted using Chelex-100 resin.

DNA extracted via the commercial kit and the Chelex-100 extraction method. In the real-time ERA assay, the commercial kit and the Chelex-100 extraction method both detected five EHP-positive and three AHPND-positive samples. The results showed

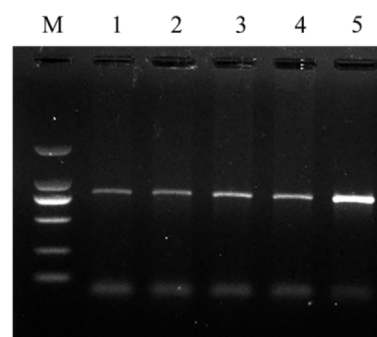


Fig. 5. Optimization of incubation time of Chelex-100 resin. Lane M: DL2000 Marker; lanes 1–5: 12 min, 14 min, 16 min, 18 min, and 20 min, respectively.

that the positive coincident rate of real-time PCR and real-time ERA using DNA extracted by both the Chelex-100 method and the commercial kit was 100%. All shrimp samples identified as positive for EHP or AHPND were sent for sequencing and tested positive (Table 2 and Table 3). The Chelex-100 extraction approach took merely 30 min to extract DNA from shrimp tissues, as

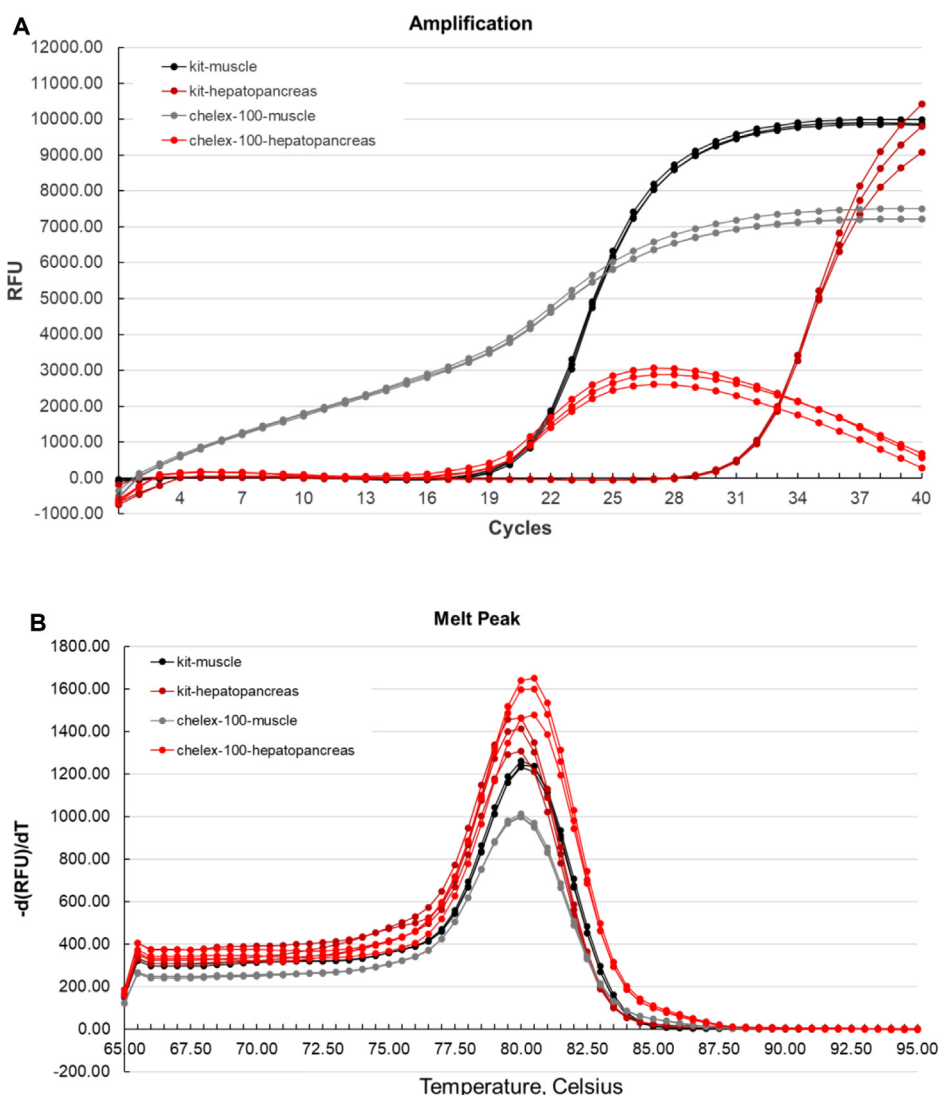


Fig. 4. Real-time PCR amplification curve (A) and melting curve (B) of shrimp β -actin gene. Experiments are conducted in three replicates run independently.

Table 2
Comparison between Kit and Chelex-100 in evaluation of EHP field samples.

Sample No.	Test kit		Chelex-100	
	real-time PCR	real-time ERA	real-time PCR	real-time ERA
1	–	–	–	–
2	–	–	–	–
3	–	–	–	–
4	–	–	–	–
5	–	–	–	–
6	+	+	+	+
7	–	–	–	+
8	–	–	–	–
9	–	–	–	–
10	–	–	–	–
11	–	–	–	–
12	–	–	–	–
13	–	–	–	–
14	–	–	–	–
15	–	–	–	–
16	–	–	–	–
17	–	–	–	–
18	–	–	–	–
19	–	–	–	–
20	+	+	+	+
21	–	–	–	–
22	+	+	+	+
23	–	–	–	–
24	–	–	–	–

Table 3
Comparison between Kit and Chelex-100 in evaluation of AHPND field samples.

Sample No.	Test kit		Chelex-100	
	real-time PCR	real-time ERA	real-time PCR	real-time ERA
1	–	–	–	–
2	+	+	+	+
3	–	–	–	–
4	–	–	–	–
5	–	–	–	–
6	–	–	–	–
7	–	–	–	+
8	–	–	–	–
9	+	+	+	+
10	–	–	–	–
11	–	–	–	–
12	–	–	–	–
13	–	–	–	–
14	–	–	–	–
15	+	+	+	+
16	+	+	+	+
17	–	–	–	–
18	–	–	–	–
19	–	–	–	–
20	–	–	–	–
21	–	–	–	–
22	+	+	+	+
23	–	–	–	–
24	–	–	–	–

opposed to the 3–5 h needed using the commercial kits. Subsequently, to validate the accuracy of clinical sample testing, the DNA samples extracted via Chelex-100 from shrimp infected with AHPND and EHP were used as templates for real-time PCR and real-time ERA replicates, and three independent replications of each experiment were conducted. The results showed that the experiment was reproducible (Fig. 6).

4. Discussion

Pacific white shrimp is a crucial species for China’s aquaculture sector. However, this species faces severe pathogenic threats.

Therefore, it is imperative to establish a precise and convenient shrimp pathogen detection system [29]. The technology for detecting shrimp pathogens is becoming more mature, with nucleic acid molecular detection methods being the primary means of pathogen detection. In recent years, rapid progress has been made in real-time PCR and isothermal amplification technologies. DNA extraction plays a vital role in the pathogen detection process, and thus, a rapid, efficient, and convenient DNA extraction method is crucial for enhancing the overall shrimp pathogen detection system. The most common method of DNA extraction for shrimp pathogen analysis is the use of commercial kits. However, most available commercial DNA kits require multiple steps to separate

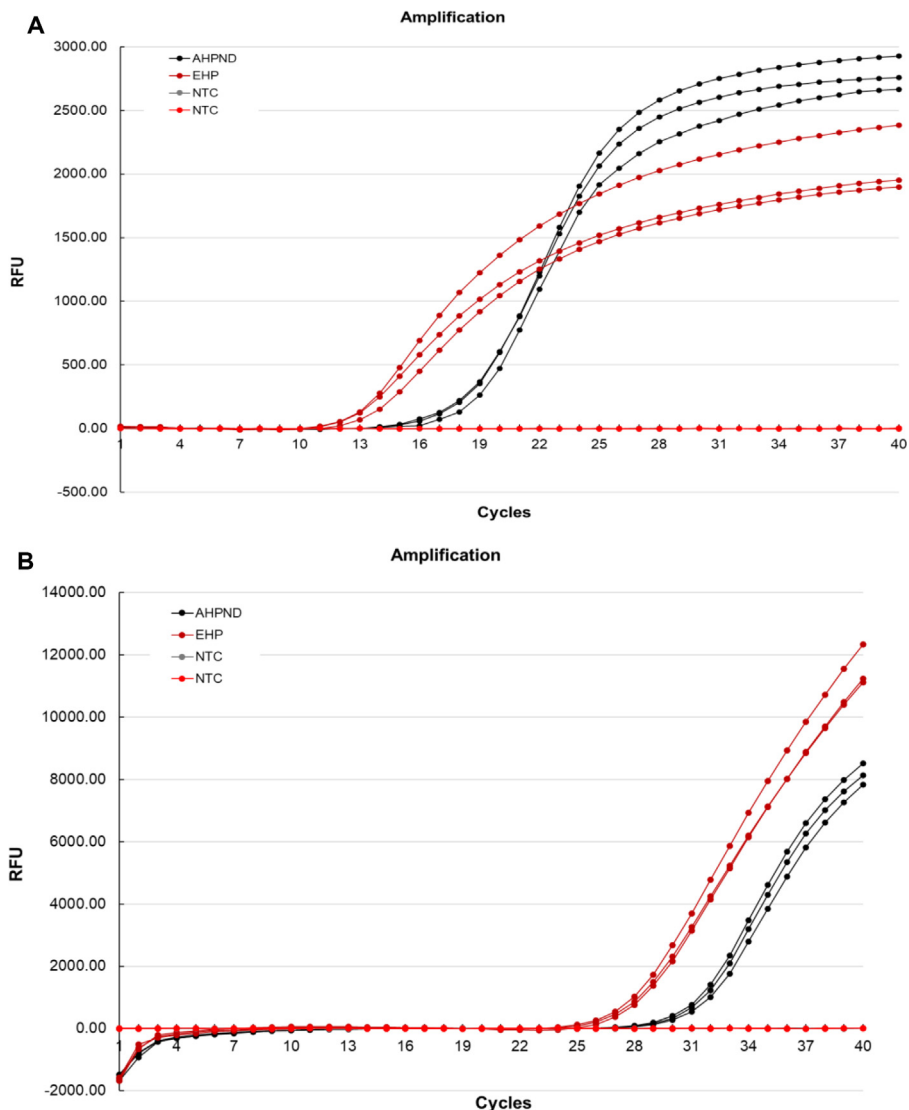


Fig. 6. Real-time PCR amplification curve of diseased shrimp (A), real-time ERA amplification curve of diseased shrimp (B). Experiments are conducted in three replicates run independently.

nucleic acids, leading to frequent sample transfer and the potential for DNA contamination. These complicated procedures may undermine the accuracy of pathogen detection [30,31]. In contrast, the Chelex-100 method requires only five steps to extract DNA from tissue, and the process takes approximately 30 min and uses only three reagents to treat the tissues [32,33,34,35].

In the present study, we used Chelex-100 and commercial kits to extract DNA from the muscle and hepatopancreas tissues of shrimp. The results showed that the concentration of the DNA extracted using the Chelex-100 method differed significantly from that extracted using the kit method. Although a higher DNA concentration was detected using the Chelex-100 method, the Chelex-100 method is a cruder method of DNA extraction, resulting in the inclusion of many contaminants in the extracted product. The commercial kit method extracted DNA at a lower concentration, but the multiple extraction steps resulted in a higher DNA purity. However, the cost of obtaining higher-purity DNA is that it takes a long time. The results revealed that agarose gel electrophoresis did not display clear and visible bands. This could be attributed to Chelex-100 being a crude method for DNA extraction. However, the DNA extracted using this method can still be utilized for further experiments. When the above products were

used to validate shrimp internal reference genes, the results were correct. The real-time PCR amplification curves of Chelex-100–muscle and Chelex-100–hepatopancreas produced less favorable results when amplifying the β -actin gene, likely due to the inhibitory effect of the Chelex-100 resin. When clinical samples were later tested, hepatopancreatic DNA extracted using either method correctly detected the incidence of disease in shrimp. Therefore, although the quality of DNA extracted by the Chelex-100 method is not as good as that of the commercial kit, it does not affect the accuracy of pathogen detection. In addition, it makes the process of pathogen detection faster and more convenient, significantly shortening the DNA extraction time. It has been demonstrated that the quality of the DNA can be improved after extraction using Chelex-100 resin via pre-precipitation with ammonium acetate and resuspension with Tris-EDTA buffer [35]. This method is expected to be more effective.

During DNA extraction, various conditions can affect the extraction quality [36]. Thus, additional optimization of the Chelex-100 method’s parameters is necessary to enhance the quality of the extracted DNA. It has been suggested that pH, temperature, and incubation time are critical to the effectiveness of DNA extraction. Therefore, while maintaining time efficiency, we optimized the

Chelex-100 method's incubation time by testing durations of 12, 14, 16, 18, and 20 min. It is possible that increasing the incubation time could yield better extraction results, but it may also decrease the efficiency of pathogen detection. Therefore, we chose not to extend the incubation time any further. We used these optimized incubation times as templates for amplifying the 18S rRNA internal reference gene and then detected the PCR products using 1.5% agarose gel electrophoresis. Our findings revealed that incubation for 20 min produced the brightest bands, indicating the extraction of the most products. Therefore, 20 min is recommended as the optimal incubation time.

In the testing of the clinical samples, the successful extraction of hepatopancreatic DNA samples from shrimp infected with *VpAHPND* and EHP was confirmed using real-time PCR and real-time ERA techniques to accurately detect the pathogens. The final test results are consistent with those found using the Chelex-100 and kit extraction methods. The results indicate that Sample 7 produced false positives in the real-time ERA assay when using DNA extracted via the Chelex-100 method as a template. This may have been caused by contamination resulting from improper handling. Nonetheless, the Chelex-100 extraction method is effective in detecting pathogens at shrimp farms.

5. Conclusions

This study presents an expedited method for extracting DNA from shrimp muscle or hepatopancreas tissues using Chelex-100 resin; the entire process takes only 30 min. It was determined that the ideal incubation duration while extracting DNA using this method is 20 min. We conducted pathogen detection tests using DNA extracted using both Chelex-100 resin and commercial kits. The results obtained from both methods were consistent. In summary, Chelex-100 extraction is an efficient method with a high extraction efficiency and simple operation, making it appropriate for the field detection of pathogens in shrimp.

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CRedit authorship contribution statement

Haoran Yang: Writing – original draft, Methodology, Investigation. **Qingqian Zhou:** Methodology, Investigation. **Jingjie Hu:** Project administration. **Zhenmin Bao:** Supervision, Funding acquisition. **Mengqiang Wang:** Writing – review & editing, Methodology, Conceptualization.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary material

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