Rapid and sensitive detection of pathogenic *Elizabethkingia miricola* in black spotted frog by RPA-LFD and fluorescent probe-based RPA

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**A B S T R A C T**

*Elizabethkingia miricola* is a highly infectious pathogen, which causes high mortality rate in frog farming. Therefore, it is urgent to develop a rapid and sensitive detection method. In this study, two rapid and sensitive methods including recombinase polymerase amplification combined with lateral flow dipstick (RPA-LFD) and fluorescent probe-based recombinase polymerase amplification (exo RPA) were established to effectively detect *E. miricola*, which can accomplish the examination at 38 °C within 30 min. The limiting sensitivity of RPA-LFD and exo RPA (10^2 copies/μL) was ten-fold higher than that in generic PCR assay. The specificities of the two methods were verified by detecting multiple DNA samples (*E. miricola, Staphylococcus aureus, Aeromonas hydrophila, Aeromonas veronii, CyHV-2* and *Edwardsiella ictaluri*), and the result showed that the single band was displayed in *E. miricola* DNA only. By tissue bacterial load and qRT-PCR assays, brain is the most sensitive tissue. Random 24 black spotted frog brain samples from farms were tested by generic PCR, basic RPA, RPA-LFD and exo RPA assays, and the results showed that RPA-LFD and exo RPA methods were able to detect *E. miricola* accurately and rapidly. In summary, the methods of RPA-LFD and exo RPA were able to detect *E. miricola* conveniently, rapidly, accurately and sensitively. This study provides prospective methods to detect *E. miricola* infection in frog culture.

1. Introduction

*Elizabethkingia miricola* is a gram-negative bacterium that can cause a variety of diseases in humans and animals [1]. *E. miricola* causes joint infections in multiple anuran (frogs and toads) species [2]. In addition, According to previous studies, *E. miricola* was proposed to cause urinary tract and native joint infections in humans [3,4]. *Pelophylax nigromaculatus* (black spotted frog) culture is booming because of delicious taste, abundant nutrition and high market value [5]. However, intensive aquaculture brings about a parallel growth trend of a variety of infectious diseases [6]. Black spotted frogs with *E. miricola* infection show the symptom of distorted head, catarracts and red lips [7]. The optimal time to control the diseases was easily missed, resulting in huge economic losses in the process of aquaculture. Therefore, it is essential to develop a rapid and convenient diagnostic method to detect potential *E. miricola* for the frog farming industry.

In general, traditional PCR has shown some advantages in rapid detection of bacteria [8,9]. However, traditional PCR keeps some defects. The traditional PCR is time-consuming and requires expensive temperature-controlled equipment. The real-time PCR and loop-mediated isothermal amplification (LAMP) have been further developed for detection of bacteria [10–12]. However, the reactions are affected by polymerase inhibitors possibly, and require temperature-controlled equipment and skilled operators. Therefore, there is an urgent need for convenient and rapid methods for clinical detection.

Recombinase polymerase amplification (RPA) technology, first introduced in 2006, is a novel isothermal nucleic acid amplification method.
technique, which mainly relies on three proteins: recombinase that binds single-stranded nucleic acids (oligonucleotide primers), single-stranded DNA-binding protein (SSB), and strand-substituting DNA polymerase [13]. RPA, a simple, rapid, and low cost without the need of sophisticated equipment or specialized skill, is of great application. For instance, RPA is used to detect genotype III grass carp reovirus [14], shrimp hemocyte iridescent virus [15] and shrimp viremia of carp virus [16]. Previous reports described that RPA was more sensitive, rapid and accurate than traditional PCR [17]. Although the traditional RPA can achieve the detection of RPA products by agarose gel electrophoresis, accurate than traditional PCR [17] . Although the traditional RPA can avoid the possibility of cross-contamination, but also eliminates the need for bulky laboratory equipment and cumbersome handling steps. The results are visualized in less than 30 min. RPA-LFD is used to detect infectious spleen and kidney necrosis virus [17], cyprinid herpesvirus 2 (CyHV-2) medium (Haibo biology, Oxoid Ltd) for 24 h at 37 °C.

2. Materials and methods

2.1. Frogs, pathogens and sampling

200 black spotted frogs were obtained from farms with and without infection history of wryneck disease in Wuhan, Hubei Province. The frogs were evenly divided into five tanks (83 cm × 60 cm × 54 cm). The tanks were kept wet. All the experimental procedures were approved by the animal protection and used Committee of Huazhong Agricultural University. The ethical number is HZAUFR-2021–0001.

E. miricola, Aeromonas hydrophila (ATCC 7966), Staphylococcus aureus (ATCC 25,923), Aeromonas veronii (LMG 9075), Edwardsiella ictalurid (DSM 13,697) and CyHV-2 were from our laboratory stock [30–32]. E. miricola strain was isolated and identified by our laboratory. The E. miricola strain was cultured to mid-log stage in brain–heart infusion (BHI) medium (Haibo biology, OxoId Ltd) for 24 h at 37 °C. Other pathogens were cultured to mid-log stage in Luria-Bertani (LB, L3027, Sigma, Shanghai, China) medium. CyHV-2 was kept at −80 °C.

Black spotted frogs with significant distorted head and cataracts were selected for the tissue bacterial load and qRT-PCR assays. Tissues from healthy black spotted frogs were used as negative control. The frogs were anesthetized with 0.4 g/L MS-222 (3-Aminobenzoic acid ethyl ester methanesulfonate) (Merck, Germany) for 20 min and their tissues (liver, lung, brain, stomach, spleen, heart, kidney, eye, and muscle) were dissected in a sterile environment.

2.2. DNA extraction

Tissue DNA was extracted from dissecting black spotted frogs. The samples were cut into mung bean size with scissors, and then grinded 5 min with 150 μL DNA extraction buffer (0.5% sodium dodecyl sulfate, 1 μg/mL, 300 mM NaCl 6 mL, 10 mM Tris-HCl, 2 mL, pH 8.0, 10 mM EDTA, 10 mL, pH 8.0). Then centrifuged slightly and added 10 μL proteinase K (150 μg/mL), and the samples were digested in 62 °C for 3 h. Then 600 μL Tris-saturated phenol was added and fully mixed for 10 min, and centrifugation was performed at 12,000 rpm for 10 min. The supernatant was transferred to a new EP tube, twice the volume of ethanol was added, precipitated at −20 °C for 30 min, and centrifuged at 12,000 rpm for 5 min. The supernatant was discarded, then the precipitate was washed twice with 70% ethanol and air-dried at room temperature. 50 μL the extracted DNA was dissolved in ultrapure water and then was stored at −20 °C for standby.
designed three forward primers and three reverse primers according to the principles of RPA primer (generally the length of RPA primer is 30–35 nt, the amplification efficiency is high, and the amplification product is less than 500 bp) and examined nine primer combinations (mutTF699/mutTR700, mutTF699/mutTR702, mutTF699/mutTR704, mutTF701/mutTR702, mutTF701/mutTR700, mutTF701/mutTR704, mutTF703/mutTR704, mutTF703/mutTR700, mutTF703/mutTR702).

The results of RPA reaction were detected with 2% agarose gel. The RPA-LFD probe was designed according to the sequence between the optimal primers. The RPA-LFD probe added a FAM on the 5′ end, a C3 spacer on the 3′ end, a dSpacer (tetrahydrofuran, THF) in the middle, and the reverse primer for RPA-LFD added a biotin on the 5′ end.

The probe for exo RPA was designed based on the sequence between the optimal primers. The exo RPA probe THF as the recognition site of exonuclease is located at least 35 nt away from the 5′ end. Then, a fluorescent group was labeled at the upstream of THF site, and a quenched group was labeled at the downstream. The distance between the two groups is 1–4 nt [33]. All primers and probes in this study were listed in Table 1, were synthesized by Tsingke Biotechnology (Beijing, China).

2.4. Establishment of basic RPA, RPA-LFD and exo RPA assays

The basic RPA was initiated by using the TwistAmp Basic kit (TwistDx, Cambridge, UK), and the reaction system contained 29.5 μL rehydration buffer, 2 μL forward primer, 2 μL reverse primer, 12 μL ddH₂O and 2 μL E. miricola DNA sample, and then 2.5 μL magnesium acetate was added immediately to start the reaction, and the reaction lasted for 30 min at 38 °C [17]. H₂O was used as a negative control. The reaction products were purified by phenol chloroform 1:1 extract and analyzed on 2% agarose gel electrophoresis subsequently.

TwistAmp nfo kit (TwistDX, Cambridge, UK) and Hybridetect 1 (Milenia Biotech GmbH, GieBen, Germany) dipsticks were used for RPA-LFD. The reaction system included 29.4 μL rehydration buffer, 2 μL forward primer, 2 μL reverse primer, 0.6 μL probe, 11.5 μL ddH₂O and 2 μL E. miricola DNA sample and was started by 2.5 μL magnesium acetate, H₂O was used as a negative control. mutT1 probe (without DNA sample) was used as a blank control. RPA-LFD reactions were performed in 38 °C for 30 min [17], and then, the products were diluted at 1:90 with ddH₂O. Dipsticks were put into the diluted samples and the results can be read in 5 min.
The exo RPA was initiated by using the TwistAmp exo kit (TwistDx, Cambridge, UK) and the reaction system included 29.4 μL rehydration buffer, 2 μL forward primer, 2 μL reverse primer, 0.6 μL probe, 11.5 μL ddH$_2$O and 2 μL E. miricola DNA sample and was started by 2.5 μL magnesium acetate, H$_2$O was used as a negative control. Fluorescent reactions were performed at 38°C for 30 min [17]. The fluorescence be observed by fluorescence imaging system (iQ5, Bio-Rad, California, USA) with fluorescence measurements recorded in the FAM channel.

2.5. Specificities of basic RPA, RPA-LFD and exo RPA assays

The genomic DNA were extracted from the pathogens (E. miricola, S. aureus, A. hydrophila, A. veronii, CyHV-2 and E. ictaluri) by a Universal Genomic DNA kit (CWBIO, China). The specificities of basic RPA, RPA-LFD and exo RPA were verified by detecting multiple DNA samples and a negative control (H$_2$O). The fluorescence value of exo RPA product was measured by the enzyme-labeled instrument (Tecan Nano Quant) (wavelength range: 485–535 nm).

2.6. Sensitivities of basic RPA, RPA-LFD and exo RPA assays

The partial sequence (545 bp) of mutT gene of E. miricola was obtained through PCR and cloned into the pMD19-T plasmid to generate recombinant plasmid pMD19-mutT. Then the plasmid was purified and converted to copy number by measuring the concentration of recombinant plasmid, DNA copies/μL = (ng/μL × 6.02 × 10$^{23}$ × 10$^{-9}$)/(Fragment length (bp) × 660) [21]. pMD19-mutT recombinant plasmid was diluted from 10$^4$ to 10$^7$ copies/μL. Detection results of PCR, RPA, RPA-LFD and exo RPA were compared, and the sensitivities of the four methods were analyzed.

2.7. Tissue bacterial load and qRT-PCR of E. miricola

Tissues (liver, lung, brain, stomach, heart, kidney, eye, and muscle) from five diseased black spotted frogs (distorted head or cataracts) were weighed and then homogenized in a sterile environment. Tissues from healthy black spotted frogs were used as negative control. Per gram of tissues were immediately homogenized in 6 mL PBS buffer by a tissue grinder (P0485, Sigma, Shanghai, China) at 28°C for 5 min, and then incubated in BHI medium at 28°C for 24 h. The colonies of E. miricola were pale yellow in color, slightly opaque in BHI medium [6]. The number of bacteria in the tissues was counted by two independent investigators.

Tissue bacterial load was calculated by dilution coated plate method [31]. Frogs euthanized by MS-222 was dissected, and the tissues were immediately gathered and placed into EP tubes containing 800 μL of Trizol. Total RNA was extracted and reversed to cDNA and stored at −80°C. The bacterial gene expression was analyzed by qRT-PCR, which was performed in a real-time PCR system (Applied Biosystems, USA). The qRT-PCR mixture contained 4 μL of cDNA sample, 3.1 μL of nuclease-free water, 7.5 μL of 2× AceQ® qPCR SYBR Green Master Mix, and 0.2 μL of each gene specific primer. Conditions for amplification were 3 min at 95°C, followed by 40 cycles of 15 s at 95°C (denaturation), 15 s at 60°C (annealing) and 20 s at 72°C (extension). 18S rRNA was used as the housekeeping gene, and the relative mRNA expression level was calculated with the $2^{-\Delta\Delta CT}$ method. All the experiments were performed in triplicate.

2.8. Practical evaluation of RPA-LFD and exo RPA assays

DNA extraction from 24 black spotted frogs with or without E. miricola were randomly selected from frog farms for testing the
practical performance of PCR, RPA, RPA-LFD and exo RPA, and DNA was prepared as previously described.

2.9. Statistical analysis

The results were expressed as the means ± standard deviation (SD) and all statistical analysis were done using SPSS 26.0 package. The experimental data were subjected by Dunn’s multiple comparison (with Bonferroni adjustment) to identify the significance.

3. Results

3.1. Screening primers and probes

The mutT sequence was selected for designing primers and probes. In the primer screening assay, we designed three forward primers and three reverse primers, and performed nine combinations for RPA test. The agarose gel electrophoresis results showed that the band from mutTF703/mutTR700 primer pair combination was the brightest and single band (Fig. 1A). Therefore, primer pair mutTF703/mutTR700 was optimal for RPA assay. Target product amplified by mutTF703/mutTR700 was 198 bp in length.

The verification of mutT1 probe and mutT2 probe based on primer pair mutTF703/mutTR700 yielded the positive results. The sample added with mutT1 probe and DNA sample showed specific band on dipstick in RPA-LFD assay, but the samples with water (negative) as template or only with mutT1 probe (blank) showed no specific bands (Fig. 1B). Compared with the negative control group, the sample added with mutT2 probe produced obvious fluorescence in exo RPA detection (Fig. 1C).

3.2. Specificities of basic RPA, RPA-LFD and exo RPA assays

The specificities of RPA, RPA-LFD and exo RPA methods were verified by DNA samples of 6 pathogens. The E. miricola DNA obtained positive results by means of gel electrophoresis (Fig. 2A), lateral flow dipstick (Fig. 2B) and fluorescence imaging system (Fig. 2C), while S. aureus DNA, A. hydrophila DNA, A. veronii DNA, CyHV-2 DNA and E. ictalurid DNA did not. The fluorescence of exo RPA product was quantified by enzyme-labeled instrument. The results showed that fluorescence intensity value of E. miricola sample was significantly higher than that of others (Fig. 2D). These results suggest that RPA, RPA-
LFD and exo RPA methods can specifically detect *E. miricola*.

### 3.3. Sensitivities of basic RPA, RPA-LFD and exo RPA assays

The $10^{10} – 10^7$ copies/μL diluted standard plasmid sample of pMD19*-mutT* were detected by PCR, RPA, RPA-LFD and exo RPA. The results showed the minimum detectable concentration of generic PCR was $10^3$ copies/μL (Fig. 3A), and that of RPA, RPA-LFD and exo RPA were $10^2$ copies/μL (Fig. 3B-3D). The sensitivity of basic RPA was the same as that of RPA-LFD and exo RPA, which was ten-fold higher than that of conventional PCR. The fluorescence intensity values of exo RPA products were proportional to the concentration of standard plasmid in the range of $10^2 – 10^7$ copies/μL. The limit of visible fluorescence of exo RPA was $10^2$ copies/μL (Fig. 3E).

### 3.4. Tissue distribution of *E. miricola*

The morphology and color of *E. miricola* were observed and determined by culturing *E. miricola* in BHI medium. This was used to distinguish *E. miricola* from commensal bacteria. We used healthy frog tissues for smearing plate in 5 replicates and did not detect *E. miricola*. However, *E. miricola* was detected in the samples of frogs with significant crooked head and cataract symptoms (Fig. S1).

The tissues of five sick black spotted frogs were collected containing eye, heart, stomach, brain, spleen, liver, kidney, lung and muscle. The bacterial load of these tissues was analyzed by dilution coated plate method and the expression of *E. miricola* gene in tissues by qRT-PCR (Fig. 4A). Colony counts indicated that the brain loaded the most abundant bacteria (Fig. 4B). The results of qRT-PCR assay were consistent with the results of tissue bacterial load assay (Fig. 4C).

### 3.5. Practicability of RPA, RPA-LFD and exo RPA assays

24 clinical samples were randomly selected for practical testing. The clinical performance of RPA, RPA-LFD and exo RPA was evaluated by comparing the PCR assay result of the same samples. The results showed that 7 positive samples were detected by the PCR assay (Fig. 5A), and 7 positive samples were also detected by RPA, RPA-LFD and exo RPA assays (Fig. 5B-5D). The four methods had the same detection positive rate. The fluorescence intensity values of exo RPA products detected by the enzyme-labeled instrument showed that only 7 samples had high fluorescence (Fig. 5E).

### 4. Discussion

Facing the detriment of bacterial diseases with high infectivity and high mortality in frogs, developing low-cost, rapid and sensitive detection methods is an urgent and highly prominent problem [34]. Rapid and reliable diagnostic techniques play an important role in efficiently detecting *E. miricola*. In the present study, we developed the RPA-LFD and exo RPA methods for rapid detection of *E. miricola*. RPA-LFD and exo RPA, which have the advantages of simple operation, sensitive detection and low cost of production, were extensively used in fundamental research and industry [18,32,33,35,36]. However, the RPA method was rarely used in amphibians. We reported the application of RPA related technology in black spotted frogs for the first time.

Improper gene sequences selection can affect the specificity of RPA reaction [37]. In previous study, a real-time PCR detection system based on *mutT* gene sequence amplification was established, which can specifically identify *E. miricola* [12]. We used the *mutT* gene sequence as the target sequence to establish the detection system of RPA-LFD and exo RPA technologies, which detected the *E. miricola* specifically.

Probes of RPA-LFD and exo RPA are important components of the probe accuracy. The distance between the dT-fluorophore and dT-fluorophore is crucial in the probe design. A normal fluorophore must be able to provide the information to identify the target sequence. Further research is needed to improve the accuracy and specificity of RPA-LFD and exo RPA in the detection of *E. miricola*.
quenching group is an important factor in the exo RPA reaction, which affects the intensity of fluorescence. The distance between the two groups was 1–4 nt, and the optimum distance was 1–2 nt [29]. Fluorescence is a luminous phenomenon. According to previous reports, the maximum excitation and maximum emission wavelengths of FAM and SYBR Green are similar, and the fluorescence emission peaks are almost completely superimposed [38, 39]. We used FAM channel to detect fluorescence. In future experiments, SYBR Green channel can also be used if conditions are limited, or a portable FAM machine (IT-IS UK) can be purchased, which is portable, lightweight and can work a whole day charging by battery.

Sensitivity and specificity assays are the basis to verify the practicability of RPA-LFD and exo RPA. In previous studies, the sensitivity of RPA-LFD and exo RPA methods are ten-fold higher than that of PCR, and the target bacteria DNA can be specifically detected in a variety of different pathogens [25, 32]. In this study, the sensitivity of RPA-LFD and exo RPA is $10^2$ copies/μL, which ten-fold higher than that of generic PCR ($10^3$ copies/μL), and E. miricola was specifically detected in different pathogens. These results showed that RPA-LFD and exo RPA are suitable methods for detecting pathogens and have potential application.

In order to sample quickly and accurately in clinical detection, we screened the tissues with high bacterial load in naturally diseased frogs by dilution coated plate method and qRT-PCR. The dilution coated plate method is a reliable method for calculating the bacterial load in tissues [40]. qRT-PCR is a classical method for detecting the expression of...
bacteria in tissues [31]. The results found that the brain was the main invading tissue of E. miricola. This is consistent with previous studies that the brain was the main target organ of E. miricola [5, 7]. In addition, among the 24 clinical samples of black spotted frog, 7 samples were positive for E. miricola by RPA, RPA-LFD and exo RPA assays, which were also positive by the PCR generic assay. The positive rates of the five detection methods were the same.

5. Conclusion

RPA-LFD and exo RPA can achieve rapid (within 30 min), accurate (long primers and probes ensure the specificity), sensitive (10^6 copies/μl), convenient (without expensive equipment), practical detection of E. miricola, which contribute to the early diagnosis in frog culture farms. The results were analyzed in practical applications, and showed that RPA, RPA-LFD and exo RPA have an excellent potential for rapid diagnosis of E. miricola.

CRediT authorship contribution statement

Meihua Qiao: Conceptualization, Investigation, Writing – original draft. Liqiang Zhang: Visualization, Investigation. Jiao Chang: Methodology, Investigation, Validation. Xiaoxuan Li: Methodology, Investigation, Validation. Jingkang Li: Data curation, Writing – original draft. Weicheng Wang: Methodology, Investigation, Validation. Gailing Yuan: Methodology, Investigation, Validation. Jianguo Su: Conceptualization, Project administration, Writing – review & editing.

Declaration of Competing 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fsirep.2022.100059.

References

[1] K.K. Kim, M.K. Kim, J.H. Lim, H.Y. Park, S.T. Lee, Transfer of Chryseobacterium meningosepticum and Chryseobacterium miricola to Elizabethkingia gen. nov. as Elizabethkingia meningoseptica comb. nov. and Elizabethkingia miricola comb. nov., Int. J. Syst. Evol. Microbiol. 55 (3) (2005) 1287–1293.
[2] J. Trimpert, I. Eichhorn, D. Vladimirova, A. Haake, A.K. Schink, R. Klopfleisch, R.X. Hu, J.F. Yuan, Y. Meng, Z. Wang, Z.M. Gu, Pathogenic Elizabethkingia miricola infection in cultured black-spotted frogs, China, 2016, Emerg. Infect. Dis. 23 (2016) 2055–2059.
[3] X.P. Lei, G. Yi, K.Y. Wang, P. OuYang, J. Chen, X.L. Huang, C. Huang, W.M. Lai, Z. J. Zhong, C.L. Huo, Z.X. Wang, Y. Yang, Elizabethkingia miricola infection in Chinese spiny frog (Quasipaa spinosa), Transbound. Emerg. Infect. Dis. 66 (2019) 1049–1055.
[4] J. Zhong, C.L. Huo, Z.X. Yang, J. Qi, J. Hu, Development of real-time reverse transcription-isothermal recombinase polymerase amplification for rapid detection of pathogenic Elizabethkingia miricola, Vet. Med. Sci. 6 (2020) 894–900.
[5] A. Vantarakis, G. Komninou, D. Venieri, M. Papapetropoulou, Development of a lateral flow dipstick recombinase polymerase amplification assay for rapid detection of infectious spleen and kidney necrosis virus by recombinase polymerase amplification combined with lateral flow dipsticks, Aquaculture 519 (2020), 734926.
[6] M.M. Cabada, J.L. Malaga, A. Castellanos-Gonzalez, R.A. Bagwell, P.A. Naeger, H. Wang, J.Q. Hu, Y. Wang, H.J. Su, H.M. Ding, X.C. Sun, H. Gao, Y. Geng, Z.C. Wang, Rapid diagnosis of cucumber mosaic virus in banana plants using a fluorescence-based real-time recombinase polymerase amplification combined with triple-labeled nucleotide probes, Mol. Cell. Probes 50 (2020), 101501.
[7] N. Srivastava, R. Kapoor, R. Kumar, S. Kumar, S.R. K, S. Kumar, V.K. Baranwal, Rapid diagnosis of Cucumber mosaic virus in banana plants using a fluorescence-based real-time isothermal reverse transcription-recombinase polymerase amplification assay, J. Virol. Methods 270 (2019) 52–58.
[8] J. Qi, J. Hu, Z. Su, Z. Su, J. Wang, Development of a real-time reverse transcription recombinase polymerase amplification for the detection of Fasciola hepatica in human stool, Acta Trop. 204 (2021) 1–6.
[9] F. Cong, F.W. Zeng, M.L. Wu, J.J. Wang, B.H. Huang, Y.Y. Wang, Q. Zhang, S. Zhang, L. Ma, P.J. Guo, W.W. Zeng, Development of a real-time reverse transcription recombinase polymerase amplification assay for rapid detection of spring viremia of carp virus, Mol. Cell. Probes 50 (2020), 101494.
[10] H.K. Li, G.L. Yuan, Y.Z. Luo, Y.Z. Yu, T.S. Ai, J.G. Su, Rapid and sensitive detection of infectious spleen and kidney necrosis virus by recombinase polymerase amplification combined with lateral flow dipsticks, Aquaculture 519 (2020), 734926.
[11] H. Wang, M. Sun, D. Xu, P. Podok, J. Xie, Y. Jiang, L. Lu, Rapid visual detection of cyanprin herbicidervis 2 by recombinase polymerase amplification combined with a lateral flow dipstick, J. Fish Dis. 41 (2018) 1201–1206.
[12] Y. Yang, X.D. Qin, X.G. Zhang, Z.X. Zhao, W. Zhang, X.L. Zhu, G.Z. Cong, Y.M. Li, Z. D. Zhang, Development of real-time lateral flow dipstick recombinase polymerase amplification assays for rapid detection of goatpox virus and sheep pox virus, Virol. J. 14 (2017) 131.
[13] J.Q. Hu, Y. Wang, H.J. Su, H.M. Ding, X.C. Sun, H. Gao, Y. Geng, Z.C. Wang, Rapid analysis of Escherichia coli O157:H7 using isothermal recombinase polymerase amplification combined with triple-labeled nucleotide probes, Mol. Cell. Probes 50 (2020), 101501.
[14] L.W. Zhao, J.C. Wang, X.X. Sun, J.F. Wang, Z.M. Chen, X.D. Xu, M.Y. Dong, Y. N. Guo, Y.Y. Wang, P.P. Chen, W.J. Gao, Y.Y. Geng, Development and evaluation of a real-time reverse transcription recombinase polymerase amplification assay for rapid detection of porcine reproductive and respiratory syndrome virus (PPRSV), J. Virol. Methods 277 (2020), 113802.
[15] H.J. Su, Z. Su, J. Wang, B.H. Huang, Y.Y. Wang, Q. Zhang, S. Zhang, L. Ma, P.J. Guo, W.W. Zeng, Development of a real-time reverse transcription recombinase polymerase amplification assay for rapid detection of infectious spleen and kidney necrosis virus, Emerg. Infect. Dis. 23 (12) (2017) 341–346.
[16] F. Cong, F.W. Zeng, M.L. Wu, J.J. Wang, B.H. Huang, Y.Y. Wang, Q. Zhang, S. Zhang, L. Ma, P.J. Guo, W.W. Zeng, Development of a real-time reverse transcription recombinase polymerase amplification assay for rapid detection of porcine reproductive and respiratory syndrome virus (PPRSV), J. Virol. Methods 277 (2020), 113802.
[17] S. Zhang, R.L. Hu, Rapid and sensitive recombinase polymerase amplification combined with lateral flow strip for detecting African swine fever virus, Front. Microbiol. 10 (2019) 1004.
[18] H. Wang, M. Sun, D. Xu, P. Podok, J. Xie, Y. Jiang, L. Lu, Rapid visual detection of cyprinid herpervirus 2 by recombinase polymerase amplification combined with a lateral flow dipstick, J. Fish Dis. 41 (2018) 1201–1206.
[19] Y. Yang, X.D. Qin, X.G. Zhang, Z.X. Zhao, W. Zhang, X.L. Zhu, G.Z. Cong, Y.M. Li, Z. D. Zhang, Development of real-time lateral flow dipstick recombinase polymerase amplification assays for rapid detection of goatpox virus and sheep pox virus, Virol. J. 14 (2017) 131.
[20] J.Q. Hu, Y. Wang, H.J. Su, H.M. Ding, X.C. Sun, H. Gao, Y. Geng, Z.C. Wang, Rapid analysis of Escherichia coli O157:H7 using isothermal recombinase polymerase amplification combined with triple-labeled nucleotide probes, Mol. Cell. Probes 50 (2020), 101501.
Y.Q. Zhang, X. Xiao, Y.Z. Hu, Z.W. Liao, W.T. Zhu, R. Jiang, C.R. Yang, Y.A. Zhang, J.G. Su, CXCL20a, a teleost-specific chemokine that orchestrates direct bactericidal, chemotactic, and phagocytosis-killing-promoting functions, contributes to clearance of bacterial infections, J. Immunol. 207 (7) (2021) 1911–1925.

Z.S. Wang, X.C. Huo, Y.Q. Zhang, Y. Gao, J.G. Su, Carboxymethyl chitosan nanoparticles loaded with bioactive protein CiCXCL20a effectively prevent bacterial disease in grass carp (Ctenopharyngodon idella), Aquaculture 549 (2022), 737745.

H.X. Li, L.Q. Zhang, Y.Z. Yu, Y.A. Zhang, J.G. Su, Rapid detection of Edwardsiella ictaluri in yellow catfish (Pelteobagrus fulvidraco) by real-time RPA and RPA-LFD, Aquaculture 552 (2022), 737976.

X.Q. Liu, Q.Y. Yan, J.F. Huang, J. Chen, Z.Y. Guo, Z.D. Liu, L. Cai, R.S. Li, Y. Wang, G.N. Yang, Q.X. Lan, Influence of design probe and sequence mismatches on the efficiency of fluorescent RPA, World J. Microbiol. Biotechnol. 35 (6) (2019) 95.

G.R. Rahim, N. Gupta, Elizabethkingia miricola: discrepancies in identification and antimicrobial susceptibilities, Diagn. Microbiol. Infect. Dis. 94 (1) (2019) 104.

Z.Y. Wang, Y. Wang, L. Lin, T. Wu, Z.Z. Zhao, B.W. Ying, L.Q. Chang, A finger-driven disposable micro-platform based on isothermal amplification for the application of multiplexed and point-of-care diagnosis of tuberculosis, Biosens. Bioelectron. 195 (2022), 113663.

R.K. Daher, G. Stewart, M. Boissinot, D.K. Boudreau, M.G. Bergeron, Influence of sequence mismatches on the specificity of recombinase polymerase amplification technology, Mol. Cell. Probes 29 (2) (2015) 116–121.

S.J. He, B. Song, D. Li, C.F. Zhu, W.P. Qi, Y.Q. Wen, L.H. Wang, S.P. Song, H. P. Fang, C.H. Fan, A graphene nanoprobe for rapid, sensitive, and multicolor fluorescent DNA analysis, Adv. Funct. Mater. 20 (3) (2010) 453–459.

M.V. Poucke, A.V. Zeveren, L.J. Peelman, Combined FAM-labeled TaqMan probe detection and SYBR green I melting curve analysis in multiprobe qPCR genotyping assays, BioTechniques 52 (2) (2012) 81–86.

C. Xu, M.H. Qiao, X.C. Huo, Z.W. Liao, J.G. Su, An oral microencapsulated vaccine loaded by sodium alginate effectively enhances protection against GCRV infection in grass carp (Ctenopharyngodon idella), Front. Immunol. 13 (2022), 848958.