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A handheld isothermal fluorescence detector for duplex visualization of aquatic pathogens via enhanced one-pot LAMP-PfAgo assay

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ABSTRACT

The expansion of large-scale aquaculture has exacerbated the challenge of aquatic diseases, resulting in substantial economic losses annually. Currently, traditional laboratory-based diagnostic methods are time-consuming and costly, hindering on-site testing for individual farmers. We address this issue by developing a state-of-the-art handheld isothermal nucleic acid amplification device (WeD-1) capable of fluorescence tracking of reactions and integrating it with an enhanced one-pot Prokaryotic Argonaute based nucleic acid detection method, enabling duplex visual detection of aquatic pathogens. WeD-1 is portable, reusable, user-friendly, and cost-effective, offering real-time smartphone interaction and enabling real-time fluorescence observation during the reaction. The enhanced one-pot Loop-Mediated Isothermal Amplification (LAMP)-PfAgo method, incorporating paraffin-encapsulated lyophilized PfAgo protein, achieves precise target-specific cleavage, significantly enhancing multiplex nucleic acid detection. This innovation streamlines on-site testing, negating the need for specialized laboratory conditions while ensuring an aerosol-free system. With newly developed and highly sensitive LAMP primer sets, our compact WeD-1/LAMP-PfAgo nucleic acid rapid testing system exhibits remarkable sensitivity, readily detecting aquatic pathogens with naked eyes from rapidly prepared fish and shrimp samples within 40 min, even when the Ct values are as high as 34.

1. Introduction

As large-scale and intensive aquaculture continues to develop, some issues related to aquatic diseases are becoming increasingly prominent. These diseases result in significant economic losses each year (Lafferty et al., 2015; Naylor et al., 2021). Currently, there are no effective treatments for common viral infections. Early diagnosis and frequent screening, along with preventing the introduction of infected seedlings into the aquaculture process, are the primary means to effectively prevent outbreaks of these diseases. Currently, it takes at least 5 days or more from sending samples to central lab to receiving results. The cost of testing a single sample is high, causing many individual farmers to be unwilling to send samples for testing. Developing a rapid and efficient virus detection method is crucial to promptly identify and prevent aquatic animals' virus transmission, and it holds great significance for epidemic prevention efforts. Therefore, there is an urgent need to develop rapid on-site testing systems, enabling pond-side farmers and aquaculture nursery farm to conduct on-site tests and contribute to the healthy development of the aquaculture industry.

The aquaculture industry has already introduced antigen rapid test kits for rapid disease detection. Nevertheless, these kits exhibit relatively low levels of specificity and sensitivity (Nakajima et al., 1995). At present, the predominant technique for testing aquatic pathogens is polymerase chain reaction (PCR), a molecular diagnostic technology (Caipang et al., 2004; Kurita et al., 1998). PCR achieves precise and sensitive detection by exponentially amplifying minute quantities of

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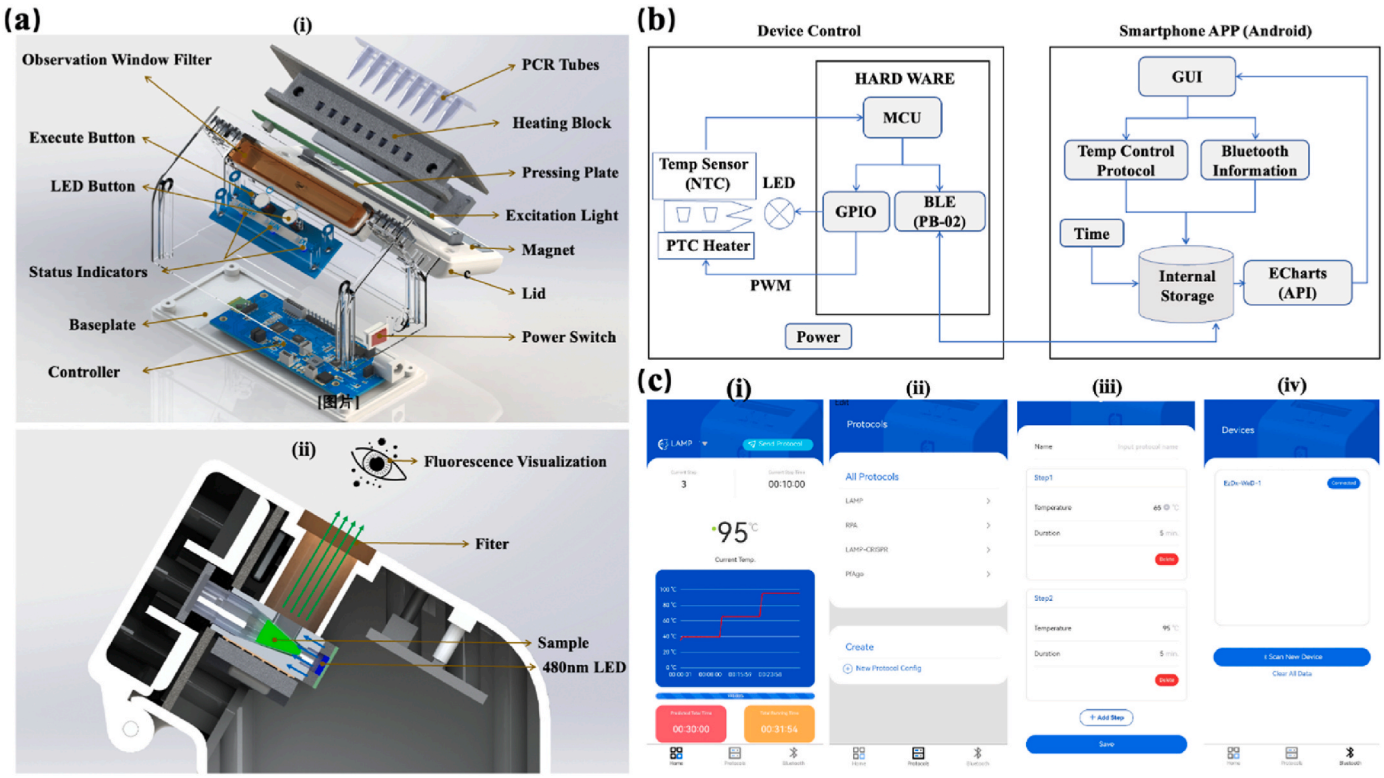


Fig. 1. Design and development of the device and smartphone app for on-site detection. (a) The schematic diagram of the handheld isothermal fluorescence detector, presenting its mechanical and electronic components. **(b)** The control and operation logic diagram of the device, detailing its software and hardware components and how they function together. **(c)** The user-friendly software interface of the smartphone app, demonstrating the ease of use of the system for on-site detection.

nucleic acids. However, PCR assays necessitate tightly controlled temperature cycling, intricate multi-step procedures, skilled technical personnel, and specialized laboratory conditions (Caipang et al., 2004; Kurita et al., 1998). These prerequisites prove less than optimal for conducting on-site testing.

Thermophilic Argonaute (Ago)-based nucleic acid detection represents an innovative approach for identifying target nucleic acids through guide DNAs (gDNAs), liberating it from the constraints of the target sequence (Hegge et al., 2018; Jolly et al., 2020; Liu et al., 2018). Leveraging the steadfastness of gDNA, the adaptability of design, and the precision of endonuclease activity, Ago-based methods have emerged as a potent means for detecting tumor and pathogen biomarkers (Bau et al., 2021; He et al., 2019; Liu et al., 2021; Song et al. 2018, 2020; Xun et al., 2021b). Notably, the Ago enzymes derived from the hyperthermophilic species *Thermus thermophilus* (TtAgo) and *Pyrococcus furiosus* (PfAgo) have proven instrumental in enabling efficient multiplex detection of rare mutations (Bau et al., 2021; Liu et al., 2021; Song et al., 2020). In the realm of virus detection, Ago's sequential cleavage mechanism has been harnessed to enable sequence-specific detection of the human papillomavirus (He et al., 2019; Xun et al., 2021b). However, these methods are still integrated with PCR in a two-step protocol, necessitating bulky instrumentation and a lid-opening process, while carrying a risk of contamination. Recently, one-pot PfAgo-based detection approaches have emerged for COVID-19 diagnosis, seamlessly integrating rapid isothermal amplification with PfAgo cleavage. Yet, these platforms demand the utilization of prefabricated capillaries (Xun et al., 2021a), specialized sealed tubes (Ye et al., 2022), and specific devices that are both costly and unsuitable for field detection.

In this study, we present the creation of a handheld isothermal fluorescence detector (dubbed WeD-1) and an enhanced one-pot PfAgo-based detection method. When amalgamated, these innovations possess the capability for duplex visual detection of aquatic pathogens. This device is a reusable, portable, user-friendly, and cost-effective nucleic acid testing tool, weighing 280 g. It enables real-time interaction with smartphones, can be conveniently carried in a backpack, and stands as an optimal choice for at-home nucleic acid testing and on-site rapid screening. The enhanced one-pot PfAgo-based detection method employs paraffin encapsulation of lyophilized PfAgo protein, enabling its reaction at the desired elevated temperature while also providing an effective paraffin seal after the reaction to prevent aerosol contamination. Integrated with the enhanced one-pot PfAgo-based detection method, the system provides simplified operation and enables real-time observation of the fluorescence color change during the reaction. Employing intricately designed gDNAs and reporters, we harnessed this novel approach to simultaneously detect aquatic pathogens. This compact nucleic acid rapid testing system obviates the necessity for centralized laboratories or specialized technicians, presenting itself as a compact solution for on-site detection of aquatic pathogen nucleic acids.

2. Materials and methods

2.1. Design and development of the device and smartphone app

(1) **The structure design of the device.** The device's structure was designed using SolidWorks software. During the prototyping phase, the complete shell was 3D-printed using nylon material on a Flashforge Guider II 3D printer to assess the feasibility of the structural design. After validation, injection molding was used to create the optimal shell using a high-temperature resistant medical-grade material polycarbonates. As depicted in Fig. 1a–i, the device features a rectangular base and a columnar body that slopes at the observation window, which allows for convenient real-time monitoring of the reaction's progress while also

securing the liquid in the test tube. The lid and main body of the device are closed using magnetic attraction. A spring compression plate on the lid applies pressure to the PCR 8-tube strips, ensuring enhanced contact with the heating block upon closure of the lid, and improving thermal conductivity.

The eight-tube heating block was constructed from aluminum alloy material, selected for its excellent heat conduction properties. This heating block undergoes shaping via a CNC machine, followed by an anodization process that lends it a black surface. This surface treatment effectively minimizes the impact of side wall reflections, enhancing the overall performance. Apertures were introduced at the lower part of the heating block to facilitate the transmission of excitation light, while additional openings on the side ensure the ease of observing test results.

The observation window filter effectively blocks excitation light at 480 nm, enabling the passage of fluorescence signals within the range of 500 nm–670 nm (Fig. 1a–ii). Positioned on the device's exterior, a side-mounted power switch serves for power connection, while two front buttons are designated for initiating the run (left) and activating the excitation light (right). Presenting three device indicators, the left one signifies the POWER status, the middle one indicates the RUN status, and the right one denotes task completion (DONE). Upon power connection, the POWER indicator emits a red glow, while the other two indicators flash in blue. Upon pressing the front right button, the RUN indicator begins its flashing pattern.

(2) **PCB design.** The core control circuit's PCB design is executed using LCEDA (China), while the embedded software programming is compiled and applied through Keil μVision5. As shown in Fig. 1b, the device's heating module is overseen by a microcontroller (MCU), which effectively manages the heating module via GPIO in accordance with pre-set or pre-stored reaction programs. The heating control employs a PID control algorithm, with the MCU adeptly adjusting the PWM value to precisely modulate the power output of the heating module. Utilizing ADC sampling, the MCU acquires the resistance value of the thermistor, thereby accurately gauging the temperature of the heating module. The pb-02 Bluetooth module enables seamless data communication between the device and a smartphone app described below. Users can send amplification protocols via the app to the device's MCU, which are then stored on the device's EEPROM. This allows the device to be utilized independently in subsequent runs without the need for a smartphone connection. The button signals are effectively collected through GPIO, and the MCU regulates LED excitation light for fluorescence visualization based on the collected signals.

(3) **Smartphone App design.** We developed the smartphone app using Java programming language with the Android Studio. The smartphone App establishes seamless communication with the device via Bluetooth, empowering users to configure a range of required temperatures and transmit them to the device. Similarly, real-time temperature data from the device is relayed to the smartphone through Bluetooth and directly displayed on the smartphone screen through ECharts (an open-source visualization chart library based on JavaScript) (Fig. 1b). To adapt to varying temperature profiles of different reaction systems, users can personally modify, add, or delete reaction protocols within the App. In terms of interface design, the software's main page is the home hub, accompanied by three tab bars beneath: Home, Protocols, and Bluetooth. Within the Home section, users can select protocols, transmit them, view temperature readings (Fig. 1c–i), and monitor the progress of reactions. The Protocols page allows users to edit reaction modes and save these modified modes for future reference (Fig. 1c–ii and 1c-iii). The Bluetooth page offers functionality to search for available Bluetooth devices, and users can establish or terminate connections with their chosen devices (Fig. 1c–iv).

2.2. Duplex visualization of aquatic pathogens

The detailed experimental procedures can be found in the **Supplemental Materials and Methods**.

3. Results and discussion

3.1. The performance of the handheld isothermal fluorescence detector (WeD-1)

The WeD-1 fluorescence detector offers highly accurate temperature control and enables real-time smartphone interaction. This is especially critical for the LAMP-PfAgo method, which involves a two-step reaction requiring two distinct reaction temperatures—typically set at 65 °C and 95 °C. With our WeD-1, users can effortlessly program heating profiles using their smartphones and closely monitor instrument temperatures in real-time, even during the elevated reaction temperature of 95 °C (Fig. 1c–i). WeD-1 displays changes in fluorescence intensity through an observation window (shown in the following figures), ensuring effortless and timely observations throughout the entire reaction process, making it an ideal choice for LAMP-PfAgo assay.

3.2. Design and select the optimal LAMP primer set for LMBV detection

Apart from conducting in silico analysis to assess the performance of the 4 newly designed LMBV MCP gene primer sets, we generated a dilution series of LMBV DNA and conducted LAMP (as shown in Supplemental Fig. 1) to do a comparison between the 4 primer sets. Our findings highlighted that primer set 4 (LMBV4 in Supplemental Table 1) demonstrated exceptional sensitivity, capable of detecting as few as 0.8 copies/μL (equivalent to 20 copies of the target sequence in a 25 μL reaction buffer) of LMBV DNA. As illustrated in Supplemental Fig. 2, this set exhibited enhanced amplification speed and sensitivity, alongside its robust repeatability and good linear range.

Moreover, to assess the specificity of primer set 4, we conducted further tests using a variety of aquatic pathogens available in our laboratory, including DIV1, WSSV, Enterocytozoon hepatopenaei (EHP), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and acute hepatopancreatic necrosis disease (AHPND). Our testing revealed that samples exclusively containing LMBV displayed a positive amplification signal on a real-time thermal cycler and characteristic electrophoretic bands on a 2% agarose gel (Supplemental Fig. 3), verifying the specificity of Primer set 4. As a result, we have selected this primer set for further use.

3.3. The performance of the LAMP-PfAgo assay

While LAMP has demonstrated good specificity and sensitivity in detecting nucleic acids, its strong amplification ability often produces a large number of amplicons, which can lead to aerosol contamination. In contrast, LAMP-PfAgo has an advantage in that, with the guidance of a DNA guide, the specific cleavage of the target can eliminate aerosols. Moreover, signal generation is not affected by primer dimer. Importantly, LAMP-PfAgo can also recognize different targets under the guidance of different guides, making it possible to achieve multiplex detection (He et al., 2019; Xun et al. 2021a, 2021b; Ye et al., 2022).

Previous work on LAMP-PfAgo has utilized either prefabricated capillaries and specific devices (Xun et al., 2021a), or specialized sealed tubes (Liu et al., 2021) to enable rapid isothermal amplification and PfAgo cleavage integration. However, these approaches are not practical for field detection due to their high cost and impracticality. In this study, we have developed an improved approach that utilizes paraffin to encapsulate lyophilized PfAgo protein. In this one-pot reaction,

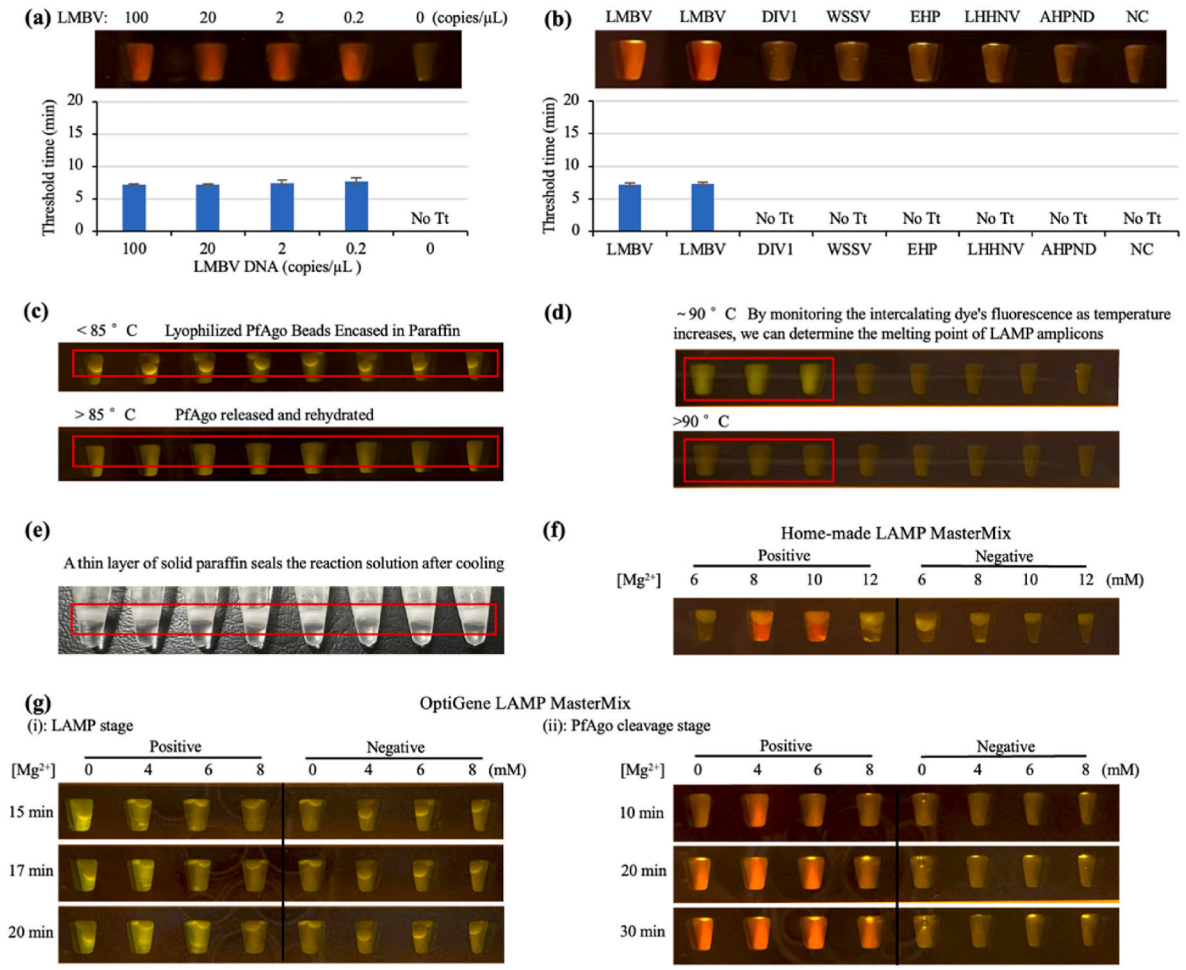


Fig. 2. Performance characteristics of the LAMP-PfAgo assay. (a) and **(b)** Investigation of the sensitivity and specificity of the LAMP-PfAgo visualization assay. All experiments were conducted in triplicate and sumarized in the histogram. 4 μL of purified DNA (~50 ng/μL) was added. NC: negative control. The threshold time (Tt) is defined as the time until signal intensity exceeds 100% of signal saturation level. **(c)** Controlled release of encapsulated lyophilized PfAgo from paraffin at temperatures above 85 °C is demonstrated, indicating the feasibility of this approach for one-tube LAMP-PfAgo reaction. **(d)** Intercalating fluorescent dye enables the observation of LAMP amplification progress and the melting point of LAMP amplicons as the temperature is increased. **(e)** The effectiveness of solid paraffin as a sealant to prevent aerosol contamination is demonstrated. **(f)** The effect of final magnesium concentration on home-made LAMP MasterMix-PfAgo assay. A final Mg^{2+} concentration of 8 mM was selected for subsequent experiments. **(g)** The effect of additional Mg^{2+} concentration on OptiGene LAMP MasterMix-PfAgo assay. The optimal LAMP-PfAgo reaction is achieved with the addition of 4 mM Mg^{2+}. We carried out all experiments in triplicate, adding 400 copies of LMBV plasmid templates to each reaction.

amplification and cleavage occur sequentially, with temperature gradually increasing. Initially, LAMP amplification takes place at 65 °C for 30 min. The reaction then progresses to 95 °C, where active PfAgo is released to cleave the amplicon. This guided cleavage is facilitated by two specific gDNAs, resulting in the generation of secondary gDNAs, which then trigger a secondary cleavage of the reporters and ultimately produce fluorescence signals that enable visualization. This paraffin encapsulation approach ensures the controlled release of PfAgo protein as needed while also providing an effective paraffin seal after the reaction to prevent aerosol contamination further.

To evaluate the performance of LAMP-PfAgo in detecting LMBV, we conducted experiments involving a dilution series of LMBV DNA, as well as samples containing nucleic acids from several other aquatic pathogens, including DIV1, WSSV, EHP, IHHNV, and AHPND. Fig. 2a and b illustrate the sensitivity and specificity of the LAMP-PfAgo visualization assay. Our experiments revealed that the assay can detect LMBV DNA as few as 0.2 copies/μL with great accuracy, even though its quantitative capacity is limited (Fig. 2a). The LMBV target produces a distinctive vivid red coloration, which is an emission light resulting from Rox dye cleavage, confirming a positive result. In contrast, other aquatic pathogens do not produce such colorization, indicating negative results (Fig. 2b). Both results demonstrate the LAMP-PfAgo visualization assay's remarkable sensitivity and specificity in detecting pathogens, underscoring its high accuracy.

Additionally, Supplemental Video 1 and Fig. 2c provide visual representation of the controlled release of the lyophilized PfAgo protein from the encapsulated paraffin at temperatures above 85 °C. Meanwhile, Supplemental Video 2 and Fig. 2d show that intercalating dye Eva Green is capable of complementing the visualization detection of LAMP-PfAgo, enabling the observation of LAMP amplification progress and the approximate melting point of LAMP amplicons as the temperature is increased. This occurs because the fluorescence of the intercalating dye fades away when the temperature exceeds the melting temperature of the amplicons. On the contrary, the fluorescence increases due to the PfAgo cleavage of the reporter at 95 °C. Remarkably, Fig. 2e showcases the effectiveness of solid paraffin as a sealant, successfully preventing aerosol contamination by forming a thin layer over the reaction solution after it has cooled.

3.4. Optimization of Mg^{2+} concentration for LAMP-PfAgo

As is well-known, Mn^{2+} is often considered a key factor influencing

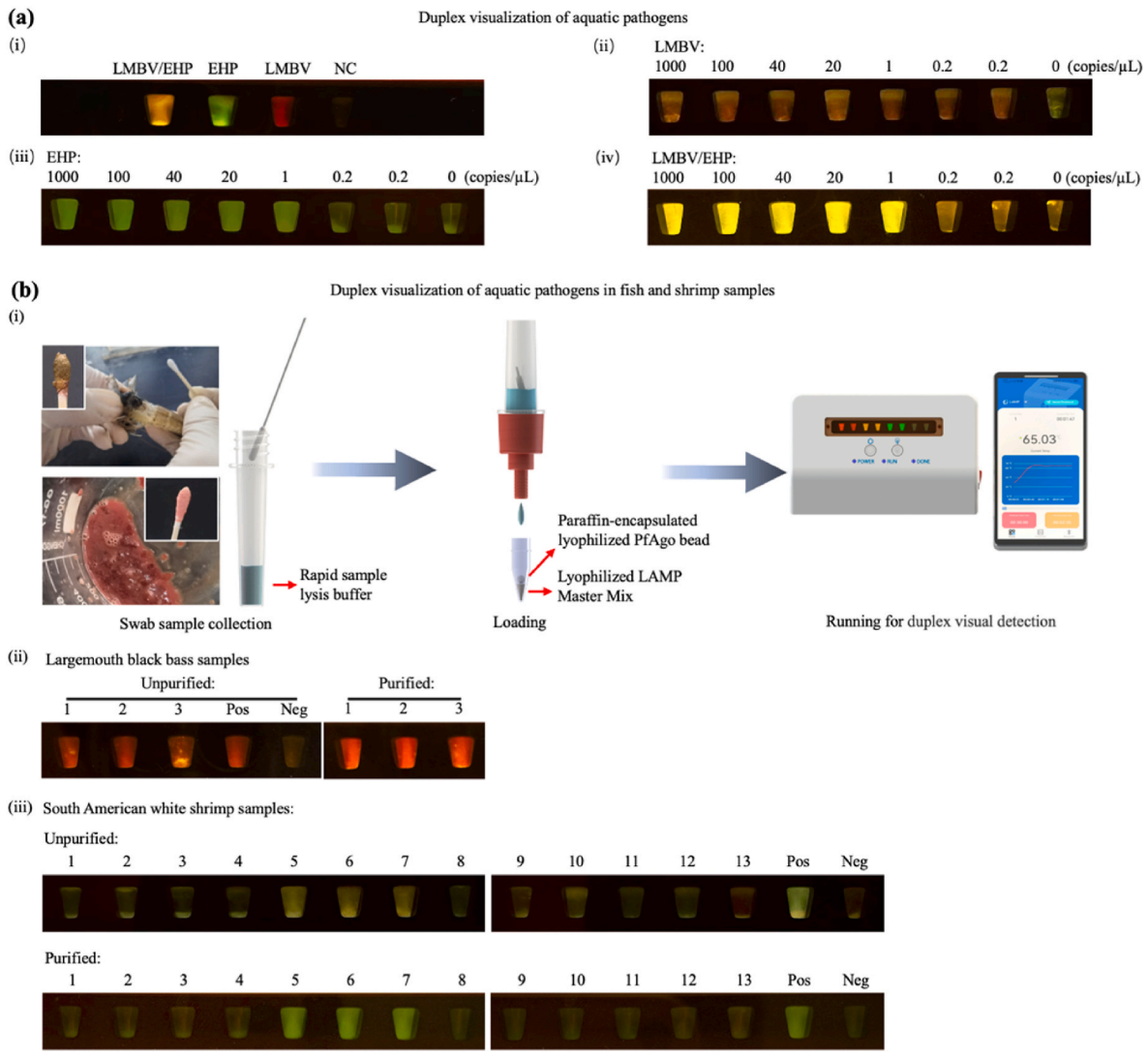


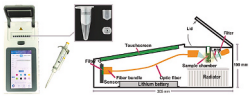
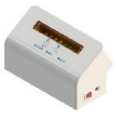
Fig. 3. (a) Duplex visualization of aquatic pathogens. (a–i) Differentiation of target pathogens LMBV and EHP using our duplex LAMP-PfAgo assay. **(a-ii)-(a-iv)** Sensitivity assessment of our duplex LAMP-PfAgo assay for detection of LMBV DNA, EHP DNA, and LMBV/EHP DNA mixture. We selected the EHP LAMP primer set from the literature (T et al., 2018). All experiments were conducted in triplicate. **(b) Duplex visualization of aquatic pathogens in fish and shrimp samples using LAMP-PfAgo. (b–i)** The workflow for real sample detection in field settings. The process involves the use of a swab for sample collection. **(b-ii)** Visual detection of LMBV in largemouth black bass samples using the newly-designed LAMP primers and the duplex LAMP-PfAgo assay. **(b-iii)** Application of the duplex LAMP-PfAgo method for the visual detection of EHP in South American white shrimp samples.

the activity of PfAgo. However, in our research, we discovered that high concentrations of Mg^{2+} can also activate PfAgo's cleavage activity. Since Mg^{2+} concentration is not only a crucial factor for LAMP nucleic acid amplification but also a key factor affecting PfAgo cleavage activity, we investigated the overall impact of Mg^{2+} concentration on LAMP-PfAgo reactions. Fig. 2f and Supplemental Fig. 4a demonstrates that LAMP MasterMix prepared with Bst polymerase from Haigene exhibits optimal LAMP amplification and PfAgo cleavage with a final Mg^{2+} concentration of 8 mM, so a final concentration of 8 mM Mg^{2+} was selected for subsequent experiments using this home-made LAMP MasterMix. On the other hand, Fig. 2g and Supplemental Fig. 4b show that for OptiGene LAMP MasterMix, optimal LAMP amplification is achieved without additional magnesium (0 mM), while PfAgo exhibits optimal cleavage activity with the addition of 4 mM additional Mg^{2+}. Due to this, a concentration of 4 mM additional Mg^{2+} was chosen for OptiGene LAMP MasterMix.

3.5. *Duplex visualization of aquatic pathogens*

Fluorescence-based detection methods using CRISPR-Cas12/13/14 proteins are typically limited to single-target detection due to the non-specific cleavage activity of the proteins on fluorescent reporters (Chen et al., 2018; East-Seletsky et al., 2016; Gootenberg et al., 2017; Harrington et al., 2018; Hu et al. 2022, 2023; Joung et al., 2020; Lu et al., 2022). However, fluorescence-based detection methods employing Argonaute proteins can specifically cleave targets, producing specific secondary guides that can, in turn, specifically cleave fluorescent reporters corresponding to their respective targets. In cases where the system contains two targets, each of which corresponds to a different color of fluorescent reporter, such as a green FAM reporter and a red Rox reporter, the detection system can exhibit up to three different colors of fluorescence in the reaction solution: green (when target A is present), yellow (when both targets A and B are present), and red (when target B is present). We demonstrated this approach using target LMBV and EHP, as shown in Fig. 3a–i, where the presence of target EHP is indicated by green fluorescence in the tube, the presence of both targets LMBV and

Table 1
Comparison of WeD-1 and other rapid test devices.

Products	Testing device list price	Capacity per test	Single testing price	Assay	Characteristic
 **Cue health**	$199	1 sample	$50/sample	LAMP Electrochemical signal read-out	1 singleplex test 2 battery-powered 3 specialized cassette 4 easy to operate 5 pocket-sized (<300 g)
 **Lucira Health**	$75	1 sample	$75/sample	LAMP PH change-based colorimetric assay	1 singleplex test 2 battery-powered 3 specialized reactor 4 easy to operate 5 pocket-sized (<300 g)
 **Circle HealthPod**	$127	1 sample	$25/sample	LAMP PH change-based colorimetric assay	1singleplex test 2battery-powered 3specialized reactor 4easy to operate 5pocket-sized (<300 g)
 **SPOT device (Xun et al. 2021a)**	NA	1 sample	$6.14 (cost)/ sample	LAMP + PfAgo Fluorescence based detection	1 duplex test 2 battery-powered 3 prefabricated capillaries 4 easy to operate 5 portable (>750 g)
 **Portable device for MULAN (Ye et al. 2022)**	$5000	8 samples	$4/sample	LAMP + PfAgo Fluorescence based detection	1 duplex test 2 power adapter required 3 specialized tubes 4 easy to operate 5 portable (>750 g)
 **EzDx WeD-1**	$500	8 samples	$2/sample	LAMP + PfAgo Fluorescence based visualization	1 duplex test 2 battery-powered 3 PCR tube adaptable 4 easy to operate 5 handheld (<300 g)

EHP is indicated by yellow fluorescence, and the presence of target LMBV alone is indicated by red fluorescence.

To evaluate the sensitivity of the duplex LAMP-PfAgo assay, we generated a dilution series of LMBV DNA, EHP DNA and LMBV/EHP DNA mixture and performed LAMP-PfAgo assays, as depicted in Fig. 3a–ii, **3a-iii** and **3a-iv**. Results show that our duplex LAMP-PfAgo assay can reliably detect LMBV DNA at a concentration as low as 0.2 copies/μL (Fig. 3a–ii) and EHP DNA at a concentration as low as 1 copy/μL (Fig. 3a–iii). Moreover, our duplex LAMP-PfAgo assay was capable of detecting both LMBV and EHP DNA at concentrations as low as 1 copy/μL when they coexist (Fig. 3a–iv). The sensitivity and repeatability of our duplex LAMP-PfAgo assay were further assessed by conducting all experiments in triplicate using a real-time quantitative Thermal Cycler (BioRad, Model CFD3240). Remarkably, the data analysis from the commercial Thermal Cycler system revealed the same sensitivity as that obtained using WeD-1, indicating excellent consistency in the duplex LAMP-PfAgo assay results (Supplemental Fig. 5). These findings underscore its potential as a powerful tool for rapid and accurate pathogen identification in various aquatic settings, ranging from shrimp and fish farming to environmental monitoring.

3.6. Duplex visualization of aquatic pathogens in fish and shrimp samples

Efficient sample preparation is essential for field detection. To this end, we have developed a convenient workflow for LAMP-PfAgo, as illustrated in Fig. 3b–i. Our workflow utilizes a swab that is used to collect samples from the crushed liver and pancreas of fish or shrimp (Supplemental Fig. 6), along with a rapid sample lysis buffer which is compatible with LAMP-PfAgo for nucleic acid extraction from aquatic pathogens. To assess the efficacy of this field detection method, we compared the duplex assay with conventional PCR for detecting two aquatic species: LMBV in largemouth black bass and EHP in South American white shrimp. Fig. 3b–ii illustrates our successful detection of LMBV using our newly-designed LAMP primers in combination with the duplex LAMP-PfAgo. Remarkably, all three largemouth black bass samples tested positive for LMBV using our approach. This detection rate was consistent with that observed using traditional PCR and LAMP alone (Supplemental Fig. 7), both of which utilized nucleic acid purification prior to amplification. Notably, one of the samples had a high Ct value of 34 (Supplemental Fig. 7a), demonstrating the remarkable sensitivity of our assay. As depicted in Fig. 3b–iii, our newly established duplex visual LAMP-PfAgo method detected 3 EHP-positive out of 13 South American white shrimp samples, exhibiting a detection rate that was fully consistent with that of traditional PCR (Supplemental Fig. 8a). Our assay demonstrated 100% sensitivity and specificity for EHP detection. Notably, both LMBV and EHP detection exhibited consistent results between the nucleic acid purified and unpurified samples, indicating the effectiveness of the rapid sample preparation method (Fig. 3b–ii and 3b-iii). In contrast, EHP LAMP alone produced a false-positive result when using purified nucleic acid compared to real-time PCR (Supplemental Fig. 8b). These findings demonstrate the utility of our LAMP-PfAgo methods in facilitating rapid and reliable diagnosis of aquatic pathogens in field settings.

4. Conclusions

In conclusion, our study has successfully developed the WeD-1, a portable and compact isothermal fluorescence detector in conjunction with an enhanced one-pot LAMP-PfAgo nucleic acid detection method. These innovations provide us with the ability to rapidly perform duplex visual detection of aquatic pathogens, enabling us to specifically target LMBV and EHP in this study. Compared to current rapid test devices (Table 1), the WeD-1 device is a remarkable achievement in terms of its reusability, portability, user-friendliness, and cost-effectiveness, weighing a mere 280 g. It enables real-time observation of fluorescence color changes during nucleic acid amplification, delivering much better sensitivity and specificity than PH-change-based colorimetric analysis. Additionally, its seamless integration with smartphones makes it an easily accessible tool for at-home nucleic acid testing and on-site rapid screening.

The developed detection system streamlines the process significantly when compared to traditional techniques such as PCR, thereby removing the requirement for bulky instrumentation, specialized laboratory conditions, and highly trained technicians. It also provides specific target cleavage, limiting the release of amplicon aerosols, avoiding primer dimer interference, and offering the potential for multiplex nucleic acid detection. Furthermore, it offers controlled PfAgo protein release as demanded, followed by a dependable paraffin seal that protects against aerosol contamination further.

Looking ahead, this technology not only opens new avenues for the field of aquaculture by offering rapid and reliable pathogen detection but also holds promise for broader applications in the field of molecular diagnostics. Through result recording and real-time sharing via smartphones, we envision a future where WeD-1 devices are widely deployed for continuous monitoring, providing invaluable insights into disease prevention and management. This interconnected surveillance approach has the potential to revolutionize the way we safeguard aquatic ecosystems and beyond.

CRediT authorship contribution statement

Feibiao Pang: Formal analysis, Investigation, Project administration, Validation. **Tao Zhang:** Formal analysis, Methodology, Validation. **Fengyi Dai:** Investigation, Software, Validation. **Kaizheng Wang:** Investigation, Software. **Tianjiao Jiao:** Investigation, Software, Validation. **Zuoying Zhang:** Validation. **Liyi Zhang:** Validation. **Mingli Liu:** Validation. **Peng Hu:** Funding acquisition, Supervision, Visualization, Writing – review & editing. **Jinzhao Song:** Conceptualization, Funding acquisition, Investigation, Supervision, Validation, Writing – original draft, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2024.116187.

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