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# A compact, palm-sized isothermal fluorescent diagnostic intelligent IoT device for personal health monitoring and beyond via one-tube/one-step LAMP-CRISPR assay

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ABSTRACT

The demand for accurate, user-friendly, and sensitive at-home nucleic acid testing solutions is rising due to occasional outbreaks of various infectious diseases and a growing desire for an improved quality of life. In response, we developed the WeD-mini, a compact, palm-sized isothermal fluorescent diagnostic IoT device that weighs just 61 g. The WeD-mini features a uniquely designed, highly sensitive optical sensing system, ultra-low power consumption, a minimalist industrial design, and an intelligent operating algorithm. It integrates real-time fluorescence detection and automatic result interpretation via a smartphone, with results seamlessly uploaded to the 'EzDx Cloud' for comprehensive health management and spatio-temporal disease mapping. The device supports various assays that operate at different temperatures and with varying fluorescence emission intensities, such as RPA (39 °C, low intensity), LAMP (65 °C, high intensity), and LAMP-PfAgo (65/95 °C, high intensity), while maintaining precise temperature control and exceptional fluorescence detection sensitivity. Additionally, we engineered a more thermostable AapCRISPR-Cas12b variant that operates effectively at 63 °C, enhancing compatibility with LAMP to create a robust One-Tube/One-Step LAMP-CRISPR assay. Adaptable for at-home testing of SARS-CoV-2 and influenza viruses, the WeD-mini achieved 100% sensitivity and specificity with the newly established One-Tube/One-Step LAMP-CRISPR assay. Furthermore, the WeD-mini shows potential applications in detecting meat adulteration, monitoring respiratory diseases in pets, and conducting wastewater surveillance, making it suitable for a wide range of personal and public health use cases.

## 1. Introduction

Occasional outbreaks of various infectious diseases, coupled with a growing desire for an improved quality of life, have led to an increasing demand for accurate, user-friendly, cost-effective, and highly sensitive at-home testing. This demand extends beyond personal health, encompassing various aspects of personal life, including food safety and pet care (Li et al., 2022; Weese et al., 2019). For instance, in the 'new normal,' there is a pressing need to differentiate between COVID-19 and influenza (Volz et al., 2021). Furthermore, testing for sexually transmitted diseases (STDs) requires a heightened level of privacy (Workowski and Bolan, 2015). Concerns related to genetically modified

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organisms and food safety issues are on the rise (González et al., 2020; Li et al., 2022). Additionally, the need for species identification (Baerwald et al., 2020), including the authentication of meat sources and Chinese herbal medicines (Han et al., 2018), as well as the health monitoring of household pets, is becoming increasingly integrated into people's daily lives (Weese et al., 2019).

At present, the primary method for at-home pathogen detection remains the Rapid Antigen Test (RAT). However, it's important to note that Nucleic Acid Amplification Tests (NAAT) offer higher accuracy compared to RAT (Lobato and O'Sullivan, 2018; Piepenburg et al., 2006). Among NAAT methods, Real-time Polymerase Chain Reaction (RT-PCR) is the most widely used due to its exceptional sensitivity, specificity, and well-established protocol, often regarded as the gold standard (Chan et al., 2020; Li et al., 2020; Vogels et al., 2020). Nevertheless, one of its drawbacks is the relatively lengthy turnaround time, typically exceeding 2 h, in addition to the sizable, costly, and cumbersome thermal cycler requirement (Kevadiya et al., 2021; Safiabadi Tali et al., 2021; Udugama et al., 2020). In contrast to PCR, isothermal amplification methods (Kaminski et al., 2021; Zhao et al., 2015) operate at a constant temperature, eliminating the need for thermal cycling systems, which makes them highly suitable for at-home testing (Chen et al., 2022; Dolgin, 2024; Joung et al., 2020; Song et al., 2016, 2017, 2018a, 2018b).

To overcome the cost and time constraints of traditional PCR-based nucleic acid detection methods, as well as the limited sensitivity and specificity of antigen tests, we developed the WeD-mini, a palm-sized isothermal fluorescent diagnostic intelligent internet of things (IoT) device, with a uniquely designed, highly sensitive optical sensing system and an ultra-compact industrial design. The WeD-mini provides real-time signal monitoring through a smartphone interface enabled by an elaborately designed connectivity module and offers automated result interpretation powered by an intelligent algorithm. As an IoT device, it uploads test results, along with personal health and location data, to the 'EzDx Cloud'(Wang et al., 2024) for comprehensive health management and spatio-temporal disease mapping, while also sending text notifications of results directly to users. Moreover, the WeD-mini is designed with a strong focus on ultra-low power consumption and can be conveniently powered through a mobile phone's Type-C interface. Weighing a mere 61 g, the device can maintain precise temperature control within the range of 25°–100 °C and is compatible with widely available, cost-effective consumable PCR tubes. Notably, this device supports various assays that operate at different temperatures and with varying fluorescence emission intensities, such as Recombinase Polymerase Amplification (RPA, 39 °C, low intensity)(Piepenburg et al., 2006), Loop-mediated Isothermal Amplification (LAMP, 65 °C, medium intensity)(Notomi, 2000), RPA-CRISPR (39 °C, high intensity) (Kaminski et al., 2021), LAMP-CRISPR (65 °C, high intensity) (Broughton et al., 2020), LAMP-PfAgo (65/95 °C, medium intensity) (Pang et al., 2024; Xun et al., 2021), and more (Zhao et al., 2015).

In addition, we engineered a more thermostable AapCRISPR-Cas12b variant to resolve the optimal temperature compatibility issue between LAMP amplification and CRISPR-specific cleavage within a single reaction system. Subsequently, we designed a One-Tube/One-Step LAMP-CRISPR assay to enable duplex detection of SARS-CoV-2 and influenza in a single-step, single-tube procedure. To evaluate the performance of the WeD-mini, we demonstrated the rapid detection of SARS-CoV-2 and influenza viruses by combining the palm-sized intelligent device with the improved One-Tube/One-Step LAMP-CRISPR system. This newly developed detection system achieved 100% sensitivity and specificity, exhibiting significantly higher sensitivity than RAT, and the measured results closely align with real-time PCR. In addition to its high sensitivity and specificity, the WeD-mini system offers accurate, user-friendly, and efficient at-home rapid genotyping capabilities, which will soon be released by our research group, further broadening its range of applications. In the event of an infectious disease outbreak, primers for NAAT can be rapidly designed and synthesized, offering greater time efficiency compared to the antibody screening required for RAT kit development. Furthermore, the WeD-mini platform utilizes 200 μL PCR tubes, making it significantly more cost-effective than disposable RAT cassettes. Together, these features highlight the WeD-mini's capability to deliver rapid and precise diagnostics, crucial for maintaining personal health and enhancing everyday life.

## 2. Materials and methods

### 2.1. Instrumentation

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

### 2.2. Exploring the compatibility of WeD-mini with recombinase assisted amplification (RAA), LAMP, and LAMP-PfAgo assays

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

### 2.3. Development of a one-tube/one-step LAMP-CRISPR assay using engineered thermostable CRISPR-Cas12b

A pioneering One-Tube/One-Step LAMP-CRISPR assay was developed, harnessing the newly engineered thermostable AapCRISPR-Cas12b variant named ScCas12b. The LAMP-CRISPR Assay encompasses a total reaction volume of 25 μL, consisting of the following components: 1X LAMP Isothermal buffer (New England Biolab, US), 1X primer mix (Supplemental Tables 1 and 2), 8U BST2.0 (SignalChem Biotech Inc., SuZhou), ScCas12b at various concentrations, 25 nM sgRNA (Supplemental Tables 1 and 2), 500 nM ssDNA reporter (Supplemental Tables 1 and 2), 6 mM MgSO4, and 1U AMV (Promega, US). Additionally, either 1 μL of RNA standard templates (procured from the National Sharing Platform for Reference Materials, China) or purified virus RNA from human nasopharyngeal swabs or wastewater, is introduced into the reaction mixture. Further insights into the reagents employed in the LAMP-CRISPR assay are available in Supplemental Table 3. The viral RNA from human nasopharyngeal swabs and 10 mL wastewater samples was extracted using the Qiagen QIAamp Viral RNA Kit and the E.Z.N.A. Water DNA Kit (Omega Bio-tek, Inc), respectively. The incubation of the reaction mixture takes place in a WeD-mini system at 65 °C for 30 min.

## 3. Results

### 3.1. Overall design and module validation

The WeD-mini was meticulously developed to meet user demands for a lightweight, compact, and on-the-go molecular detection device, giving users the flexibility to perform molecular testing anytime and anywhere. To achieve this, we engineered a remarkably compact design, similar in size and appearance to a True Wireless Stereo (TWS) power charging case. This device can function as a full nucleic acid fluorescence detection device, typically used in central labs, by being seamlessly controlled and interacted with through a smartphone (Supplemental Video 1). The WeD-mini was designed as shown in Fig. 1a and Supplemental Fig. 1 and fabricated following the methods outlined in the Supplemental Materials and Methods. Consisting of four integral modules, the WeD-mini is efficiently integrated and controlled by a main control PCB board (refer to Fig. 1b), featuring an ultra-low power consumption design (Supplemental Fig. 2). These modules include an optical unit for highly-sensitive fluorescence sensing and signal amplification, a thermal module for precise temperature regulation, a power management unit optimized for energy efficiency, and a connectivity module enabling IoT functionality. The exceptional performance of the optical, thermal, and power modules is illustrated in

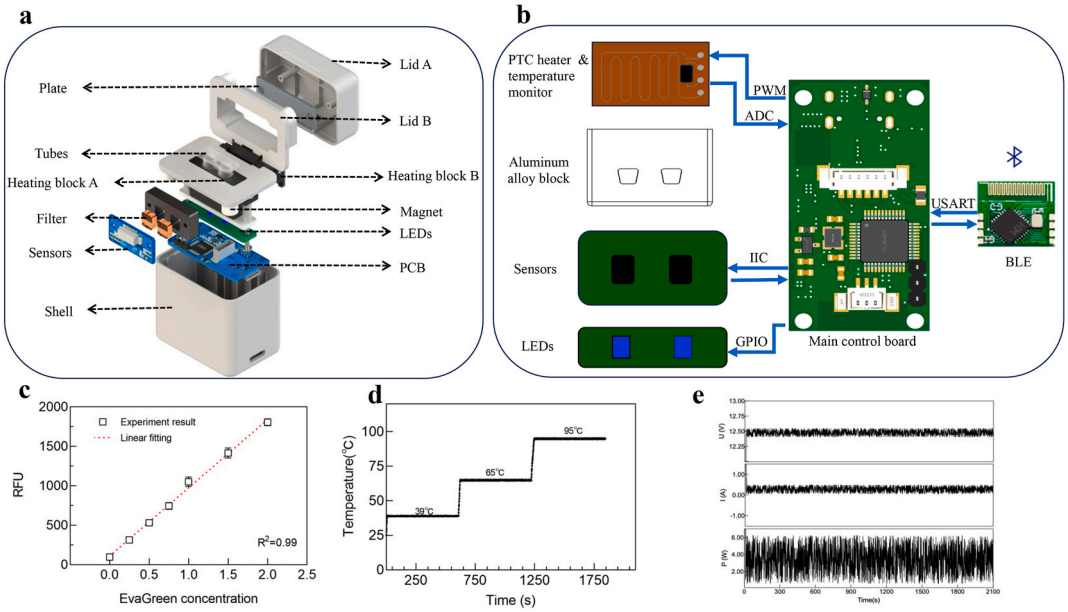



**Fig. 1.** Overall design and module validation. **(a)** The 3D module exploded view of the device, which is composed of a control circuit, a heating module, an excitation light module, a fluorescence sensor module and a heating block. **(b)** PCB architecture diagram of the control system. **(c)** The mean and standard deviation of the relative fluorescence unit (RFU) as a function of dsDNA-intercalating dye concentration. The concentration of *E. coli* DNA is fixed at 50 ng/ul. The WeD-mini fluorescence sensing function was evaluated by determining the RFU of dsDNA-EvaGreen (20x) at varying concentrations ranging from 0x to 2x. **(d)** Assessing heating rate and temperature stability of WeD-mini. The temperatures were set to three commonly utilized points for isothermal amplification or detection—39 °C, 65 °C, and 95 °C—and maintained for 10 min each. **(e)** Power consumption characterization for a complete test.

Fig. 1c–d, and Fig. 1e, respectively, and further detailed in the Supplemental Results.

### 3.1.1. *APP development*

The smartphone App was developed using the Java language in Android Studio, and is compatible with Android 8 and later operating

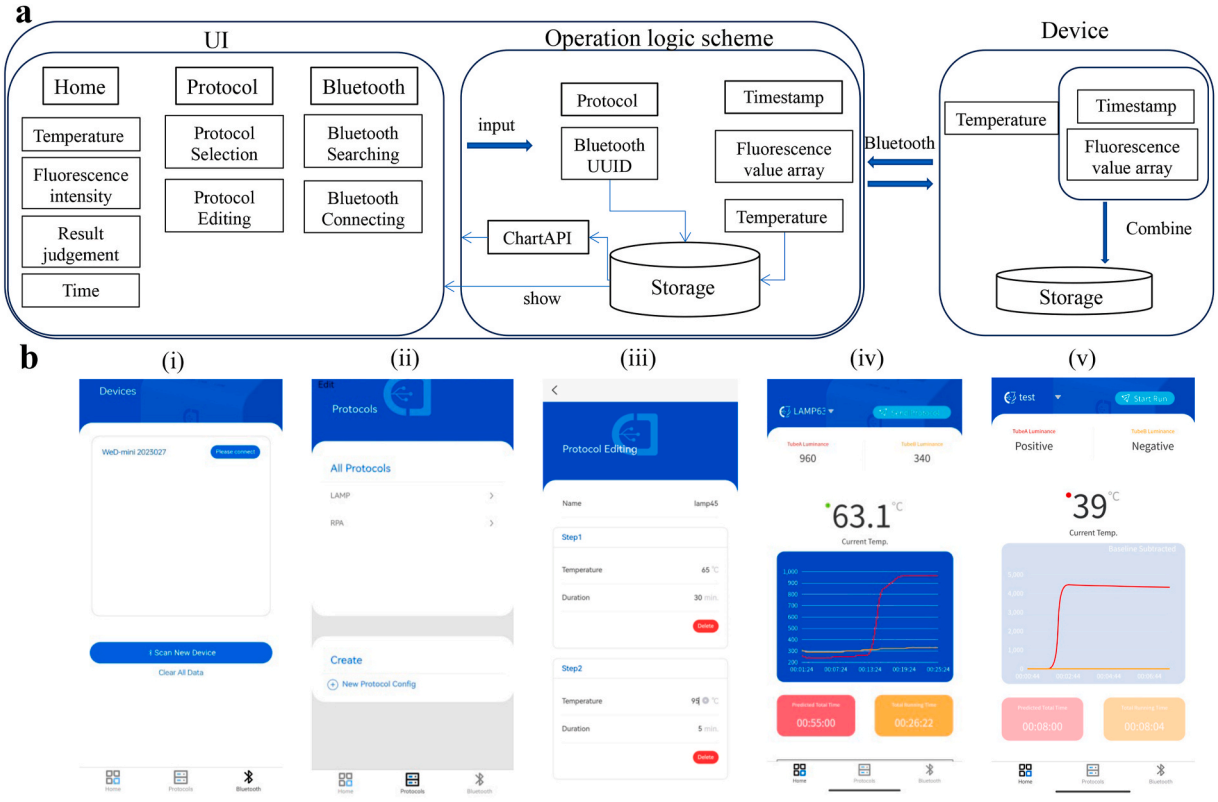



**Fig. 2.** Overview of the Smartphone App for Device Control and Data Analysis. **(a)** The architecture of the App includes the user interface (UI), operational logic scheme, and communication with devices. **(b)** Screenshots of the App interface, featuring: (i) The Bluetooth connection page, (ii) Protocol setting page, (iii) Protocol editing page, (iv) Home page displaying real-time data, and (v) Interface demonstrating automatic result judgment after the reaction.

systems. The program interface comprises three primary sections: Home, Protocol, and Bluetooth, depicted in Fig. 3a. To initiate device connection, users must locate our device via the Bluetooth page during the first use. Upon successful connection, the program automatically records the matched Bluetooth UUID in the mobile phone, enabling seamless device recognition for subsequent uses (refer to Fig. 2b–i). To accommodate various reaction protocols, we offer preset heating profiles such as RPA and LAMP (Fig. 2b–ii). Users also have the flexibility to customize their own heating profiles, adjusting parameters like temperature, duration, and adding steps (as demonstrated in Fig. 2b–iii). Throughout the experiment, the device's Bluetooth functionality transmits real-time fluorescence signals and temperature data. Simultaneously, a real-time curve is displayed on the smartphone's Home page (Fig. 2b–iv). The threshold value was determined using the algorithm/logic outlined in Supplemental Fig. 3. Post-reaction, our WeD-mini intelligent device autonomously evaluates the results, displaying a positive or negative outcome based on the preset threshold (Fig. 2b–v). To enhance device usability and avoid excessive reliance on the mobile phone, the Bluetooth link can be disconnected while the amplification process is ongoing. During this time, fluorescence and corresponding time information are temporarily stored on the device (Fig. 2a) and will synchronize back to the mobile phone upon reconnection. The curve data will be redisplayed on the home page (as shown in Fig. 2b–v and Supplemental Video 3) for further analysis.

#### 3.1.2. *WeChat mini-program development*

To ensure compatibility across various smartphones operating on different systems such as iOS and Android, and to eliminate the need for app downloads and installations, we developed a WeChat mini-program using the Uniapp framework. This framework streamlines development by enabling the utilization of JavaScript for deploying the mini-program across various social media platforms. Illustrated in Supplemental Fig. 4i-iv, our mini-program mirrors the functionality of our Android App, encompassing essential features for device control and data collection, including Home, Protocols, and Bluetooth pages. However, distinct from our Android App, the WeChat mini-program introduces a Recording page (Supplemental Fig. 4v and 4vi), facilitating seamless data recording and sharing on our 'EzDx Cloud' platform (Supplemental Fig. 4vii and 4 viii). Through the WeChat mini-program, users can conveniently edit, modify, and transmit amplification protocols (heating profiles) to the device's MCU, ensuring secure storage within the device, similar to our Android App.

### 3.2. *Exceptional platform versatility: Supporting diverse assays and detection of varied targets for various applications*

Detection of nucleic acids for personal use encompasses a wide array of applications, extending beyond personal health to include areas such as food safety, pet care, and the detection of pathogens relevant to animal and fish breeding. Given this broad scope, it is imperative that our device demonstrates compatibility with assays operating at varying temperatures and exhibiting different levels of fluorescence intensity for the detection of diverse targets. To illustrate its versatility, we conducted a thorough evaluation of the device's performance with different assays. Specifically, we assessed its efficacy with RAA at a working temperature of 39 °C, as depicted in Fig. 3a, where the fluorescence intensity was measured at approximately 200 units. Subsequently, we evaluated its performance with LAMP at a working temperature of 65 °C, as shown in Fig. 3b, where the fluorescence intensity significantly increased to approximately 3320 units. Additionally, we tested the device with LAMP-PfAgo across a temperature range of 65 °C–95 °C, as illustrated in Fig. 3c, where the fluorescence intensity remained consistently high at approximately 9200 units. Notably, Fig. 3c underscores the device's exceptional capability in facilitating PfAgo cleavage even at the elevated temperature of 95 °C.

We evaluated the performance of RAA, LAMP, and LAMP-PfAgo by detecting various nucleic acid targets, namely RAA with the respiratory pathogen SARS-CoV-2 (Fig. 3a), LAMP with meat sources from donkey and horse (Fig. 3b), and LAMP-PfAgo with the aquaculture pathogen Largemouth bass ranavirus (LMBV) (Fig. 3c). Our results indicate that the device is compatible with various assays that operate at different temperatures and emit light with varying intensities, making it capable of effectively accommodating diverse target applications with high levels of sensitivity.

### 3.3. *Enhancing one-tube/one-step LAMP-CRISPR assay through engineering thermostable CRISPR-Cas12b for simultaneous detection of SARS-CoV-2 and influenza*

Ever since the development of SHERLOCK, DETECTR, and HOLMES (Kaminski et al., 2021), several research groups have been working to develop one-tube CRISPR-based methods. However, it is worth noting that, despite being described as a one-tube method, these approaches still require a step to separate the amplification and CRISPR-Cas cleavage (Hu et al., 2022, 2023). Furthermore, although some one-tube, one-step LAMP-CRISPR-Cas assays have recently been developed, the thermostability (Cao et al., 2023; Fuchs et al., 2022; Joung et al., 2020; Li et al., 2019; Nguyen et al., 2022, 2023) of CRISPR-Cas and compatibility (Nguyen et al., 2022) between nucleic acid isothermal

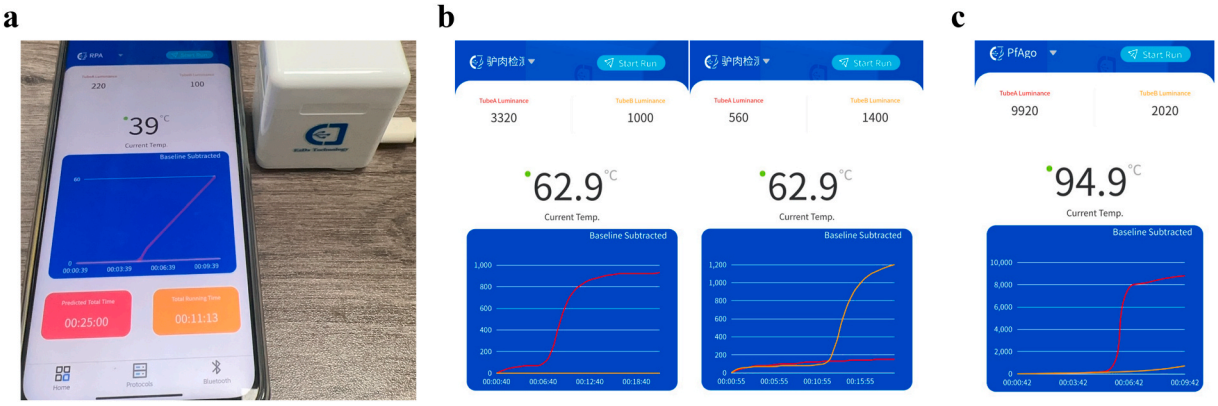



**Fig. 3.** The Device's Versatile Compatibility with Various Assays and Applications for Different Targets. **(a)** RAA Assay for SARS-CoV-2 Detection. **(b)** LAMP Assay for Differentiation between Meat from Donkey and Horse. **(c)** LAMP-PfAgo Assay for LMBV Virus Detection. Evaluation was conducted using 100 copies/reaction of SARS-CoV-2 RNA and LMBV DNA, as well as meat swab samples. All experiments were performed in triplicate.

amplification and CRISPR-specific cutting remain problematic. The Yin group endeavored to enhance compatibility between nucleic acid isothermal amplification and CRISPR-specific cleavage by employing strategies such as utilizing suboptimal PAM or engineering the protospacer-adjacent motif-interacting domain of Cas12b (Tong et al., 2024). However, it is important to note that the optimal temperature for the established assay is no more than 60 °C, which does not fully align with the optimal temperature required for Bst DNA polymerase and LAMP. To overcome these limitations, we engineered AapCRISPR-Cas12b to be more thermostable and utilized it to develop a newly designed One-Tube/One-Step LAMP-CRISPR assay that operates effectively at 63 °C. As illustrated in Fig. 4a, we streamlined the procedure of the two-step LAMP-CRISPR assay (Broughton et al., 2020) by integrating the LAMP reaction with the thermostable CRISPR-Cas12b into one reaction. During the reaction, the LAMP amplicon generated is rapidly recognized by the guide RNA, initiating the CRISPR-Cas12b′s trans-cleavage activity and emitting a fluorescence signal (Fig. 4b). Research from the Hsing group indicates that CRISPR-Cas12b′s cis-cleavage can help eliminate amplicons, thereby reducing the risk of aerosol contamination (Cao et al., 2023). We believe that our assay also offers this capability. Additionally, their CoLAMP method (CRISPR-based one-pot loop-mediated isothermal amplification) incorporates a PAM-containing loop primer. Integrating a PAM-containing loop primer in our One-Tube/One-Step LAMP-CRISPR assays would probably not only simplify the design by eliminating the need for precise PAM positioning but also further improve efficiency in reducing the risk of aerosol

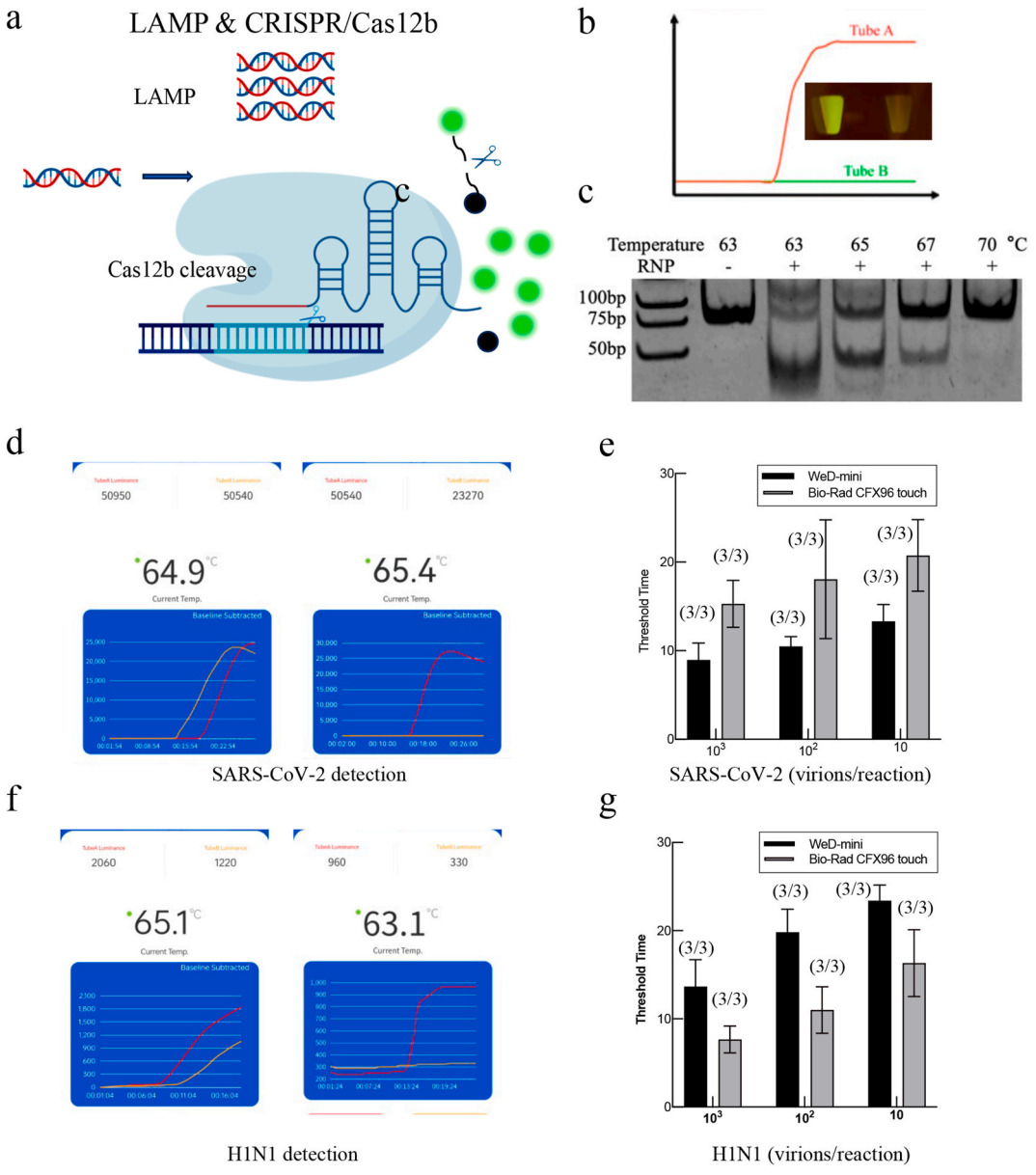



**Fig. 4.** Development and Evaluation of a One-Tube/One-Step LAMP-CRISPR Assay for Co-detection of SARS-CoV-2 and Influenza. **(a)** Schematic representation of the streamlined One-Tube/One-Step LAMP-CRISPR assay integrating LAMP reaction with thermostable CRISPR-Cas12b. **(b)** Illustration of the reaction mechanism where LAMP amplicons are recognized by guide RNA, initiating CRISPR-Cas12b trans-cleavage activity and fluorescence signal emission. **(c)** Evaluation of the heat resistance capability of thermostable CRISPR-Cas12b. Cleavage of a 75 bp double-stranded DNA (dsDNA) template was performed in an incubator for 20 min at temperatures ranging from 63 °C to 70 °C. **(d–g)** Representative results showing that the One-Tube/One-Step LAMP-CRISPR lyophilized reagents reliably detect 10 SARS-CoV-2 and H1N1 virions with high repeatability and sensitivity, highlighting robust performance in identifying respiratory viruses. The simulated swab samples were created by adding intact SARS-CoV-2 and H1N1 virions to our LAMP-compatible rapid lysis buffer. Experiments were quintuply repeated for reproducibility and consistency.

contamination.

### 3.4. Simultaneous detection of SARS-CoV-2 and influenza with the WeD-mini/one-tube/one-step LAMP-CRISPR detection system

To assess the heat resistance capability of thermostable CRISPR-Cas12b, its cleavage efficiency was tested across various temperatures. Fig. 4c and Supplemental Fig. 5 demonstrate that thermostable CRISPR-Cas12b maintained a cleavage efficiency of over 95% at temperatures between 45 °C and 63 °C. However, the cleavage efficiency dropped to below 50% at temperatures of 37 °C and above 65 °C. Furthermore, the concentration of thermostable CRISPR-Cas12b and the length of the reporter were optimized, as shown in Supplemental Fig. 6. Our experiments (Supplemental Figs. 6a–c) indicated that concentrations of thermostable CRISPR-Cas12b greater than 24 nM resulted in higher fluorescence emission. Additionally, a 10 nt reporter yielded the highest fluorescence increase (Supplemental Figs. 6e–f). Based on these findings, we selected a concentration of 24 nM thermostable CRISPR-Cas12b and a 10 nt reporter for subsequent experiments, as these conditions were determined to be optimal.

Distinguishing between COVID-19 cases and other respiratory illnesses, such as influenza, is of paramount importance. In the case of influenza, Oseltamivir has proven notably effective in controlling the replication of both type A and type B virus strains (Kawai et al., 2006, 2008). Consequently, we developed an assay capable of detecting both SARS-CoV-2 and influenza. The WeD-mini/One-Tube/One-Step LAMP-CRISPR detection system exhibited 100% consistency in detecting SARS-CoV-2 and H1N1 compared to the qPCR instrument/LAMP-CRISPR (Supplemental Table 4), underscoring the device's robust performance in identifying prevalent respiratory viruses. Additionally, compared to conventional LAMP, the newly established method effectively eliminates non-specific amplification, as evidenced during the detection of SARS-CoV-2 (Supplemental Fig. 7).

Moreover, for convenient application in home and field settings, it is preferable to eliminate the necessity for a cold chain. To achieve this objective, we lyophilized the One-Tube/One-Step LAMP-CRISPR reagent. Notably, the performance of the lyophilized reagents matched that of our liquid reagents, as demonstrated in Supplemental Fig. 8. When incubated with our WeD-mini intelligent device, the One-Tube/One-Step LAMP-CRISPR lyophilized reagents consistently detect as few as 10 SARS-CoV-2 and H1N1 virions in rapidly prepared simulated swab samples, demonstrating high repeatability (Fig. 4d-g) and specificity (Supplemental Fig. 9). These results demonstrate the seamless compatibility between LAMP amplification and CRISPR-specific cleavage within a single reaction system, facilitated by the newly engineered thermostable CRISPR-Cas12b.

### 3.5. Monitoring the health of household pets with the WeD-mini/one-tube/one-step LAMP-CRISPR detection system

The ability to conduct molecular diagnosis of respiratory diseases in dogs and cats at home holds significant importance. It empowers pet owners to promptly and conveniently assess their pets' health status, eliminating the need for frequent visits to the veterinary clinic. To exemplify this concept, we demonstrate the capability of the WeD-mini/One-Tube/One-Step LAMP-CRISPR detection system in detecting feline respiratory pathogens spiked in cat nasal swab elutions, including Chlamydophila felis (CF), Mycoplasma felis (MF), Bordetella bronchiseptica (Bb), Feline calicivirus (FCV), and Feline herpesvirus (FHV). As illustrated in Fig. 5, the device reliably detects as few as 100 copies of CF, MF, Bb, FCV, and FHV, providing pet owners with a valuable tool for home-based diagnosis and monitoring of their pets' respiratory health.

### 3.6. Detection of SARS-CoV-2 and influenza viruses in patients with respiratory disease symptoms and in wastewater

In Fig. 6a, we illustrate the workflow of the WeD-mini/One-Tube/One-Step LAMP-CRISPR detection system. To detect SARS-CoV-2, oral swab samples were collected from patients with respiratory symptoms and added to our rapid lysis buffer, which has been confirmed to have no adverse effect on our lyophilized LAMP-CRISPR reagents (Supplemental Fig. 10). Subsequently, 25 μL of the sample was transferred to the tube containing the lyophilized reagents. Testing these samples with the WeD-mini intelligent device revealed 8 positive SARS-CoV-2 cases out of 26 patients, demonstrating 100% consistency with the commercial qPCR kit (Fig. 6b). The results were uploaded to our 'EzDx Cloud' for health management, treatment guidance analysis, and disease mapping. All patients received text message notifications with their test results (Supplemental Fig. 4viii).

Given the importance of wastewater surveillance in supporting clinical surveillance efforts, we evaluated the performance of the WeD-mini/One-Tube/One-Step LAMP-CRISPR detection system for detecting SARS-CoV-2 and influenza viruses in wastewater. Nucleic acids from 10 mL of wastewater were purified and concentrated using a commercial Omega kit. Fig. 6c shows 4 positive SARS-CoV-2 cases out of 7 wastewater samples and 1 positive H1N1 case out of 8 wastewater samples, also demonstrating 100% consistency with the commercial qPCR kit.

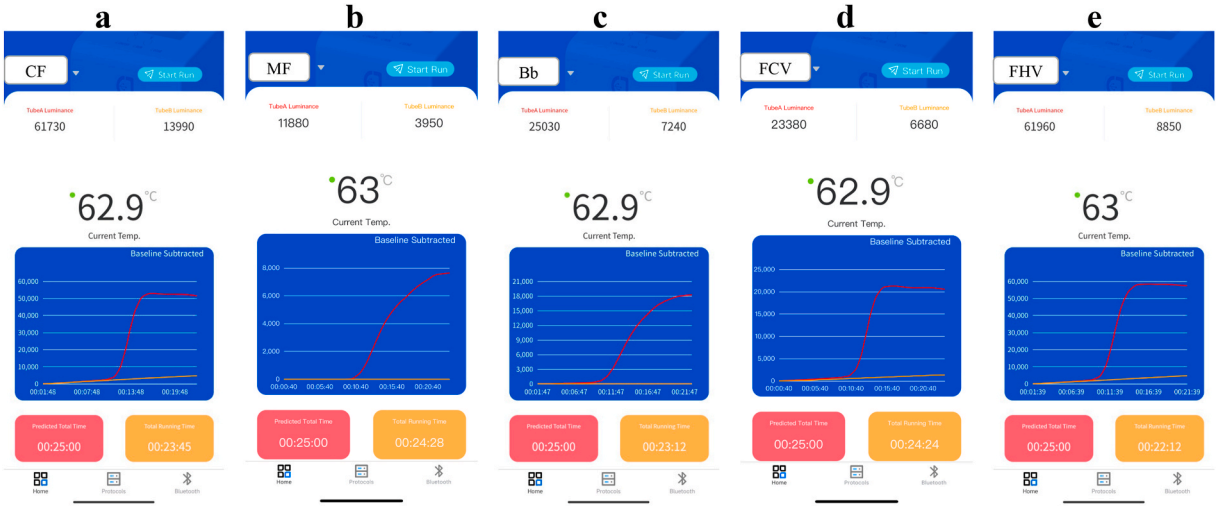
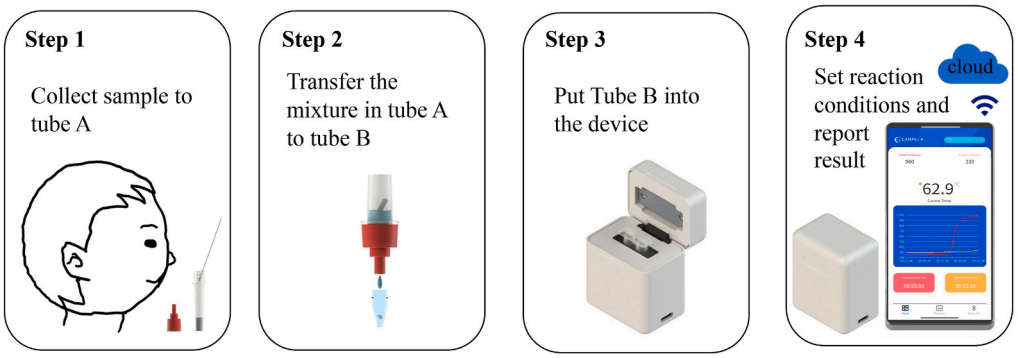



**Fig. 5.** Detection of Feline Respiratory Pathogens Using the WeD-mini/One-Tube/One-Step LAMP-CRISPR Detection System. The assay was performed using 100 copies/reaction of CF DNA **(a)**, MF DNA **(b)**, Bb DNA **(c)**, FCV RNA **(d)**, and FHV DNA **(e)**.

**a**




**b**

**Detection of SARS-CoV-2 in patients with respiratory disease symptoms**

| Sample ID (SARS-CoV-2) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PCR result | N | N | N | N | N | P | N | P | P | N | N | P | N |
| Ct Value | NA | NA | NA | NA | NA | 34.31 | NA | 32.34 | 31.49 | NA | NA | 30.27 | NA |
| WeD-mini result | N | N | N | N | N | P | N | P | P | N | N | P | N |

| 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | P | N | P | N | N | P | N | P | N | N | N | N |
| NA | 33.83 | NA | 32.69 | NA | NA | 33.46 | NA | 35.64 | NA | NA | NA | NA |
| N | P | N | P | N | N | P | N | P | N | N | N | N |

**c**

**Detection of SARS-CoV-2 and influenza viruses in wastewater**

| Sample ID (SARS-CoV-2) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | NC | Sample ID (H1N1) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | NC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PCR result | N | N | N | P | P | P | P | N | PCR result | P | N | N | N | N | N | N | N | N |
| Ct Value | NA | NA | NA | 26.79 | 26.89 | 26.07 | 25.59 | NA | Ct Value | 41.01 | NA | NA | NA | NA | NA | NA | NA | NA |
| WeD-mini result | N | N | N | P | P | P | P | N | WeD-mini result | P | N | N | N | N | N | N | N | N |

**Fig. 6.** Detection of SARS-CoV-2 and influenza viruses in patients presenting with respiratory disease symptoms and in wastewater. **(a)** Workflow of the WeD-mini/One-Tube/One-Step LAMP-CRISPR detection system for detecting viruses in patients with respiratory disease symptoms. **(b)** Detection of SARS-CoV-2 in patients with respiratory disease symptoms. **(c)** Detection of SARS-CoV-2 and influenza viruses in wastewater.

## 4. Conclusions

In this study, we showcased the WeD-mini as an innovative nucleic acid detection platform that combines compactness and lightweight design with precise temperature control (±0.3 °C) and highly sensitive fluorescence detection capability. Compared to existing rapid test devices (Supplemental Table 5), the WeD-mini stands out for its exceptional portability, reusability, IoT capabilities, real-time quantification, user-friendliness, and cost-effectiveness, all while weighing just 61 g. The WeD-mini intelligent IoT device offers real-time fluorescence detection and automatic result interpretation via a connected smartphone interface. The WeChat mini-program mirrors the functionality of our Android app, eliminating the need for app downloading and installation. Results can be uploaded to our 'EzDx Cloud' for health management, treatment guidance analysis, and disease mapping. Additionally, with its ultra-low power consumption design, the device can be powered by a mobile phone charger or power bank, ensuring uninterrupted testing and promoting user safety and health by enabling nucleic acid testing anytime, anywhere. Our experiments demonstrated the versatility of the WeD-mini, showing its compatibility with various assays requiring different working temperatures and emitting diverse fluorescence light intensities, including RPA, LAMP, and LAMP-PfAgo.

Additionally, we engineered a more thermostable AapCRISPR-Cas12b variant that functions effectively at 63 °C, improving its compatibility with LAMP and enabling the development of a more robust One-Tube/One-Step LAMP-CRISPR assay. This newly established assay, integrated with the WeD-mini device, achieved 100% sensitivity and specificity for at-home testing of the SARS-CoV-2 virus. Furthermore, our experiments demonstrated the potential application of this system for at-home monitoring of feline respiratory diseases. In the future, we aim to develop at-home and on-site nucleic acid purification methods to facilitate rapid testing of complex samples, such as stool, wastewater, and sputum.

## CRediT authorship contribution statement

**Fengyi Dai:** Formal analysis, Investigation, Validation, Visualization, Writing – original draft. **Tao Zhang:** Formal analysis, Investigation, Validation. **Feibiao Pang:** Investigation, Methodology. **Tianjiao Jiao:** Investigation, Software. **Kaizheng Wang:** Software, Validation. **Zhanfang Zhang:** Investigation, Validation. **Nuo Wang:** Formal analysis, Methodology. **Zhiwei Xie:** Methodology, Resources. **Yanchong Zhang:** Methodology, Validation. **Zihao Wang:** Methodology, Software. **Zhiguang Chen:** Investigation, Methodology. **Mingxia Yu:** Resources, Validation, Writing – review & editing. **Hongping Wei:** Formal analysis, Methodology, Resources, Writing – review & editing. **Jinzhao Song:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jinzhao Song reports financial support was provided by This study was supported by the National Key Research and Development Program of China (2023YFD2402100) and Zhejiang Leading Innovation and Entrepreneurship Team Program (2022R01006). Jinzhao Song has patent #Jinzhao Song, Feibiao Pang, Fengyi Dai, Kaizheng Wang and Tianjiao Jiao are coinventors on a patent related to this manuscript (application number, CN202210983377.8A, applied August 16, 2022; international application number, PCT/CN2023/109464, applied July 27, 2023; Publication/Patent Number: CN218860746U, granted April 14, 2023). The remaining authors declare no conflicts of interest. Issued to Hangzhou EzDx Technology Co., Ltd. If there are other authors, they 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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## Appendix. ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2024.116945.

## Data availability

No data was used for the research described in the article.

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