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Field-deployable FCVD: A contamination-free dual-mode diagnosis platform for the simultaneous detection of sexual co-infection

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ABSTRACT

Rapid and accurate identification of sexual pathogens is crucial for effectively controlling the spread of infections and facilitating timely treatments. However, the gold standard PCR technique typically requires centralized laboratories and well-trained professionals, limiting the possibilities for comprehensive screening and efficient prevention. In response, a field-deployable contamination-free visual detection platform (termed FCVD) has been established in this study. The CRISPR-powered dual-channel detection strategy with contamination-free LAMP-based amplification is firstly designed for the simultaneous identification of co-infection sexual viruses HIV and HPV. Armed with a home-made portable, connected, and real-time signal readout device, a detection sensitivity of 10^2 copies/μL (HIV-1) and 10 copies/μL (HPV-16) is achieved, respectively. The versatility of the platform is demonstrated by the detection of HIV and HPV in clinical samples, which agree well with the results of the PCR method. Our findings highlight the outstanding performance of the established FCVD platform, demonstrating the potential for rapid and accurate diagnostics in both clinical and resource-limited settings. With good compatibility to various sexual virus diagnosis, this portable platform holds significant potential for enhancing comprehensive screening and facilitating the effective prevention of sexually transmitted diseases.

1. Introduction

Sexually transmitted infections (STIs) represent a significant and growing global public health challenge, driven by hidden, rapid, and widespread transmission, as well as their persistently rising prevalence across the worldwide [1]. Among these, HIV and HPV are particularly notable due to their severe health consequences. According to the World Health Organization (WHO), HIV has led to approximately 42.3 million deaths to date, with an estimated 39.9 million individuals living with the virus by the end of 2023. Similarly, high-risk HPV infections are the primary cause of cervical cancer, which ranked as the fourth most common cancer and the fourth leading cause of cancer-related deaths among women globally in 2022, with around 660,000 new cases and 350,000 deaths reported that year [2]. Limiting rapid and accurate testing at clinical setting fail to achieve timely identification and effective intervention, resulting in an interruption delay of pathogen transmission risk and worsening health outcomes. In addition, fear of confidentiality also discourages clinical diagnosis, leading to widespread unrealized STIs, which highly call for the user-friendly testing at home.

Traditional detection methods, such as standard cytology (e.g., Pap smear) for HPV [3] and antibody tests for HIV [4], are characterized by relatively low sensitivity and specificity. In contrast, nucleic acid testing (NAT) demonstrates significant potential for viral load detection, with polymerase chain reaction (PCR)-based strategies established as the gold standard for viral nucleic acid testing, including for HIV and HPV [5,6],

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owing to their versatility, stability, and high sensitivity. However, these techniques necessitate expensive instrumentation, highly skilled personnel, and extended processing time [7]. Recently, simplified isothermal amplification methods, such as loop-mediated isothermal amplification (LAMP), have emerged as promising alternatives for detecting HIV and HPV, given their simplicity, sensitivity, and reduced resource requirements [8,9]. Although LAMP eliminates the need for thermal cycling, its susceptibility to non-specific amplification [10], primer-dimer formation [11] and aerosol contamination will compromise detection specificity and lead to false-positive results, limiting its practical application for accurate identification of sexual pathogens.

Recently, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas-based diagnostics have attracted considerable attention as a cutting-edge tool for molecular diagnosis characterized by their rapid, high-specific, sensitive, and simple reaction requirements, making them ideally suited for point-of-care testing (POCT) [12,13]. However, the sensitivity of CRISPR/Cas system usually ranges from picomolar to femtomolar, fail to directly detect sexual pathogens. This limitation promotes researchers to combine nucleic acids amplification such as loop-mediated isothermal amplification (LAMP) with CRISPR to develop sensitive molecular diagnostic platforms, such as the SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) system [14]. However, due to the limited compatibility between the LAMP and CRISPR systems, the procedures often require sequential reactions, which increases the risk of aerosol contamination and complicates the operational process [15,16].

To address above challenge, a novel platform termed Field-Deployable Contamination-Free Visual Detection (FCVD) is developed in this study to realize rapid and visual contamination-free detection of sexual co-infection, enabling comprehensive screening, early prediction and effective prevention. Utilizing an engineered *Zst* polymerase developed in our lab, directly reverse transcription without additional reverse transcriptase enable the simultaneous detection of DNA/RNA targets. In addition, the *Zst* polymerase can efficiently polymerize four oligonucleotides (dATP, dCTP, dGTP, and dUTP), enabling contamination-free target amplification through eliminating aerosol interference with the help of Uracil-DNA Glycosylase (UDG). Coupled with CRISPR/Cas detection, the high specificity of CRISPR further mitigates the risk of false-positive results from non-specific amplification from primers. With a home-made portable heating device, the point-of-care, rapid and connected detection of co-infection of sexual pathogens from clinical samples are performed, which is consisted well with the gold-standard PCR method. These results demonstrate the FCVD as a potential tool for supporting comprehensive screening and strengthening preventive strategies in STIs.

2. Materials and methods

2.1. Reagents and instruments

$(NH_4)_2SO_4$, KCl, KOH, Tween 20, Tris-HCl, NaCl, $MgCl_2$, EBT, agarose powder, TAE buffer, Triton X-100, betaine, glycerol, and DTT were purchased from Sangon Biotech (Shanghai, China). The 2× Taq Master Mix (Dye Plus) was obtained from Vazyme Biotech Co., Ltd. (Nanjing, China). RNAPol reaction buffer, rNTP mix, T7 RNA polymerase, DNase I (RNase-free), $MgSO_4$ (100 mM), dNTP mix, dATP, dCTP, dGTP, dTTP, and dUTP (100 mM), isothermal amplification buffer (10×), *Bst* 2.0 WarmStart DNA polymerase (8000 U/mL), Antarctic thermolabile UDG (1000 U/mL), and NEBuffer™ r2.1 were purchased from New England Biolabs (NEB, USA). EnGen® LbaCas12a (Cpf1) nuclease (100 μM) was acquired from New England Biolabs (USA). Nuclease-free water was purchased from Thermo Fisher Scientific (USA). Eva Green dye was obtained from Coolaber Science Co., Ltd. (Beijing, China). *Zst* polymerase was prepared in-house. Cas12/13-specific nucleic acid test strips were purchased from Suzhou Lemeda Biotechnology Co., Ltd. (China). DNA plasmids and synthetic sequences were provided by Tsingke Biotechnology Co., Ltd. (Beijing, China). A biological safety cabinet (Labconco, USA), BS224 analytical balance (Sartorius, Germany), Roche LightCycler 480 qPCR system (Roche, Switzerland), Mini Dancer Plus benchtop centrifuge (Sangon Biotech, China), Milli-Q Ultrapure Water System (Millipore, USA), MiniT-100H mini metal bath (Aosheng Instruments, China), Tanon EPS 300 gel electrophoresis system (Tanon Science, China), IKA vortex mixer (IKA, Germany), and Sorvall Legend Micro 21 centrifuge (Thermo Fisher Scientific, USA) were used. All other chemicals were of analytical grade or higher. Real-time LAMP and CRISPR/Cas12a reactions were performed using the Roche LightCycler 480 PCR system (Roche, USA).

2.2. Real-time LAMP assay

For the LAMP reaction, a 15 μL mixture was prepared containing 4.8 μL of homemade buffer, 1 μL LAMP primer mix (10×), 2.1 μL dNTP mixture (10 mM), 0.5 μL Eva Green (20×), 0.9 μL *Zst* polymerase (8000 U/mL), 0.6 μL uracil DNA glycosylase (UDG) (1000 U/mL), 1.2 μL MgSO4 (10 mM), 2.4 μL nuclease-free H_2O, and 1.5 μL template. The reaction tube was incubated at 25 °C for 10 min and at 65 °C for 40–60 min. The fluorescence value was recorded every 60 s, and the kinetic curve of the reaction was monitored using the Roche LightCycler 480 PCR (LC480) system (F. Hoffmann-La Roche Ltd., USA) or a home-made portable heating device.

2.3. Agarose gel electrophoresis of LAMP amplicons

Agarose gel electrophoresis (AGE) was performed to analyze the formation of UDG-treated and UDG-untreated LAMP amplicons. A 1.5 % agarose gel was prepared by dissolving 1.5 g of agarose in 100 mL of 1× TAE buffer (40 mM Tris-acetate, 2.0 mM EDTA, pH 8.5). The solution was heated until completely clarified, and GelRed was added as the nucleic acid dye. For electrophoresis, 10 μL of the sample-loading complex was loaded into each lane, and the gel was run at 150 V for 40 min at room temperature. After electrophoresis, the gel was visualized and captured using the Gel Doc XR+ system (Bio-Rad).

2.4. Real-time and lateral-flow CRISPR/Cas12a detection

The reaction mix for Cas12a activity was prepared by adding 1 μL NEB 2.1 buffer (10×), 0.2 μL LbaCas12a protein (10 μM), 0.2 μL crRNA (10 μM), 0.8 μL FAM-BHQ1 ssDNA reporter (10 μM) or FAM-Biotin ssDNA reporter (10 μM), 6.8 μL nuclease-free H_2O, and 1 μL LAMP amplicons, which were then added to the mix. For the lateral flow assay, 10 μL Cas12a reaction solution was mixed evenly with 40 μL nuclease-free H_2O. The lateral flow strip was inserted into the tube and incubated for 5–15 min at room temperature, followed by lateral flow reading. For real-time fluorescence detection, the reaction tubes were placed into the Roche LightCycler 480 PCR (LC480) system (F. Hoffmann-La Roche Ltd., USA) or a home-made portable heating device and incubated at 37 °C. Fluorescence was measured every 30 s for 10–30 min.

2.5. Image analysis and statistics

The hue values of the captured images were extracted using software (e.g., Adobe Photoshop 2021 or our custom Android app, "Hue Analyzer") and analyzed using GraphPad Prism 8 with an unpaired one-way ANOVA test. The hue values of each image were extracted three times. The results are presented as mean ± standard error. Significance thresholds were defined as follows: NS ($p > 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$).

2.6. Clinical specimen detection

Human clinical samples were provided by Renji Hospital, Shanghai

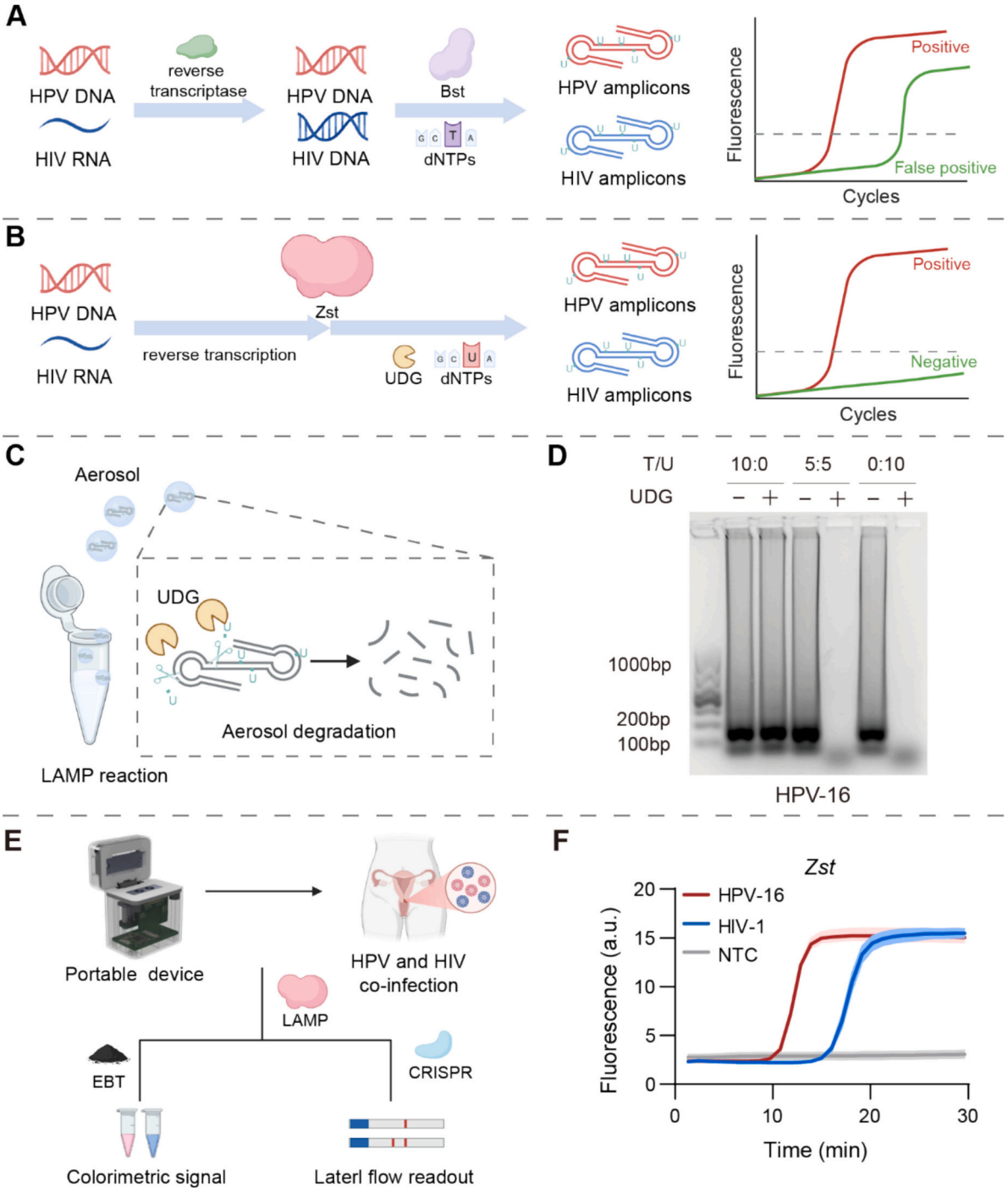

Fig. 1. The proposed principle of the Field-Deployable Contamination-Free Visual Detection (FCVD) Platform. (A) Schematic illustration of the traditional LAMP reaction using *Bst* polymerase. (B) Working principle of the FCVD platform, highlighting its capability for simultaneous detection of DNA and RNA targets. (C) Mechanism of the UDG-dUTP system in eliminating aerosol contamination from amplicons. (D) Simulation of aerosol contamination elimination efficacy under different dTTP-to-dUTP ratios. (E) Schematic illustration of the dual signal readout strategies, including colorimetric analysis and visual lateral flow strip. (F) Validation of target amplification using *Zst* polymerases. Note: NTC-no template control.

Jiao Tong University School of Medicine (IRB Protocol number: KY2021–111-B-CR-01 was approved by the Ethics Committee). The sample is stored at −80 °C to ensure the integrity of nucleic acid. The clinical samples were thoroughly mixed with lysis buffer at 1:1 ratio and then heated at 95 °C for 5 min. The lysed nucleic acid sample is directly added to the reaction system for detection.

2.7. Experiment with home-made portable heating device

The experiment was conducted using a homemade portable heating device. First, the supporting mobile application (APP) was downloaded and installed. 200 μL PCR tube was placed in the instrument, and the device was powered by Type-C power supply. The APP was launched, and Bluetooth was enabled to establish a connection with the device. The heating program and result interpretation threshold were set and saved in the APP. To initiate the operation, the “Start Run” button was

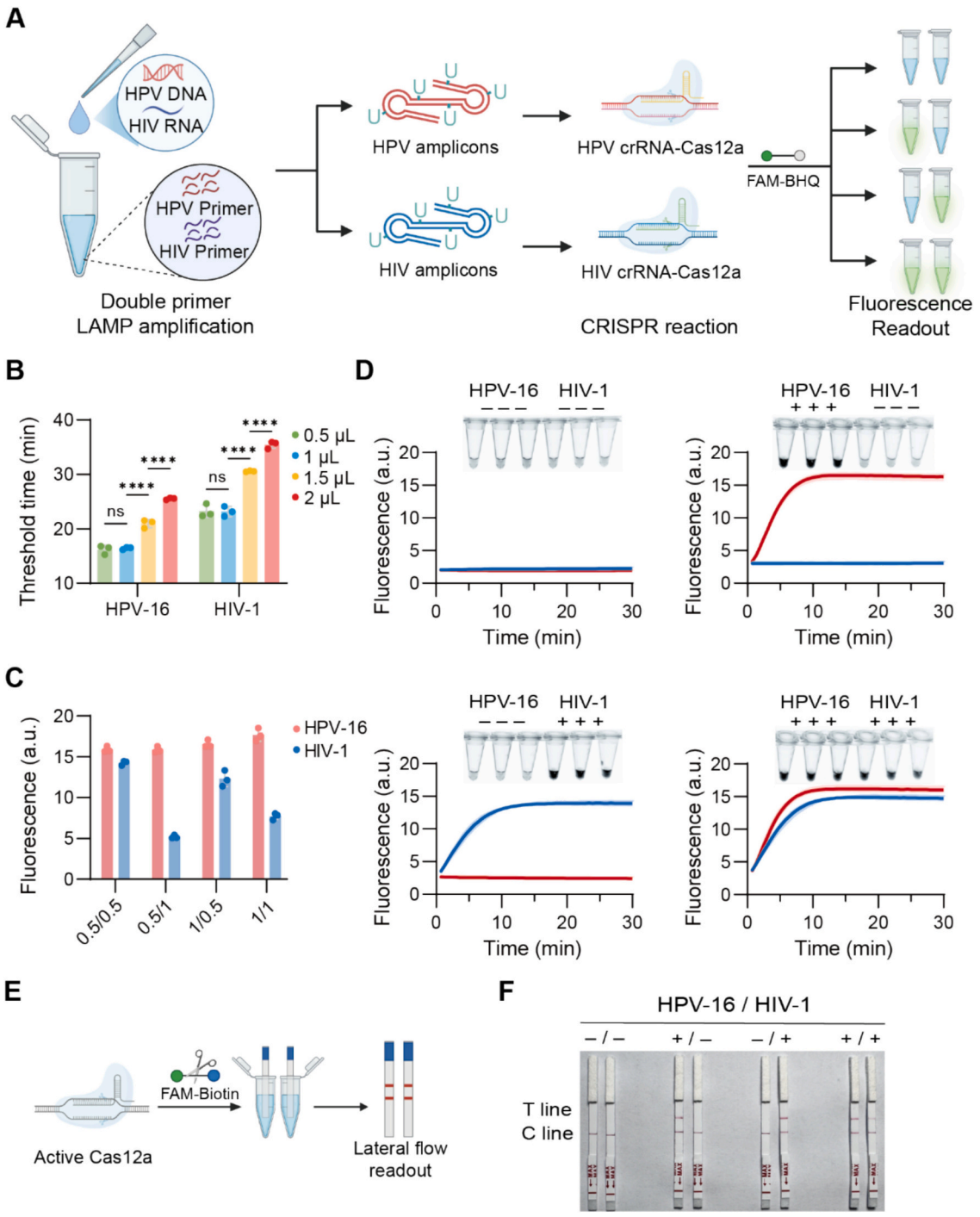

Fig. 2. FCVD platform for simultaneous detection of HPV and HIV. (A) Workflow of the FCVD assay, integrating LAMP amplification and CRISPR/Cas12a-based detection. (B) Threshold time of the LAMP reaction at different volumes of 10× primers for HPV or HIV targets. (C) Fluorescence intensity of CRISPR/Cas12a reaction at different 10× primer volume ratios for HPV and HIV targets. (D) Real-time fluorescence curves and endpoint fluorescence diagrams of the CRISPR/Cas12a reaction for no target, HPV, HIV, and HPV + HIV targets. (E) Schematic of lateral flow detection. (F) Lateral flow strip detection results for different target combinations.

clicked. Real-time fluorescence detection signals and heating status were monitored directly through the mobile phone interface.

3. Results and discussion

3.1. The overall design strategy of FCVD for sexual virus detection

Accurate detection of sexually transmitted pathogens under different conditions, particularly testing at home or in resource-limited settings, is essential for the effective control of STIs. To address this challenge, we designed a Field-Deployable Contamination-Free Visual Detection Platform (FCVD). The engineered *Zst* polymerase, which possesses reverse transcriptase activity, enables simultaneous detection of the sexually transmitted viruses HPV and HIV without requiring additional reverse transcriptase. (Fig. 1A-B) [17]. In addition, with higher polymerization efficiency for all four nucleotides (dATP, dCTP, dGTP, and dUTP) than commercial *Bst* polymerase [18], the sensitive detection on FCVD using *Zst* polymerase was free of aerosol contamination (Fig. 1C),

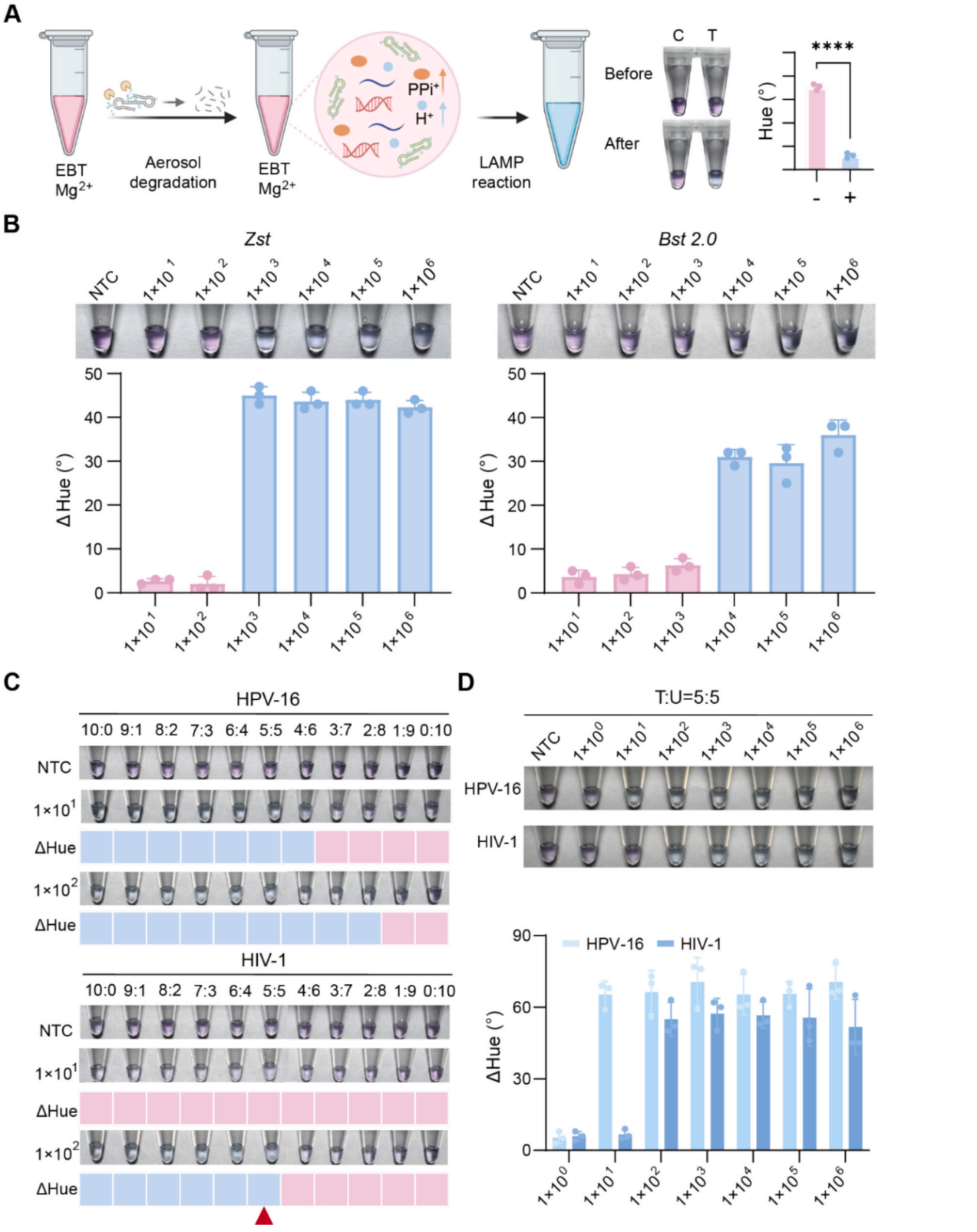

Fig. 3. FCVD Platform for colorimetric detection (A) Schematic illustration. (B) Comparison of HPV-16 detection sensitivity using *Zst* and *Bst* 2.0 polymerases under pure dUTP conditions. Note: NTC-no template control. (C) Colorimetric results under varying dTTP-to-dUTP ratios (10:0 to 0:10). (D) Sensitivity of the detection of HPV-16 and HIV-1 at a dTTP: dUTP ratio of 5:5 using FCVD Platform.

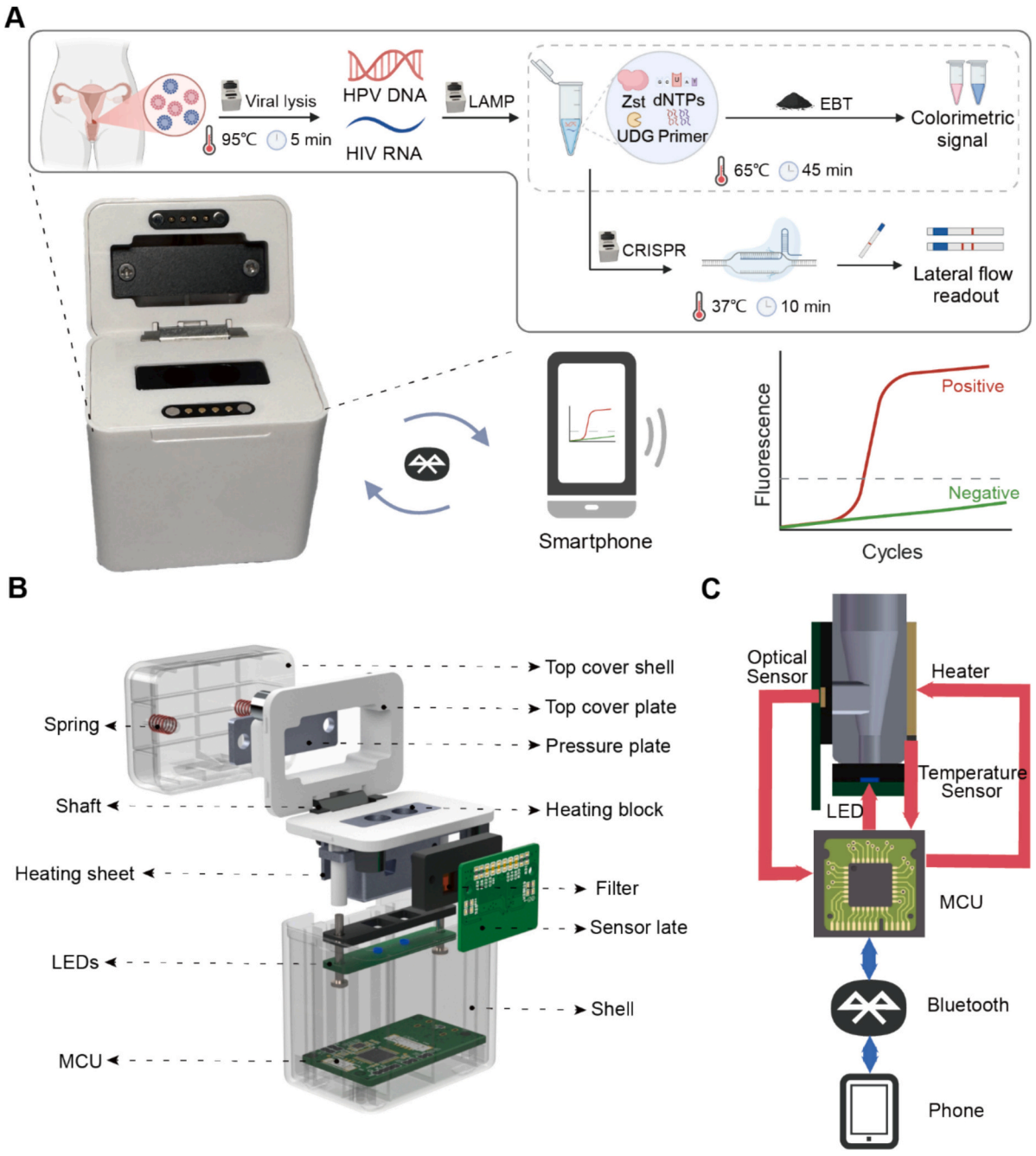

Fig. 4. Portable device for field-deployable FCVD. (A) Schematic of the on-site testing process for sexual HPV and HIV infection using FCVD platform. (B) Exploded view of the internal structure of the portable device, highlighting core components. (C) Diagram of the PCB architecture, illustrating the four functional modules: optical unit, temperature control module, power management unit, and IoT connectivity module.

due to the dUTP-based amplicons could be degraded by UDGs. To achieve a balance between amplification efficiency and anti-contamination capability, the LAMP reaction using dTTP-to-dUTP ratios from 10:0 to 0:10 was investigated, respectively (Fig. S1). The results revealed that decreasing the dTTP-to-UTP ratio from 10:0 to 5:5 had no significant effect on the amplification efficiency, but the threshold time increase when the ratio falls below 5:5. In addition, the agarose gel electrophoresis results further confirmed that the amplicons from LAMP reaction under dTTP-to-dUTP ratio of 5:5 could be almost cleared in the presence of UDG enzyme, which is similar to the results using pure dUTP (Fig. 1D; Fig. S2). In addition, we have evaluated the long-term risk of post-amplification contamination and observed no contamination within two months of continue experiments (Fig. S3). Therefore, a dTTP to UTP ratio of 5:5 was chosen in the following experiments to ensure efficient amplification while minimizing the false-positive results from LAMP contamination. In addition, the portable and connected platform could incorporate dual signal readout strategies, including LAMP-CRISPR lateral flow strips and colorimetric LAMP analysis, enabling instrument-free visual interpretation (Fig. 1E). In contrast to commercial *Bst* 2.0 polymerase, the engineered *Zst* polymerase could successfully amplify both HPV-16 and HIV-1 targets without additional reverse transcription (Fig. 1F), whereas *Bst* 2.0 was only capable of amplifying HPV-16 (Fig. S4). These features make the FCVD platform highly suitable for on-site, point-of-care applications, aligning with the goals of rapid, accurate, and contamination-free detection of sexual virus such as HPV and HIV.

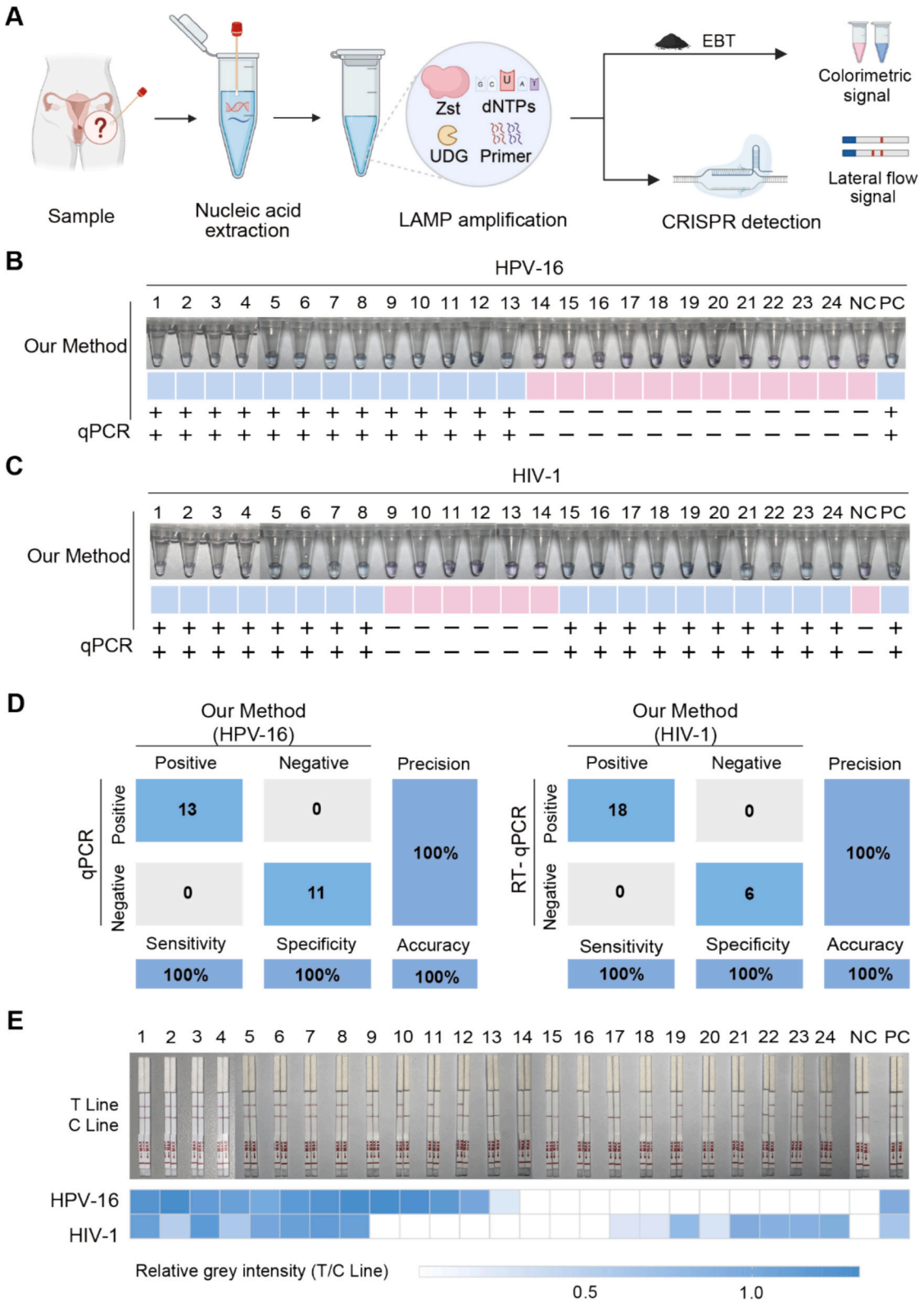

Fig. 5. Detection performance of the FCVD platform for real clinical samples. (A) Workflow of clinical samples testing on FCVD platform. (B—C) Colorimetric detection results of real clinical samples and HIV-1 pseudovirus spiked samples, B for the detection of HPV-16, C for the detection of HIV-1. (D) Performance comparison of the developed method with the qPCR. (E) Lateral flow strip results for the simultaneous detection of co-infection samples, with a heatmap showing the relative gray intensity ratio of the test line (T line). Note: NC-negative control; PC-positive control.

3.2. Optimization of the FCVD platform

The established contamination-free FCVD platform was investigated for the simultaneous detection of sexual viruses HPV and HIV by integrating LAMP amplification with CRISPR/Cas12a-based signal readout, which could not only achieve high specificity but also enable user-friendly visual recognition. As shown in Fig. 2A, the HPV and HIV were simultaneously amplified by the *Zst*-mediated LAMP reaction, followed by the CRISPR/Cas12a-based detection, enabling specific target discrimination while solving the problem of non-specific amplification caused by primer dimers [19,20]. Since the performance of CRISPR/Cas12a-based detection could be significantly influenced by the crRNA sequence, the crRNA for HPV-16 and HIV-1 detection was optimized by designing three different crRNAs to achieve the best efficiency of FCVD (Fig. S5).

To further improve the performance of the established FCVD platform through addressing the potential challenges including competitive inhibition with the simultaneous amplification of targets [21], the key parameters including the concentration of dNTP (Fig. S6) and *Zst* polymerase (Fig. S7), and the specific primers (Fig. 2B) were first optimized, respectively. In addition, the effects of HPV to HIV primer ratios on the amplification efficiency of LAMP reaction were also investigated (Fig. 2C), which indicated that the optimal primer concentration was 0.5/0.5 of 10× primers. Under the optimal reaction conditions of 1.4 mM dNTPs, 0.48 U/mL *Zst* polymerase, both the HIV and HPV targets can be well amplified to trigger the CRISPR/Cas12a detection (Fig. 2D).

To further improve the clinical utility of the FCVD platform for point-of-care diagnostics, we established a simplified visual readout system, with lateral flow assay strip integrated as the detection module (Fig. 2E). After CRISPR/Cas12a reaction, the test strip was inserted into the reaction tube, enabling rapid visual determination within minutes (Fig. 2F). Thus, this FCVD platform provides an efficient and user-friendly approach for simultaneous detection of sexual viruses HPV and HIV, meeting the goals of the rapid, accurate, and contamination-free molecular diagnosis at clinical or field settings.

3.3. The simple colorimetric detection of FCVD platform

While the integration of CRISPR/Cas12a into the FCVD system enables the simultaneous detection of HPV and HIV, the multi-step workflow leads to increased complexity. To streamline the procedure, a contamination-free colorimetric LAMP assay was developed using the FCVD platform. In the conventional LAMP reaction, the colorimetric detection was typically limited due to the efficiency compromise of *Bst* polymerase when utilizing dUTP [22]. To address this challenge, the FCVD platform utilized the high efficiency of *Zst* polymerase with a homemade LAMP solution. The byproducts PPi^{4-} and H ions during the LAMP reaction could synergistically induce the color change of indicator EBT, enabling sensitive colorimetric detection (Fig. 3A).

Colorimetric LAMP assays based on *Zst* polymerase were performed under traditional dTTP conditions, with detection limits of 10 copies/μL for HPV-16 and 10^2 copies/μL for HIV-1, respectively (Fig. S8). The sensitivity was improved compared to previous methods using conventional *Bst* polymerase [23]. To minimize the risk of false positive from aerosol contamination, pure dUTP was used and its efficacy was evaluated in comparison with the commercially available *Bst*-2.0 polymerase for the detection of HPV-16. As shown in Fig. 3B, the detection limit of the reaction using *Zst* polymerase was 10-times better than that of using *Bst* 2.0, underscoring the sensitivity advantage of *Zst* polymerase. However, the sensitivity was significantly decreased under pure dUTP condition, which is consistent with previous study [22]. To achieve a balance between the contamination-free and high-sensitivity, the LAMP reaction using dTTP-to-dUTP ratios from 10:0 to 0:10 was investigated. The results showed that increasing dUTP ratio could reduce the detection sensitivity (Fig. 3C). However, at a 5:5 dTTP: dUTP ratio, both HPV and HIV reached the detection limit that using pure dTTP (Fig. 3D), which also exhibited excellent contamination-free ability (Fig. 1D). In addition, other sexually transmitted pathogens (e.g., *Neisseria gonorrhoeae* and *Chlamydia trachomatis*) have been detected using the developed platform. As shown in Fig. S9, our developed method owned excellent detection specificity. These results highlight the superiority of the FCVD platform, offering a rapid, accurate, simple, and contamination-free colorimetric detection approach.

3.4. The portable heating device and feasibility testing

Designed with a homemade portable heating device that supports nucleic acid lysis, LAMP amplification and CRISPR/Cas12a reactions, the FCVD platform could rapidly and accurately detect sexual viruses at home or in resource-limited settings (Fig. 4A). The colorimetric results can be directly recognized after the reaction was complicated, or coupled with CRISPR-based lateral flow assay. The device is powered by a standard mobile phone charger via a USB interface and equipped with Bluetooth connectivity for real-time monitoring of fluorescence curves using a smartphone, allowing precise tracking of the reaction process. Parallel experiments conducted using a conventional fluorescent PCR system showed comparable performance (Fig. S10), confirming the reliability of our device.

The internal structure of the device is illustrated in Fig. 4B, highlighting its core components, including the sensor plate, filter, filter mounting plate, reaction bearing seat, heating plate, excitation light mounting plate, excitation light plate, and lower shell mounting plate. As shown in Fig. 4C, the device integrates four functional modules: (1) an optical unit for high-sensitivity fluorescence sensing and signal amplification, (2) a precise temperature control module, (3) an energy-efficient power management unit, and (4) an IoT-enabled connectivity module for remote data transmission. The portable device costs under 300 dollars and reagent expenses below 3 dollars per test. We employed lyophilization for reagent preservation to facilitate field deployment, and the results confirmed the excellent stability of lyophilized reagents after 4 weeks of storage (Fig. S11). These features ensure robust performance and user-friendly operation in field settings, providing a comprehensive solution for on-site testing and enabling reliable detection of sexual co-infection of HPV and HIV in resource-limited environments.

3.5. Detection performance of the FCVD platform for clinical samples

To evaluate the clinical feasibility of the developed FCVD platform, we conducted a systematic evaluation with clinical samples under IRB Protocol number: KY2021–111-B-CR-01 (Fig. 5A). Samples were mixed with nucleic acid lysis buffer [24] and heated at 95 °C for 5 min. Subsequently, both colorimetric detection and lateral flow strip detection were performed, and the results were compared with those obtained by qPCR/RT-qPCR.

A total of 35 cervical swab samples were collected from Renji Hospital, Shanghai Jiao Tong University School of Medicine, including 4 co-infected (HPV-16 and HIV-1) samples, 9 HPV-16 positive samples, 11 samples positive for other HPV subtypes, 4 positive HIV-1 clinical samples and 15 negative samples. In addition, 12 spiked HIV-1 pseudovirus samples with graded concentrations (samples 13–24, viral titers ranging from 10^1 to 10^6 copies/μL) were prepared in-house. All these samples were detected using FCVD Platform. As shown in Fig. 5B - C, the colorimetric detection results were completely consistent with those of qPCR/RT-qPCR. Additionally, we performed specificity testing against multiple HPV subtypes, and the results demonstrated high specificity without cross-reactivity (Fig. S12). As shown in Fig. 5D, the detection of clinical samples on FCVD platform achieved a sensitivity of 100 %, specificity of 100 %, and accuracy of 100 % for HPV-16 and HIV-1 detection. The sexual co-infection samples of HPV and HIV were simulated by mixing HPV-16 and HIV-1 samples in sequence, as shown in Fig. 5E. In addition, to address the possibility of multiplex detection, we

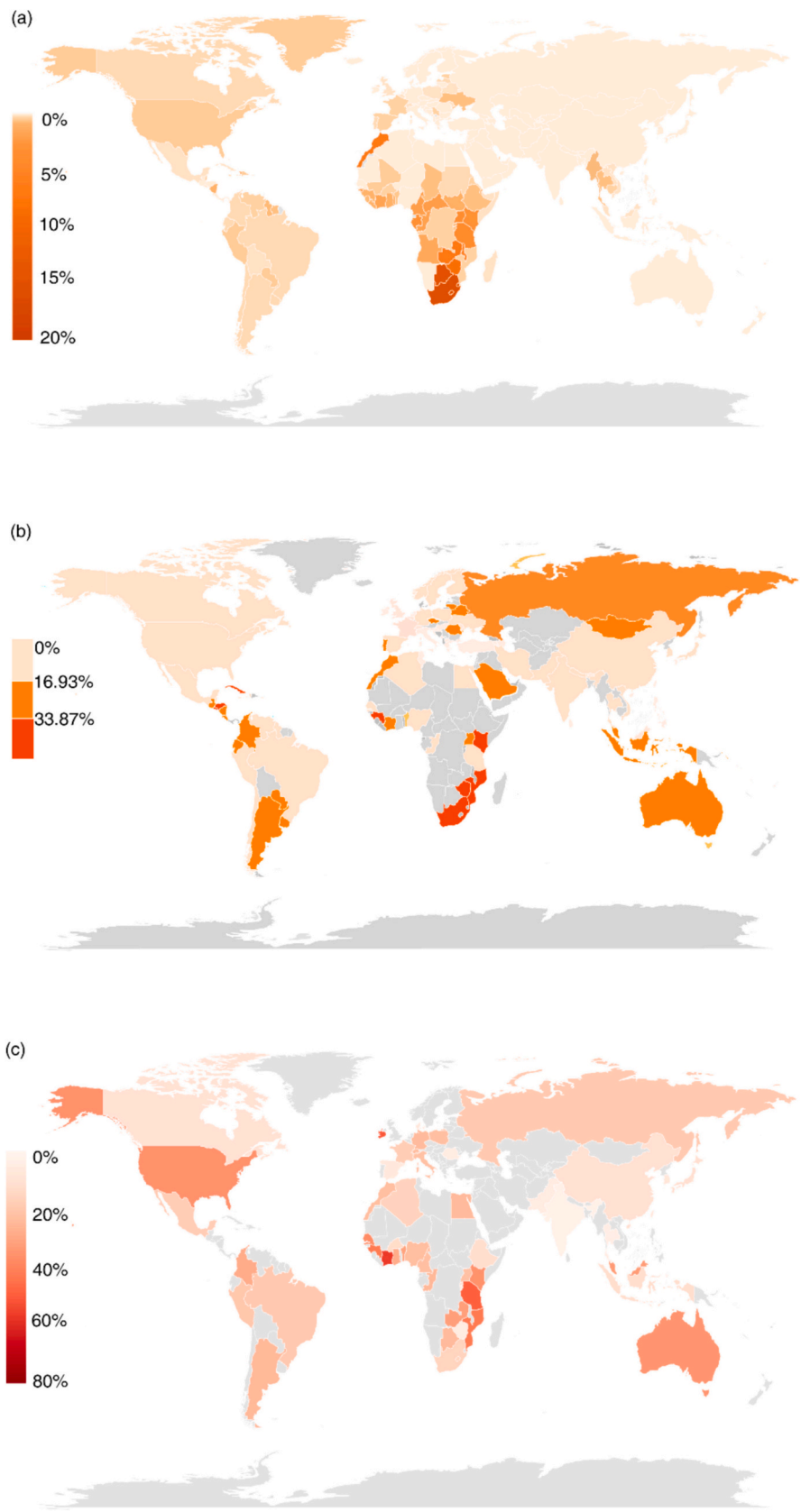

Fig. 6. Global epidemiological patterns of HIV, HPV and HIV-HPV co-infection prevalence. A. Global Geographic Distribution of HIV Infection Rates; B. Global Geographic Distribution of HPV Infection Rates; C. Global Geographic Distribution of HIV/HPV-16 Co-infection Rates.

performed the simultaneous detection of spiked HPV and HIV samples using a dual-test-line lateral flow strip following previous study [25]. As shown in Fig. S13, multiplex detection assay of HPV and HIV could be successfully realized using the developed method, which showed high consistency with those obtained by conventional qPCR. The LAMP amplification was performed in a multiplexed manner, both targets were co-amplified in a single tube. When HPV and HIV were co-existed, the assay consistently produced clear and accurate results, demonstrating the robustness of the multiplexed reaction ability. These above results were consistent with the expected outcomes, successfully detecting the co-infection. These data covering the clinical HPV and HIV samples demonstrate the excellent accuracy and robustness of the FCVD platform. The FCVD platform demonstrated excellent accuracy and robustness in detecting both HPV and HIV across all clinical specimens. Further validation with additional co-infection samples will be conducted in the future to reinforce these findings.

4. Future perspectives

The global epidemiological patterns of HIV, HPV, and their co-infections (Fig. 6, see Supplementary Materials for data collection methodology) emphasize the urgent need for decentralized diagnostic solutions with high sensitivity and specificity. The FCVD platform, with its unique integration of CRISPR-Cas12a specificity, isothermal amplification efficiency, and portable connectivity, represents a significant advancement in STI diagnostics. Its potential to reduce cervical cancer mortality in high-burden regions, such as Southern Africa where HIV prevalence exceeds 15 %, highlights its global significance. Future work will focus on expanding the platform's capabilities to detect additional STIs, thereby enhancing its versatility and diagnostic capacity. This expansion, coupled with targeted deployment in high-prevalence areas, aligns with the WHO's 2030 Sustainable Development Goals for STI management and underscores the platform's potential to transform global health outcomes.

5. Conclusions

In this study, we have developed a field-deployable contamination-free visual detection (FCVD) platform that enables rapid and accurate detection of sexual co-infection of HPV and HIV. The FCVD platform demonstrated high sensitivity, achieving detection limits of 10 copies/μL for HPV-16 and 10^2 copies/μL for HIV-1. With a home-made portable heating device, the point-of-care, rapid and connected detection of HPV and HIV from clinical samples were successfully performed, which is consisted well with the gold-standard PCR method. The FCVD platform provides a transformative solution for STD screening. Future work will focus on extending the application of the platform to other sexually transmitted pathogens and optimizing its usability for broader accessibility to further increase its potential to revolutionize diagnostics in various fields.

CRediT authorship contribution statement

Junyu Wang: Writing – original draft, Methodology, Formal analysis, Data curation. **Jun Feng:** Formal analysis, Data curation. **Hui Yin:** Resources, Methodology. **Mengxi Wang:** Methodology. **Xuetong Zhao:** Resources, Methodology. **Qinqin Hu:** Resources. **Liqin Cao:** Software, Resources. **Feibiao Pang:** Software, Methodology. **Jinzhao Song:** Software, Resources. **Jian Ma:** Visualization, Methodology. **Kun Yin:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2025.169967.

Data availability

Data will be made available on request.

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