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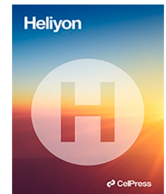

Highly sensitive and naked-eye detection of herpes simplex virus type 1 using LAMP- CRISPR/Cas12 diagnostic technology and gold nanoparticles

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ABSTRACT

Herpes simplex virus type 1 (HSV-1) Keratitis (HSK) is a highly prevalent eye disease worldwide, characterized by lifelong recurrent episodes and a major risk of leading to blindness. Detecting HSV-1 promptly and accurately can initiate a timely and appropriate therapeutic regimen, minimizing tissue damage and preventing vision impairment. Currently, PCR is the most reliable method for identifying HSV-1, but its utilization for point-of-care (POC) HSV-1 detection is limited due to the need for sophisticated equipment, particularly in areas with limited resources. Here, we propose a new method for on-site HSV detection by using LAMP-Cas12 diagnostic technology and gold nanoparticles. This technique possesses comparable sensitivity to qPCR, and its detection results could be easily read and interpreted without the need for complex equipment. In detecting HSV in clinical tear specimens, this strategy achieved a 93.9 % consistency in positive detection and a 100 % consistency in negative detection compared to qPCR. Our strategy innovates the technique of current HSV-1 detections and is expected to play a crucial role in POC diagnosis of HSK in the future.

1. Introduction

Herpes simplex keratitis (HSK), caused by herpes simplex virus type 1 (HSV-1) infection in the cornea, is one of the most common and blindness-leading eye diseases worldwide [1,2]. It was estimated in 2016 that there were about 1.7 million people suffered from HSK worldwide, among whom 230,000 people may have newly acquired uniocular vision impairment annually [3]. HSK typically begins with a subclinical HSV infection in the cornea and thereafter the virus establishes a latency in the trigeminal ganglia, or possibly other sites. Recurrent HSK occurs when the virus is reactivated from latency, leading to an inflammatory reaction and destruction of the corneal tissues [4,5].

Accurate and rapid diagnosis of HSK is essential to start the proper treatment for preventing tissue destruction, corneal scarring, and opacity. The clinical diagnosis of HSK mainly relies on physicians' observation, personal experience and logical analysis to identify lesion characteristics. Misdiagnosis in clinics is common because other non-HSV pathogens may present with similar symptoms and

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signs, making the discrimination difficult [6]. Laboratory tests for HSV detection include virus culture, enzyme-linked immunosorbent assay, immunofluorescent antibody, and polymerase chain reaction (PCR) [7–14]. However, viral culture is time-consuming and needs a week or longer time for successful culture. Immunological assays for detecting antigens or antibodies are commonly less sensitive and specific. Currently, qPCR is the most commonly used technique for detecting HSV due to its high sensitivity and specificity in identifying HSV DNA in tears and tissues [15]. However, qPCR assay requires advanced equipment and skilled technicians, which means clinical specimens need to be sent to a specialized laboratory. This delay in presenting diagnosis results to clinics hinders timely and precise treatment. The limitation of qPCR significantly impedes its broader use on HSK diagnosis in areas with limited resources. Therefore, it is necessary to create accessible HSV detection methods that have similar sensitivity to qPCR and can be applied in areas with limited resources.

The recently discovered clustered regularly interspaced short palindromic repeats (CRISPR)-Cas (CRISPR-associated proteins) system, which is an adaptive immune system that exists in most bacteria and all archaea, provides a new technology for nucleic acid detection [16–18]. Among currently discovered CRISPR/Cas systems, CRISPR/Cas12 system attracts much attention because Cas12 protein can recognize and cleave target double-stranded DNA under the guidance of crRNA, and its non-specific enzyme activity could be triggered to cleave nearby single-stranded DNA [19]. The CRISPR/Cas12 system is most effective when combined with an isothermal amplification method, due to the Cas12 proteins can recognize and cleave specific sequences without the need for temperature cycling. This combination could maximize convenience and increases assay sensitivity. The most well-known technique is DETECTR [20], which combined recombinase polymerase amplification (RPA) and Cas12a to create a CRISPR-based diagnostic tool for the targeted identification of DNA sequences. This method achieved attomolar sensitivity without the need for a complex instrument.

In this report, we developed a method called CRISPR/Cas12 powered colorimetric assay for sensitive detection of HSV-1 DNA in clinical specimen by using loop-mediated isothermal amplification (LAMP)- Cas12 diagnostic technology and gold nanoparticles. The LAMP reaction was used to amplify HSV-1 DNA extracted from clinical tear specimens, followed by CRISPR/Cas12-based amplicon recognition and cleavage of linker ssDNA. The results could be directly read by the naked eye using AuNP probes as the signal output. The assay's accuracy was confirmed by blindly testing 90 tear specimens from ocular surface patients, and the results showed that this assay could achieve 93.9 % detection sensitivity and 100 % detection specificity when compared to the qPCR test. Observation of the correlation between the test results and the development of clinical assumptive HSK in real patients undergoing antiviral drugs or topical steroid regimens demonstrated the potential applicability of the CRISPR/Cas12 powered colorimetric assay for point-of-care HSK diagnosis.

2. Materials and methods

2.1. *Collection of ocular surface tear specimens from the clinical assumptive HSK patients*

The tear specimens were collected from the clinical assumptive HSK patients using Schirmer test papers (tear production measuring strips; Meizilin Drug Co, Liaoning, China) without topical anesthesia and mydriasis. The test papers of 10 mm in length were placed in the lower conjunctival fornix of the eye. Then, the test paper was removed until it was soaked with tear. The collected specimens were transported immediately to a sterile tube and stored at −80 °C.

2.2. *Synthesis of 20 nm AuNP*

20 nm AuNP was synthesized using the sodium citrate reduction method as previously reported [21,22]. In brief, 100 mL of 1 mM $HAuCl_4$ solution was added into a glass flask and heated to boiling. Then, the solution was stirred and 8 mL of 38.8 mM sodium citrate solution was quickly added to the solution. Keep stirring and heating for 20 min. Finally, the solution was cooled down to room temperature with stirring. The prepared AuNP solution was stored at 4 °C in the dark.

2.3. *Construction of AuNP-DNA probes using the freeze-thaw method*

AuNP-DNA probes were constructed according to the previously reported freeze-thaw method [23]. Firstly, 1 mL of AuNPs solution was mixed with 100 μL of 100 μM DNA probes. Next, the mixture was frozen at −20 °C for 1 h and thawed at room temperature. Finally, the particles were washed three times with 0.01 M phosphate buffer with 0.1 M NaCl through centrifugation (10,000×*g*) for 20 min. The resulting conjugates were resuspended in 0.01 M phosphate buffer with 0.3 M NaCl and stored at 4 °C in the dark.

2.4. *DNA extraction and qPCR assay*

DNA extraction was performed using PureLink Viral RNA/DNA Mini Kit (Thermo Fish Scientific Inc, America) according to the manufacturer's protocol. A total of 20 μL of the qPCR reaction mixture was prepared with TB Green® Fast qPCR Mix (Takara Bio Inc, Dalian). The final concentration of forward and reverse primers was 200 nM. The qPCR thermal cycling program is as follows: 95 °C for 10 s, 58 °C for 40 s with the reaction cycle of 45. Fluorescence signals were recorded by a Roche LightCycler480.

2.5. Loop-mediated isothermal amplification assay

LAMP reaction was performed in 20 μL volume consisting of 2 μL target DNA, 6.4 U Bst 2.0 DNA polymerase, 1 mM/μL dNTP mixture, 1x isothermal reaction buffer (1.5 M Tris-HCl, 2.5 M KCl, 1 M $(NH_4)_2SO_4$, 1 M $MgSO_4$, and 20 % Tween 20), 2 μM FIP and BIP, 0.25 μM FOP and BOP, 1 μM LF and LB. The LAMP reaction mixture was incubated at 65 °C for 40 min.

2.6. Cas12/crRNA cleavage assay

Cas12/crRNA cleavage reaction was performed in 20 μL volume with 400 nM crRNA, 80 nM Cas12a protein, 1x NEBuffer 3.1 (10 mM NaCl, 5 mM Tris-HCl, 1 mM $MgCl_2$, 10 μg/mL), 400 nM fluorescence probes and 2 μL LAMP product. The reaction mixture was incubated at 37 °C for 30 min. The fluorescence single was monitored at 1 min intervals.

2.7. Colorimetric detection assay using CRISPR/Cas12a cleavage and AuNP probes

A 10 μL reaction system containing 2 μL LAMP product, 80 nM Cas12a, 400 nM crRNA, 1x NEBuffer 3.1, and 1 μM linker ssDNA substrate was incubated in a metal bath for 10 min at 37 °C. Then 3 μL of the above mixture was added to the solution containing 25 μL of 10 nM AuNP-DNA probe1 and 25 μL of 10 nM AuNP-DNA probe2. After incubation at room temperature for 5 min, the solution was centrifuged in a tabletop mini centrifuge (DLAB Scientific Co., Ltd.) at 7000 rpm for 1 min. The supernatant was used for colorimetric detection or absorption spectra analysis.

3. Results

3.1. Schematic overview of the Cas12-powered colorimetric assay

In the current study, we developed an HSV-1 DNA detection technique to assist the clinical diagnosis of HSK, by making full use of the *trans*-cleavage ability of CRISPR/Cas12a. Fig. 1a showed the scheme of CRISPR/Cas12-powered colorimetric assay for HSV-1 DNA detection. DNA samples extracted from ocular surface tear specimens were firstly pre-amplified by LAMP reaction. Then, Cas12a/crRNA identified the DNA amplicons, and its *trans*-cleavage ability could be activated to degrade special linker ssDNA probes. The linker ssDNA probe was designed to hybridize with AuNP probes to induce its cross-linking. In the absence of HSV-1 DNA, Cas12a/

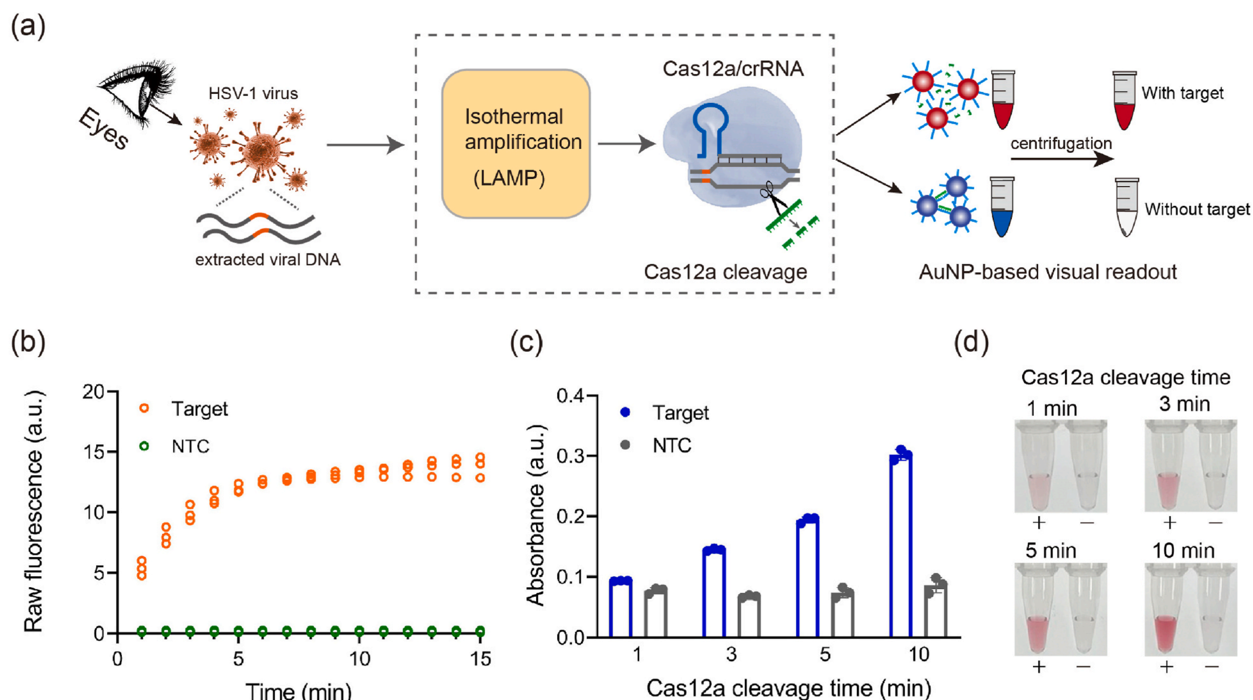

Fig. 1. CRISPR/Cas12 powered colorimetric assay for detection of HSV-1. (a) Schematic diagram of the workflow of CRISPR/Cas12 powered colorimetric assay for detection of HSV-1. (b) Real-time monitoring the fluorescence signal of LAMP amplification followed by Cas12a/crRNA cleavage with or without target HSV-1 DNA. (c) Absorbance analysis of Cas12-powered colorimetric assay in the present or absent of target HSV-1 DNA at different Cas12a cleavage time. (d) photographs of Cas12-powered colorimetric assay in the present (+) or absent (−) of target HSV-1 DNA at different Cas12a cleavage time.

crRNA would not be activated, the linker ssDNA remained intact and the AuNP probes would be cross-linked. When Cas12a/crRNA recognizes DNA amplicons, it would cleave linker ssDNA and the AuNP-probes were non-crosslinked. Visualizable detection was performed after quick low-speed centrifugation of cross-linked and dispersed AuNP-probes.

3.2. Evaluation of detection feasibility of Cas12-powered colorimetric assay

In this assay, we chosen the glycoprotein B gene (GB) of HSV-1 as the target gene since its sequence is extremely conserved [24]. The crRNA was designed to recognize the corresponding LAMP amplicons and the *trans*-cleavage efficiency of Cas12a/crRNA was firstly evaluated. As shown in Fig. S1, the fluorescence increased rapidly with time in the presence of LAMP amplicons while no fluorescence changed in the absence of LAMP amplicons, suggesting the high cleavage efficiency of Cas12a/crRNA. When the concentration of crRNA and Cas12a protein was 400 nM and 80 nM, respectively, the fastest fluorescence increase could be observed. Therefore, 400 nM crRNA and 80 nM Cas12a protein were chosen as the optimized reaction condition. Subsequently, we verified the feasibility of an AuNP-based visual reaction system and optimized the concentration of linker ssDNA. It could be seen that the color of AuNP quickly changed from red to purple in the presence of linker ssDNA (Fig. S2b). After quick low-speed centrifugation, black-purple AuNP sediment stayed at the bottom of the tube, leaving a clear liquid supernatant. While the liquid supernatant remained red in the absence of linker ssDNA. This result means that the linker ssDNA could successfully hybridize with AuNP-DNA probes, and the color change of AuNP could represent the presence of linker ssDNA. We also optimized the concentration of linker ssDNA and the result showed that 50 nM linker ssDNA was sufficient to induce complete aggregation of 10 nM AuNP probes (Fig. S2c).

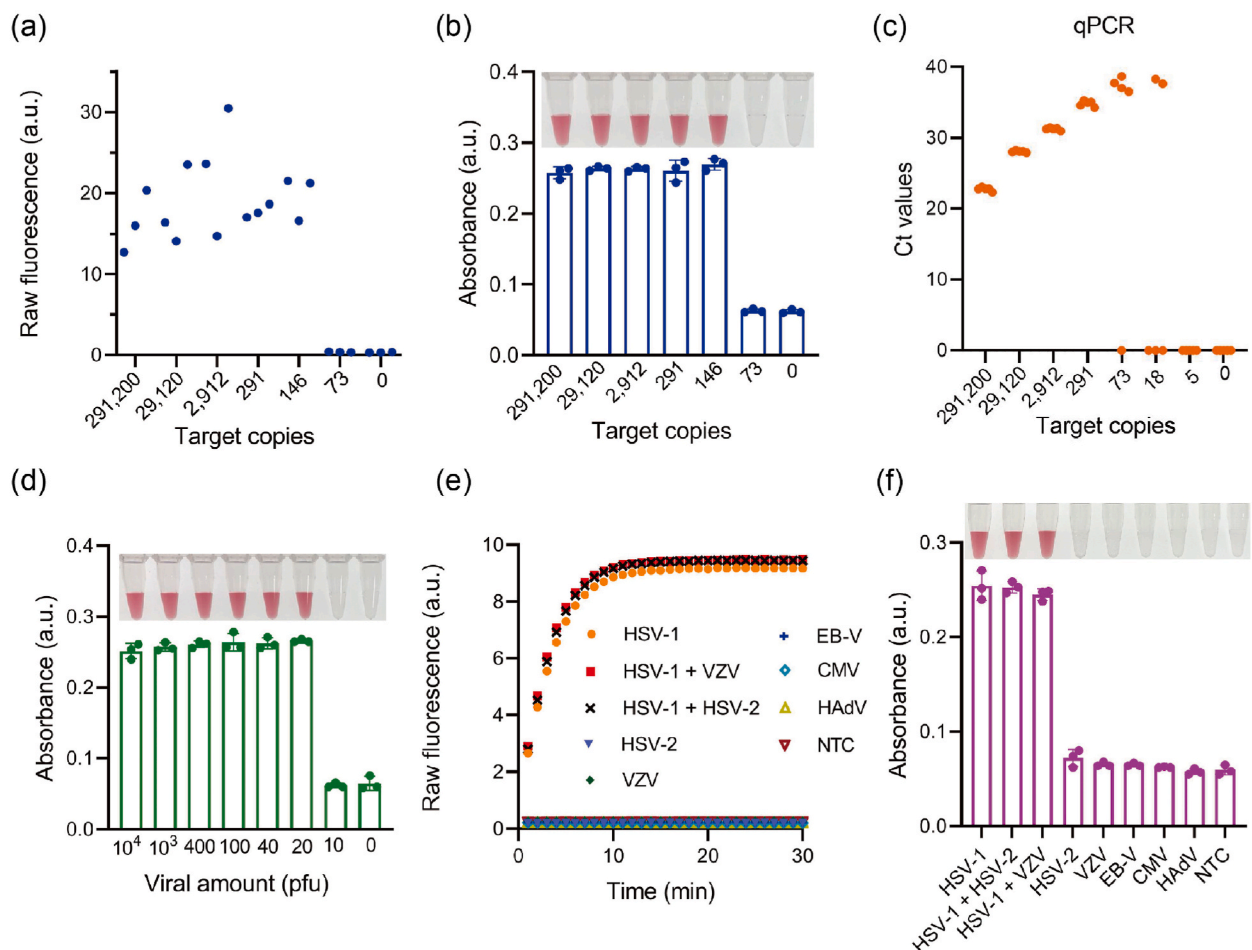

Fig. 2. Evaluation of the detection sensitivity and specificity of CRISPR/Cas12 powered colorimetric assay. (a–b) Sensitivity analysis of CRISPR/Cas12 powered colorimetric assay using various amount of synthetic plasmid containing GB gene of HSV-1. 146 copies of synthetic DNA plasmid could be reliably detected by fluorescence analysis, naked-eye observation, and absorption analysis. (c) sensitivity analysis of qPCR assay for detecting synthetic plasmid containing GB gene of HSV-1. (d) Sensitivity analysis of CRISPR/Cas12 powered colorimetric assay using various amount of DNA extracted from cultured HSV-1. (e–f) Specificity evaluation of CRISPR/Cas12 powered colorimetric assay by fluorescence analysis, naked-eye observation, and absorption analysis using extracted DNA from cultured HSV-1, HSV-2, CMV, EB-V and HAdV. Error bars represent mean ± standard error of the mean (SEM), with n = 3 technical replicates.

After establishing an optimal Cas12a/crRNA cleavage reaction condition and AuNP-based visual detection system, the feasibility of the CRISPR-Cas12 powered colorimetric assay was evaluated by analyzing DNA extracted from cultured HSV-1. We firstly monitored the process of LAMP reaction and Cas12/crRNA cleavage using fluorescence. It was found that the fluorescence signal increased with time in the presence of HSV-1 DNA, while the control without HSV-1 DNA had no increased fluorescence signals (Fig. 1b). Then, the naked-eye detection system that integrates LAMP reaction, Cas12/crRNA cleavage, and AuNP-based readout together was tested. As shown in Fig. 1c,d, the color signal was increased with the cleavage reaction time of Cas12a/crRNA in the presence of HSV-1 DNA, while the controls without HSV-1 DNA did not produce any color signals. We also found the max color difference could be obtained when the cleavage time reached 10 min, indicating that 10 min is sufficient for Cas12a/crRNA cleavage. These results confirmed the Cas12-powered colorimetric assay could be successfully applied for naked-eye HSV-1 detection, generating an easy-to-interpret qualitative readout for the presence or absence of the HSV-1 DNA.

3.3. Evaluation of detection sensitivity and specificity of Cas12-powered colorimetric assay

Generally, the amount of target virus is very low in clinical samples and the collected samples always contain various other nucleic acids like genes of other pathogens or human genes. Therefore, before applying the Cas12-powered colorimetric assay for clinical

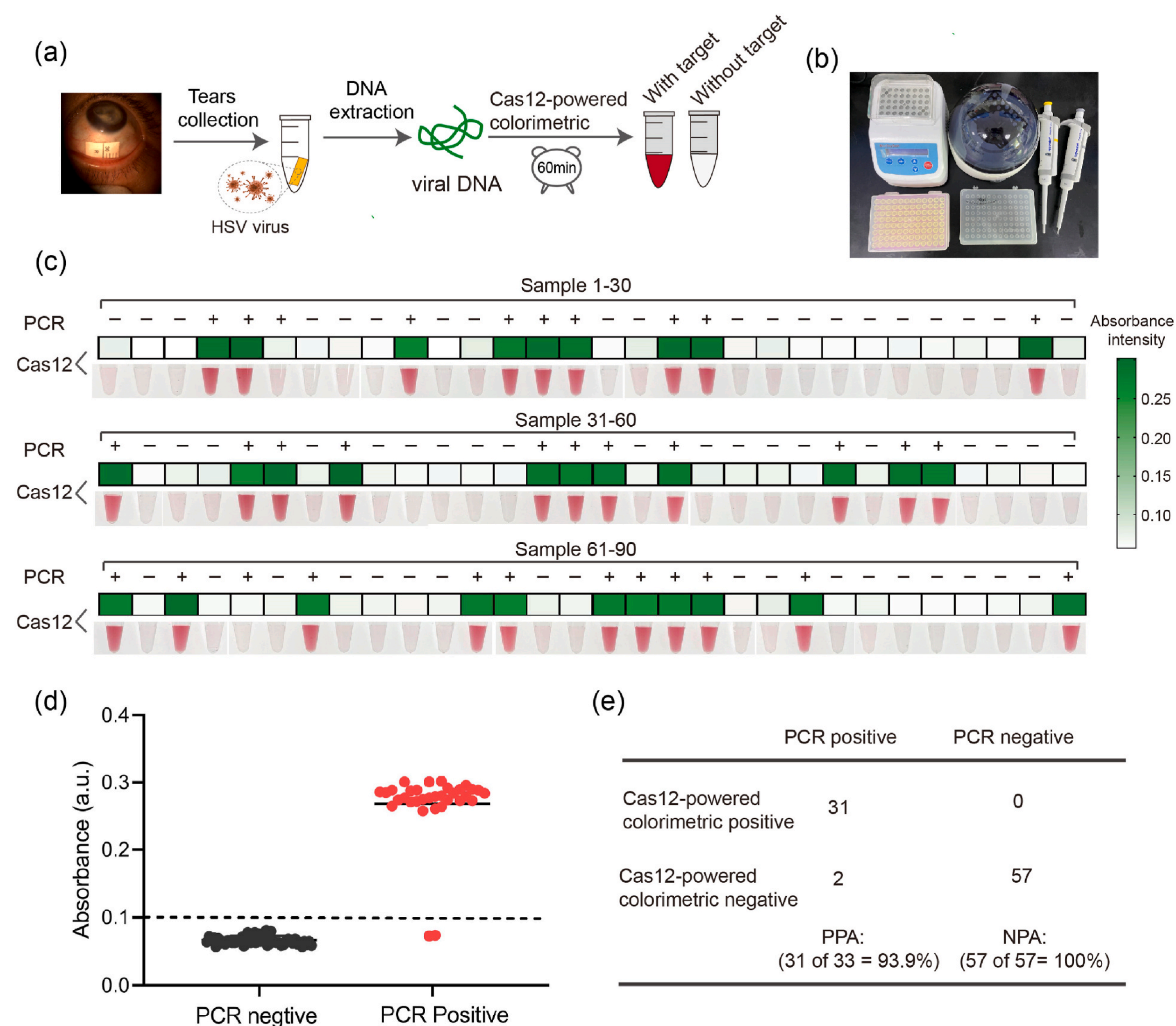

Fig. 3. Application of CRISPR/Cas12-powered colorimetric assay in clinical detection. (a) Workflow of detecting HSV-1 in clinical tear specimens for on-site diagnosis of HSK with CRISPR/Cas12 powered colorimetric assay. (b) Equipment needed for the CRISPR/Cas12 powered colorimetric assay. (c) Testing 90 of tear specimens from the clinically suspected HSK patients using CRISPR/Cas12 powered colorimetric assay and qPCR assay. (d) Scatter plot of the absorbance values and detection results of 90 clinical tear specimens detected by CRISPR/Cas12 powered colorimetric assay and qPCR assay, respectively. (e) Performance characteristics of CRISPR/Cas12 powered colorimetric assay.

detection, we firstly evaluated its analytical sensitivity and specificity. We tested the detection sensitivity using synthetic DNA plasmid containing the GB gene of HSV-1 and extracted DNA from cultured HSV-1 virus, respectively. As shown in Fig. 2a and 2b, 146 copies (namely 7.3 copies/μL) of synthetic DNA plasmid could be reliably detected by fluorescence analysis, naked-eye observation, and absorption analysis. We further utilized the qPCR method for comparison and the result showed the detection limit was 73 copies (namely 3.65 copies/μL) (Fig. 2c), which is comparable to previous reports [15]. This result showed that our Cas12-powered colorimetric assay possesses comparable sensitivity to the qPCR method. Next, we also analyzed the detection sensitivity of our assay by using extracted DNA from cultured HSV-1, and the result showed that our assay could reach the detection limit of 20 pfu by naked-eye observation, and absorption analysis (Fig. 2d). Furthermore, we tested the detection specificity of Cas12-powered colorimetric assay by analyzing highly homologous HSV-2 and other viruses that could infect the human body including VZV, CMV, HAdV, and EBV. As shown in Fig. 2e and f, there was a fast fluorescence increase with target HSV-1, while HSV-2 and other viruses could not induce any fluorescence increase, indicating that our assay possesses high specificity. Besides, when adding HSV-2 and other viruses' DNA to target HSV-1 DNA, the fluorescence increase was not changed, suggesting that the detection efficiency of this Cas12-powered colorimetric assay could not be disturbed by other DNA.

3.4. Application of Cas12-powered colorimetric assay in clinical HSV-1 detection

Subsequently, we utilized this assay to detect HSV-1 in ocular surface tear samples of clinically suspected HSK patients. The diagnosis process was shown in Fig. 3a. The ocular surface tear of the patients was collected by putting a Schirmer test paper of in 10 mm length on the lower conjunctival-fornix surface of the eye. After the paper was soaked with tears, the DNA of the virus was extracted from collected tears and detected using a Cas12-powered colorimetric assay. Fig. 3b showed the apparatus used in this assay, including a mini dry bath and a miniature centrifuge, both of which are very easily accessible devices. Then, we blindly tested 90 clinical ocular surface tear samples by using Cas12-powered colorimetric assay. These 90 ocular surface tear samples were from 70 patients, all of whom were clinically diagnosed as HSK according to the typical clinical manifestations and disease history. Among them, 16 samples from 10 patients were clinically diagnosed as quiescent HSK, and 74 samples from 60 patients were clinically diagnosed as active HSK. As shown in Fig. 3c, the detection results could be identified by the naked eye and there were 31 samples from 24 patients showed HSV-1 positive and 59 samples showed HSV-1 negative. To verify the detection accuracy of this assay, we simultaneously tested the same 90 samples by the qPCR, and the results showed that 33 samples from 26 patients were HSV-1 positive and 57 samples were HSV-1 negative. In comparison with the qPCR assay, the positive agreement and negative agreement of Cas12-powered colorimetric assay were calculated to be 93.9 % and 100 % (Fig. 3d and e), respectively. The consistency in the results indicated that our assay possesses high accuracy for testing clinical specimens. It was worth pointing out that the patient diagnosed with HSV-1 infection tested by Cas12-powered colorimetric assay at the first clinical visit with active manifestation of HSK could be gradually subsided till to complete cure with antiviral or plus topical steroid regimen, and HSV-1 DNA could not be detected anymore when the cornea became scarring (Table 1). These results showed that the application of Cas12-powered colorimetric assay in HSK patients may not only help with the pathogenic diagnosis of HSK but also may guide clinical treatment.

4. Discussion

HSK is a globally spreading eye disease that leads to lifelong recurring blindness with a high incidence rate. HSV infection causes inflammation and tissue damage in the cornea, resulting in HSK [1]. Research has shown that HSK may begin with a subclinical HSV infection of the cornea, followed by the establishment of a latent infection primarily in the trigeminal ganglia, as well as in the sensory ganglia and the cornea. Recurrent infections may happen when the HSV is reactivated from latency and travels to the cornea, causing an inflammatory response and damaging the corneal tissues [4]. It is undeniable that prompt and accurate identification of HSK was essential in initiating an efficient course of treatment, reducing harm to tissues, and averting vision impairment. While clinical diagnosis is mainly based on physicians' observations, it can result in a significant misdiagnosis rate due to the resemblance of clinical symptoms across various illnesses and differences in doctors' expertise, understanding, and logical deduction capabilities.

In clinic, detecting the presence of HSV in tissue is a more precise diagnostic method for HSK. Among the currently developed

Table 1
Symptom, dosage regimen and HSV-1 detection results of clinically suspected HSK patients.

Time	Clinical symptom	Medication	QPCR	Cas12-powered colorimetric
First visit	epithelial ulcer, epithelial defects with terminal bulbs, dense cellular infiltration	Valaciclovir, Ofloxacin eye ointment	Ct value: 24.08 HSV-1 positive	HSV-1 positive
Second visit after 7 days	epithelial healing, corneal scar, cellular infiltration, hyperemia	Valaciclovir, Ofloxacin eye ointment, 0.02 % Fluorometholon eye drops	Ct value: 34.18 HSV-1 positive	HSV-1 positive
Third visit after 27 days	Epithelial intact, corneal scar, without hyperemia	Acyclovir, 0.02 % Fluorometholon eye drops, Ofloxacin eye ointment	HSV-1 negative	HSV-1 negative

methods for detecting HSV, PCR is the most accurate technique due to its ability to directly detect HSV DNA with high sensitivity and specificity. Research has shown that PCR technology has much higher sensitivity and specificity than immunofluorescence and virus isolation in detecting HSV in clinical corneal tissues and tear specimens [12]. However, the use of PCR-based assays is greatly hindered by the need for expensive laboratory equipment such as a thermocycler and a fluorescence reader, making it difficult to implement in resource-limited areas with inadequate laboratory infrastructure. Researchers have attempted to use LAMP method for HSV-1 detection [25,26], and it achieved comparable detection sensitivity to that of PCR with constant temperature conditions. However, the previous method utilized fluorescent dyes and electrophoresis to identify amplicons, which is time-consuming and needs complicated operation. Hence, it is necessary to create a novel approach for detecting HSV, which is user-friendly, has a straightforward output, is as accurate as PCR, and specifically suitable for medical facilities without sophisticated equipment.

Recently discovered clustered regularly interspaced short palindromic repeats (CRISPR-associated (Cas12) systems have provided a cheering tool for nucleic acid diagnosis [17], which may help solve the issue of detection requirements for point-of-care diagnosis of HSV. It was reported that the CRISPR-Cas12 system could recognize the double-stranded DNA (dsDNA) with protospacer adjacent motif (PAM)-dependent mode. Once the recognition process occurs, Cas12a/crRNA activates its *trans*-cleavage ability to nonspecifically cleave nearby single-stranded DNA probes under the isothermal condition with a high turnover rate, where one activated Cas12a could induce thousands of non-specific ssDNA degradation [20]. Based on this unique cleavage ability of CRISPR/Cas12, a variety of rapid pathogen detection methods have been developed in recent years, and successfully applied to detect Dengue virus (DENV), Zika virus (ZIKV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), African swine fever virus., and others [27–30]. By integrating nanoparticles or lateral flow strips as signal output, CRISPR/Cas12-based detection methods exhibited superior detection ability on sensitivity, specificity, simplicity, cost, and speed, which is suitable for POC diagnosis in resource-limited areas.

Therefore, we use the CRISPR/Cas12 technique and gold nanoparticles to create a new method that can accurately detect HSV, thereby enhancing the diagnostic abilities for HSK. In this strategy, we use LAMP and CRISPR/Cas12 to ensure high detection sensitivity and specificity. Our results have shown that this strategy can distinguish between HSV-1 and other viruses such as HSV-2, VZV, CMV, HAdV, and EBV. Additionally, the detection sensitivity is comparable to qPCR, demonstrating the excellent specificity and sensitivity of our strategy. Furthermore, we utilize gold nanoparticles to enable naked-eye visual diagnosis. The presence of HSV-1 is indicated by a clearly visible red color in our assay, so that the detection results can be easily interpreted without the need for signal-output equipment. The entire reaction can be conducted at a constant temperature, so our assay does not require any complicated thermocycling instruments. Additionally, the detection results can be obtained in less than an hour, which is faster than qPCR and considerably quicker than other methods such as virus culture and ELISA [31].

It is noteworthy that the LAMP-Cas12 technique is utilized for the first time to detect HSV and to be applied to diagnose HSK in clinical tear samples. The results showed that this strategy achieved 93.9 % consistency in positive detection and a 100 % consistency in negative detection compared to qPCR. Significantly, this study found correlations between the test results and the development of clinical assumptive HSK in real patients undergoing antiviral drugs or topical steroid regimens. It means the application of Cas12-powered colorimetric assay in HSK patients may not only help with the pathogenic diagnosis of HSK but also may guide clinical treatment.

5. Conclusion

In summary, our developed novel method for detecting HSV has high sensitivity and excellent specificity, does not rely on complex instruments, and is less time-consuming. It is more suitable for point-of-care HSV detection than current methods such as qPCR, ELISA, virus culture, and others. This assay will serve as an important test to supplement clinical diagnosis of HSK and provides a research tool for further studies on HSV infection. The website at hand allows for the convenient design of LAMP primers and crRNA, making it possible to quickly adapt this test for diagnosing other pathogens associated with eye disease. Currently, our laboratory is investigating this possibility. We believe that the Cas12-powered colorimetric assay will have a significant impact on the clinical diagnosis of HSK and other pathogen-associated eye diseases, thereby enhancing the diagnostic capabilities for infectious keratitis in the future.

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Ethical approval

Ethical approval for this study was obtained from the Institutional Ethics Committee at Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Mengqi Huang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Yihong Chen:** Data curation, Methodology. **Libin Zheng:** Data curation, Methodology. **Yu-Feng Yao:** Conceptualization, Funding acquisition, 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22146.

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