CRISPR-Based Diagnostic Method For Chlamydia Trachomatis in Patients With Non-Gonococcal Urethritis

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Research Article

Keywords: CRISPR-Cas13a, Chlamydia trachomatis, Nucleic acid detection, On-site detection

Posted Date: February 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-201659/v1>

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Abstract

Non-gonococcal urethritis (NGU) is a type of urethritis that is transmitted by sexual contact. About 20%–50% of NGU cases are caused by *Chlamydia trachomatis*. Most NGU cases are asymptomatic, but failure to intervene promptly can lead to severe sequelae of the reproductive system and other complications. Here, we aimed to develop a technique to routinely screen for *C. trachomatis* infections to prevent such adverse consequences. The proposed approach involves the use of clustered regularly interspaced short palindromic repeats (CRISPR) RNA (crRNA) that is complementary to the cryptic plasmid fragments of *C. trachomatis* (*i.e.*, the target sequence). In the presence of the target sequence, the RNase activity of the Cas13a protein is activated, and it cleaves a quenched RNA fluorescent probe such that fluorescence will be emitted. The proposed molecular diagnostic method based on the CRISPR-Cas13a technology leverages the high DNA-amplification efficiency of recombinase polymerase amplification (RPA) and the high specificity of crRNA. The sensitivity of the *C. trachomatis*-dsDNA detection based on CRISPR-Cas13a was 10 fM. A one-pot method based on CRISPR-Cas13a detection and qPCR was also developed. The results obtained by the two methods were closely correlated: $\chi^2 = 81.798$ based on a Chi-squared test ($P < 0.001$), $\kappa = 0.975$ based on a Cohen's kappa test ($P < 0.001$), and the area under the receiver operating characteristic curve area was 0.991 (95% confidence interval: 0.970–1.000 $P < 0.001$) with a sensitivity of 0.982 (95% confidence interval: 0.890–0.999) and specificity of 1.000 (95% confidence interval: 0.863–1.000). Further, the method can be carried out at room temperature and yields results within 1 hour. The developed technique does not require expensive instruments and, thus, can meet the needs of community hospitals and other institutions for screening. Future research to develop lyophilized reagents could enable the application of this technique to point-of-care testing.

Highlights

- CRISPR-Cas13a can be used for *in vitro* nucleic acid-based detection of bacteria.
- The high specificity of the CRISPR-Cas13a system depends on the design of the crRNA, which is easier than the design of Taqman probes for PCR.
- The method based on CRISPR-Cas13a can be carried out at room temperature.

Introduction

Non-gonococcal urethritis (NGU) is a type of urethritis that is transmitted by sexual contact. About 20%–50% of NGU cases are caused by *Chlamydia trachomatis*¹. *C. trachomatis* is classified into 19 serovars, of which serovars D through K are associated with genitourinary tract infections^{2,3}. According to the World Health Organization, there are 127 million new cases of *C. trachomatis* worldwide each year⁴. About 50% of men and 75% of women are asymptomatic after infection, and failure to intervene promptly can result in severe sequelae of the reproductive system and other complications such as ectopic pregnancy, pelvic inflammation, infertility, premature delivery, and abortion^{1,5–9}. The infection can also pass from a pregnant woman to her fetus, leading to neonatal pneumonia and conjunctivitis¹⁰.

Therefore, it is necessary to develop a detection method that is suitable for routine screening for *C. trachomatis* infections to prevent such adverse consequences.

Cell culture can be used to detect the pathogen, but it is rarely used today as it requires a time-consuming and labor-intensive process¹¹. Serological diagnosis is not recommended for screening because it has poor sensitivity and can return false negatives during the window period after infection when the specific antigen or antibody cannot be detected¹¹. Most commercial one-pot rapid detection kits for *C. trachomatis* also have low sensitivity, which can easily lead to misdiagnosis¹². The current gold standard for diagnosing *C. trachomatis* infections is nucleic acid detection, which is highly sensitive and specific but may take several hours, requires complex instruments, and can only be carried out by professionally trained technicians^{13,14}. However, there are many novel methods developed to replace traditional analysis of nucleic acid of DNA and RNA¹⁵⁻¹⁷. The clustered regularly interspaced short palindromic repeats (CRISPR) system has become the most commonly used gene-editing tool in recent years¹⁸. The CRISPR-Cas system can further expand its application scope by modifying the Cas protein¹⁹. Previous studies have shown that the CRISPR-Cas13a system can be used for in vitro detection of DNA and RNA²⁰⁻²³ by combining it with recombinase polymerase amplification (RPA) to amplify target dsDNA in the sample or reverse transcription amplification to identify RNA in the sample^{24,25}. The obtained target dsDNA is then transcribed into ssRNA. The complementary pairing of the ssRNA to a designed crRNA activates the RNA-cleavage activity of Cas13a^{21,23} to cleave a quenched RNA fluorescent probe, thereby releasing a measurable signal (Figure 1)^{26,27}.

In this study, we proposed and evaluated a method for detecting *C. trachomatis* based on the use of the CRISPR-Cas13a system to target the constant region in the cryptic plasmid of *C. trachomatis*. This method is simple, rapid, sensitive, and inexpensive, making it a suitable screening method to detect *C. trachomatis*, which can potentially prevent chronic infection and the associated complications and improve clinical outcomes.

Materials And Methods

pC013-Twinstrep-SUMO-huLwCas13a was purchased from Zoman Biotechnology Co., Ltd (Beijing, China). Rosetta2 (DE3) pLysS competent cells were purchased from Tolo Biotech Co., Ltd (Shanghai, China). HiScribe T7 Quick was purchased from New England BioLabs Inc. (Ipswich, MA, USA). RPA DNA amplification kit was purchased from TwistAmp (United Kingdom). Ni Sepharose High Performance was purchased from General Electric company (Boston, MA, USA). TB Green® Premix Ex Taq™ was purchased from Takara Biomedical Technology Co., Ltd (Beijing, China). Small ubiquitin-like modifier (SUMO) Protease was purchased from Solarbio (Beijing, China). RNase inhibitor and Isopropyl-beta-D-thiogalactopyranoside (IPTG) were purchased from yuanye Bio-Technology Co., Ltd (Shanghai, China). RNA fluorescent probe was synthesized by Bio-lifesci (Guangzhou, China). Between January 2019 and August 2019, cervical swab samples and throat swab samples were randomly collected from the Zhuhai

Center for Maternal and Child Health Care. All samples were stored at -80°C . The study was approved by the Ethics Committee of the Zhuhai Center for Maternal and Child Health Care.

Expression and purification of LwCas13a protein

Rosetta2 (DE3) pLysS competent cells were thawed in ice water. 50 ng of pC013-Twinstrep-SUMO-huLwCas3a plasmid was added to a centrifuge tube containing 100 μl ($1-2 \times 10^9$ cells/ml) of competent cells; the suspension was mixed gently and incubated in a 42°C water bath for 90 seconds. The sample was then returned to the ice bath for 30 minutes. Next, 900 μl of Lysogeny broth (LB) medium was added to the centrifuge tube, and the cell suspension was placed on a shaker at 37°C for 45 minutes. Then, the suspension was centrifuged at 4,000 RPM for 2 minutes. After the supernatant was aspirated, the bacteria were resuspended in 100 μl of the remaining medium then spread on an ampicillin (Amp⁺) plate using a sterile coating rod. The plate was placed in a 37°C incubator overnight.

After cultivation, a single colony was selected and inoculated in 6 ml of Amp⁺ LB liquid medium at 37°C and shaken at 220 RPM for 5 hours. Then, all the cultures were inoculated in 1 L Amp⁺ LB liquid medium and shaken at 220 RPM at 37°C until the optical density 600 (OD₆₀₀) reached 0.6. IPTG was added to the solution to a final concentration of 0.5 mM, and the culture was shaken at 200 RPM at 20°C for 16 hours. The bacteria were collected by centrifugation at 5,500 RPM for 10 minutes. The supernatant was discarded, and the cells were lysed by resuspending them in a lysis buffer comprising 20 mM Tris-HCl pH 8.0, 500 mM NaCl, and 1 mM dithiothreitol (DTT) followed by ultrasonic disruption in an ice bath. The suspension was centrifuged at 18,000 RPM for 10 minutes to obtain the lysate. The lysate was evaluated by SDS-PAGE electrophoresis followed by Coomassie brilliant blue staining.

The synthesized fusion protein was purified by Ni-Sepharose affinity chromatography. Briefly, the supernatant was first filtered through a 0.22 μm filter membrane. The pre-packed Ni-Sepharose was balanced with five column volumes of binding buffer, and the supernatant was loaded into the column at a flow rate of 1.5 ml/minutes. The impurities were eluted with an elution buffer A (20 mM NaH_2PO_4 , 0.5 M NaCl, and 50 mM imidazole), and the protein was eluted with an elution buffer B (20 mM NaH_2PO_4 , 0.5 M NaCl, and 100 mM imidazole). The eluate was added to a 50 KDa centrifugal ultrafiltration tube (Millipore), and the protein was concentrated by centrifugation at 5,500 RPM for 10 minutes at 4°C .

After the protein concentration was analyzed using a bicinchoninic acid assay, 10 U SUMO protease per 1 mg protein was added to digest the SUMO, six histidine, and strap[®] protein tags overnight at 4°C . The enzymatically cleaved protein was concentrated by passing it through a 50 KDa centrifugal ultrafiltration tube (Millipore) twice then resuspended in a storage buffer (600 mM NaCl, 50 mM Tris-HCl pH 7.5, 5% Glycerol, and 2 mM DTT) to be stored at -80°C . Samples were collected before and after digesting the protein tags for SDS-PAGE electrophoresis followed by Coomassie brilliant blue staining.

***C. trachomatis* target RNA and crRNA preparation**

The cryptic plasmid sequences of *C. trachomatis* serovars D through K were downloaded from the National Center for Biotechnology Information (NCBI) database. DNAMAN software (DNAMAN 9, Lynnon Biosoft, San Ramon, CA, USA) was used to compare the full-length sequence of the cryptic plasmid of each sequence to find the constant region (Figure 2). Then, Primer Premier 5 software (Primer Premier 5, Premie) was used to design RPA primers (Table 1) of 25–35 nt in length. The design principle was such that the length of the amplified product was 80–140bp, the melting temperature was between 54 °C and 67 °C, and the T7 RNA polymerase promoter sequence was appended to the 5'end of the upstream primer (Table 1). A plasmid containing a fragment of the *C. trachomatis* cryptic plasmid was amplified by PCR, and the PCR products were purified using a DNA recovery kit and transcribed into ssRNA using the HiScribe T7 Quick kit, which was subsequently purified

To minimize the cost, *in vitro* transcription (IVT) methods were used to prepare the crRNA. The CRISPR-RT website (<http://bioinfolab.miamioh.edu/CRISPR-RT/index.php>) was used to design the crRNA sequence (Table 1), obtain the DNA sequence complementary to the crRNA sequence, and append the promoter sequence to the DNA sequence as a template (Table 1). Denatured and annealed crRNA template and T7 RNA polymerase promoter primer (final concentration of 10µM) were used with the HiScribe T7 Quick kit to transcribe and purify the annealing products of the previous step into crRNA.

LwCas13a protein activity verification

With the existing detection method ²¹, the concentrations of crRNA and LwCas13a protein were 135 nM and 270 nM, respectively. The reaction buffer also contained 100 nM target RNA, 250 nM quenched fluorescent RNA probe, and 1 µl RNase inhibitor. As negative controls, the designed crRNA was replaced with false crRNA, or the LwCas13a protein was replaced with Cas9 protein. The positive control was 0.2 µl RNase (OMEGA) mixed with 250 nM quenched fluorescent RNA probe. The samples and controls were loaded into a 384-well black microplate, placed in a fluorescence plate reader, and incubated at 37 °C for 30 minutes to detect the fluorescence signal (excitation wavelength: 492 nm; emission wavelength: 518 nm).

Sensitivity evaluation of the CRISPR-Cas13a detection system without RPA

To optimize the detection system, LwCas13a protein and crRNA were diluted to obtain the minimal concentrations by 108 nM for LwCas13a and 90 nM for crRNA. Then, different concentrations of target ssRNA were combined with 108 nM LwCas13a, 90 nM crRNA, 250 nM quenched fluorescent RNA probe, and 1 µl RNase inhibitor to determine the lower limit of detection. All readings were carried out after incubation at 37 °C for 90 minutes in 384-well black microplates using a fluorescence plate reader (excitation wavelength: 492 nm; emission wavelength: 518 nm).

Sensitivity evaluation of the one-pot CRISPR-Cas13a detection method

C. trachomatis dsDNA fragments were inserted into a pUC57 plasmid and diluted to different concentrations as standard plasmid solutions. The assay system including RPA and T7 RNA polymerase

IVT reagents was as follows: 0.5 μ M forward primer, 0.5 μ M reverse primer, 108 nM LwCas13a, 90 nM crRNA, 59 μ l Rehydration Buffer (from TwistAmp Basic kit), 110 U RNase inhibitor, 250 nM quenched fluorescent RNA probe, 100 U T7 RNA polymerase, 8 mM NTP mixture, 2 μ M MgCl₂, and 5 μ l magnesium acetate (from TwistAmp Basic kit), all combined in RNase-free water to reach a total volume of 95 μ l. The reagent was loaded into a PCR tube containing TwistAmp recombinase polymerase freeze-dried microspheres to form the reaction mixture. 18 μ l of the reaction mixture was combined with 2 μ l of the standard plasmid solution, and the fluorescence signal was measured in 384-well black microplate using a fluorescence plate reader after incubation at 37 °C for 120 minutes (excitation wavelength: 492 nm; emission wavelength: 518 nm).

Sample evaluation by real-time PCR and the one-pot CRISPR-Cas13a system

The dsDNA from a cervical swab specimen was extracted as follows: the sample was diluted in 1 ml of PBS, shaken slightly, and centrifuged for 5 minutes at 12000 RPM; after discarding the supernatant, the pellet was resuspended in 50 μ l RNase-free water, incubated at 100 °C for 10 minutes, and centrifuged at 12,000 RPM for 5 minutes. The supernatant contained the dsDNA. As indicated in the Takara TB Green Premix Ex Taq II instructions, 2 μ l of the dsDNA sample, 400 nM primer pair, 10 μ l of TB Green Premix ExTaq II, 0.4 μ l of ROX Reference Dye II, and 6 μ l of sterilized water. The product was evaluated using an Applied Biosystems 7500 real-time PCR system. For the proposed one-pot CRISPR-Cas13a method, 2 μ l of the dsDNA sample was mixed well with the reaction mixture; the fluorescence signal was measured in a 384-well black microplate using a fluorescence plate reader after incubating at 37 °C for 40 minutes (excitation wavelength: 492 nm; emission wavelength: 518 nm).

Results And Discussion

The SDS-PAGE results showed that the LwCas13a protein was successfully expressed in the prokaryotic cells. To verify the solubility of the LwCas13a protein, the protein concentration was measured in the supernatant and sediment of the bacterial lysate using SDS-PAGE. Results indicated that the LwCas13a protein was present in the supernatant solution (Figure 3A). The LwCas13a protein was purified using nickel ion affinity chromatography. After the tagged protein was digested by the SUMO enzyme, the molecular weight of the LwCas13a protein was determined to be 138.5 kDa (Figure 3B). The theoretical molecular weight of the fusion protein was about 155.8 kDa. This finding confirmed the successful synthesis of the LwCas13a protein.

To analyze the activity of the LwCas13a protein, three controls were tested: false crRNA was used instead of specific crRNA, and Cas9 protein was used instead of LwCas13a protein, and only RNase and the quenched fluorescent RNA probe were reacted in RNase-free water. Figure 4 shows that, in the complete detection system, the LwCas13a protein exhibited enzymatic activity under the guidance of crRNA.

C. trachomatis ssRNA in different concentrations was added to the detection system, and the fluorescence was measured every 5 minutes. The data showed that the fluorescence intensity increases

with incubation time. Further, this method can be used to detect as little as 10 nM *C. trachomatis* ssRNA in 5 minutes and as little as 1 nM *C. trachomatis* ssRNA in 10 minutes (Figure 5A).

To improve the detection rate, RPA and IVT were added to the CRISPR-Cas13a reaction mixture to build a one-pot assay method. Standard solutions of different plasma concentrations were added to the detection system, and the fluorescence was measured every 5 minutes. These results indicated that the one-pot CRISPR-Cas13a assay can be used to detect 10 fM *C. trachomatis* dsDNA in 40 minutes (Figure 5B).

To demonstrate specificity, CRISPR-Cas13a detection system was challenged with other pathogen specimens including *Chlamydia pneumoniae*, *Mycoplasma*, and *Neisseria gonorrhoeae*. The results shows that there was almost no fluorescence signal observed in the *Mycoplasma hominis*, *Ureaplasma Urealyticum*, *Neisseria gonorrhoeae*, or *Chlamydia pneumoniae*. However, the fluorescence intensity of *Chlamydia trachomatis* was over 20-fold higher than that of other pathogen specimens (Figure 5C). The results also showed that the developed detection system could distinguish *Chlamydia trachomatis* from other subspecies of *Chlamydia* and pathogens. To further investigate the effect of background human DNA in CRISPR-Cas13a detection system, the total DNA of extracted JHH-7 cells and 10 fM standard plasmid of *Chlamydia trachomatis* was added to the detection system. The results indicated that the background human DNA from 1,000 JHH-7 cells, 10,000 JHH-7 cells, or 100,000 JHH-7 cells has no significant effect on the test (Figure 5D).

To verify the performance of the proposed detection method, 86 cervical swab samples were assayed, and the results were compared with those obtained by real-time PCR as the control method. Samples in which the fluorescent signal was observed after less than 35 thermal cycles ($C_t < 35$) were defined as *C. trachomatis*-positive, and those in which the fluorescent signal was observed after 35 or more thermal cycles ($C_t > 35$) were defined as *C. trachomatis*-negative. Of the samples evaluated, 55 were *C. trachomatis*-positive, and 31 were *C. trachomatis*-negative. Based on the fluorescence values obtained by the CRISPR-Cas13a method for 30 *C. trachomatis*-negative samples, the fluorescence threshold was set as three times the standard deviation above the mean value (1250; Figure 6A). Thus, samples with fluorescence values of 1250 or higher were defined as *C. trachomatis*-positive, and those with fluorescence values less than 1250 were defined as *C. trachomatis*-negative. Based on this cutoff, 54 samples were *C. trachomatis*-positive, and 32 samples were *C. trachomatis*-negative (Figure 6B). Therefore, of the 86 cervical swabs that were evaluated by the two methods, the results of only one sample were inconsistent (Table 2). This sample was further evaluated using a commercial nucleic acid detection kit (DAAN GENE), and it was confirmed that it was *C. trachomatis*-positive, as indicated by qPCR. Comparing the results of the two detection methods, the Chi-squared test showed that the two methods had good correlation ($\chi^2 = 81.798$; $P < 0.001$), and this finding was confirmed by a Cohen's kappa test ($\kappa = 0.975$; $P < 0.001$). The area under the receiver operating characteristic curve was 0.991 (95% confidence interval: 0.970–1.000; $P < 0.001$), the sensitivity was 0.982 (95% confidence interval: 0.890–0.999), and the specificity was 1.000 (95% confidence interval: 0.863–1.000) (Figure 7B).

The gold standard for the diagnosis of *C. trachomatis* infection is nucleic acid detection, which depends on laboratory facilities, expensive equipment, and professional technicians. However, more widely accessible screening methods are needed because most patients infected with *C. trachomatis* do not experience clinical symptoms before they develop chronic infections, which are associated with severe complications.

In this study, we propose a novel method for detecting *C. trachomatis* dsDNA. This method takes advantage of the high amplification efficiency provided by RPA technology and specificity to a target sequence provided by crRNA ²⁸. We designed crRNA that is complementary to *C. trachomatis* cryptic plasmid fragments to create a rapid, specific, and sensitive method based on the CRISPR-Cas13a system that can be carried out at a constant temperature without equipment to amplify the dsDNA. Using this technique, results can be obtained within 1 hour. Experimental results from cervical swab specimens were closely correlated with those obtained by qPCR.

RPA is not suitable for clinical diagnostics because there is a risk of non-specific amplification ²⁹. In contrast, the CRISPR-Cas13a method offers high specificity (depending on the design of the crRNA), so that even if non-specific amplification occurs, it will not affect the detection performance. Further, while the fluorescent probes used with qPCR methods need to be redesigned for each target, those used for the proposed CRISPR-Cas13a method do not need to be redesigned, which simplifies the design and reduces the cost to synthesize reagents. Although the proposed method reduces the need for instruments, it is still necessary to use a fluorescence microplate reader to detect the fluorescence signal. Therefore, in future studies, we aim to develop a colorimetric method based on CRISPR-Cas13a technology in which the results can be evaluated by naked eye. This method is expected to be useful for disease screening at different ranks of hospitals to better detect genitourinary diseases and mitigate the complications and long-term consequences caused by chronic infections.

Conclusions

In conclusion, the CRISPR-Cas13a-based diagnostic method we constructed can detect Chlamydia trachomatis nucleic acid at room temperature for about 1 hour. The advantage of this method is to avoid the use of expensive instruments. By validating 86 clinical samples, it is concluded that this method will have good performance and can meet the screening needs of community hospitals and other institutions. The method also requires an instrument to read the fluorescent signal. In the future, the lyophilized reagent can be combined with lateral immunochromatography technology, which can be used for real-time inspection of the visual reading results.

Declarations

Ethical Approval

The study was approved by the Ethics Committee of the Zhuhai Center for Maternal and Child Health Care.

Authors Contributions

Tian-Cai Liu contributed to the conception of the study;

Kaichen Huang performed the experiment;

Hailing Yu and Guanfeng Lin contributed significantly to analysis and manuscript preparation;

Kaichen Huang and Hailing Yu performed the data analyses and wrote the manuscript;

Yingsong Wu, Zhigao Zhang, Xiaoyan Zhang, Hong Chen and Zhen Zhang helped perform the analysis with constructive discussions.

Funding

The National Natural Science Foundation of China (Grant No. 21575058);

The National High Technology Research and Development Program ("863" program) of China (Grant No. 2014AA020904);

The Major National Science and Technology Project of China (Grant No. 2017ZX10302201002002).

Competing Interests

We declare that the funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

Acknowledgements

The authors thank the committee of the National Natural Science Foundation of China and the Ministry of Science and Technology of China. The work was financially supported by grants from the National Natural Science Foundation of China (Grant No. 21575058), the National High Technology Research and Development Program ("863" program) of China (Grant No. 2014AA020904), and the Major National Science and Technology Project of China (Grant No. 2017ZX10302201002002).

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Tables

Table 01

Name	Sequence
T7 promoter	GAAATTAATACGACTCACTATAGGG
crRNA IVT template	TCTGAGGATAAGTTATAATAATCCGTTTTAGTCCCCTTCGTTTTTGGGGTAGTCTAAATC CCCTATAGTGAGTCGTATTAATTC
crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGGAUUUAUUUAACUUAUCCUCAGA
RPA-F	TAATACGACTCACTATAGGGGCGATTTCCTTAACCCACCATTTTTCCG
RPA-R	TTTCCAACAAGCTACCATTTCTTTCTCCCA
Quenched fluorescent RNA probe	/5-FAM/UrUrUrUrU/3BHQ

Table 02

Method		Real-Time PCR		
		Positive	Negative	total
CRISPR-Cas13a	Positive	54	0	54
	Negative	1	31	32
	total	55	31	86

Figures

Figure 1

Cell culture can be used to detect the pathogen, but it is rarely used today as it requires a time-consuming and labor-intensive process 11. Serological diagnosis is not recommended for screening because it has poor sensitivity and can return false negatives during the window period after infection when the specific antigen or antibody cannot be detected 11. Most commercial one-pot rapid detection kits for *C. trachomatis* also have low sensitivity, which can easily lead to misdiagnosis 12. The current gold standard for diagnosing *C. trachomatis* infections is nucleic acid detection, which is highly sensitive and specific but may take several hours, requires complex instruments, and can only be carried out by professionally trained technicians 13, 14. However, there are many novel methods developed to replace traditional analysis of nucleic acid of DNA and RNA 15-17. The clustered regularly interspaced short palindromic repeats (CRISPR) system has become the most commonly used gene-editing tool in recent years 18. The CRISPR-Cas system can further expand its application scope by modifying the Cas protein 19. Previous studies have shown that the CRISPR-Cas13a system can be used for in vitro detection of DNA and RNA 20-23 by combining it with recombinase polymerase amplification (RPA) to amplify target

dsDNA in the sample or reverse transcription amplification to identify RNA in the sample 24, 25. The obtained target dsDNA is then transcribed into ssRNA. The complementary pairing of the ssRNA to a designed crRNA activates the RNA-cleavage activity of Cas13a 21, 23 to cleave a quenched RNA fluorescent probe, thereby releasing a measurable signal (Figure 1) 26, 27.

Figure 2

The cryptic plasmid sequences of *C. trachomatis* serovars D through K were downloaded from the National Center for Biotechnology Information (NCBI) database. DNAMAN software (DNAMAN 9, Lynnon Biosoft, San Ramon, CA, USA) was used to compare the full-length sequence of the cryptic plasmid of each sequence to find the constant region (Figure 2). Then, Primer Premier 5 software (Primer Premier 5, Premie) was used to design RPA primers (Table 1) of 25–35 nt in length. The design principle was such that the length of the amplified product was 80–140bp, the melting temperature was between 54 0C and 67 0C, and the T7 RNA polymerase promoter sequence was appended to the 5'end of the upstream primer (Table 1). A plasmid containing a fragment of the *C. trachomatis* cryptic plasmid was amplified by PCR, and the PCR products were purified using a DNA recovery kit and transcribed into ssRNA using the HiScribe T7 Quick kit, which was subsequently purified.

Figure 3

The SDS-PAGE results showed that the LwCas13a protein was successfully expressed in the prokaryotic cells. To verify the solubility of the LwCas13a protein, the protein concentration was measured in the supernatant and sediment of the bacterial lysate using SDS-PAGE. Results indicated that the LwCas13a protein was present in the supernatant solution (Figure 3A). The LwCas13a protein was purified using nickel ion affinity chromatography. After the tagged protein was digested by the SUMO enzyme, the

molecular weight of the LwCas13a protein was determined to be 138.5 kDa (Figure 3B). The theoretical molecular weight of the fusion protein was about 155.8 kDa. This finding confirmed the successful synthesis of the LwCas13a protein.

Figure 4

To analyze the activity of the LwCas13a protein, three controls were tested: false crRNA was used instead of specific crRNA, and Cas9 protein was used instead of LwCas13a protein, and only RNase and the quenched fluorescent RNA probe were reacted in RNase-free water. Figure 4 shows that, in the complete detection system, the LwCas13a protein exhibited enzymatic activity under the guidance of crRNA.

Figure 5

C. trachomatis ssRNA in different concentrations was added to the detection system, and the fluorescence was measured every 5 minutes. The data showed that the fluorescence intensity increases with incubation time. Further, this method can be used to detect as little as 10 nM *C. trachomatis* ssRNA in 5 minutes and as little as 1 nM *C. trachomatis* ssRNA in 10 minutes (Figure 5A).

Figure 6

To verify the performance of the proposed detection method, 86 cervical swab samples were assayed, and the results were compared with those obtained by real-time PCR as the control method. Samples in which the fluorescent signal was observed after less than 35 thermal cycles ($C_t < 35$) were defined as *C. trachomatis*-positive, and those in which the fluorescent signal was observed after 35 or more thermal cycles ($C_t > 35$) were defined as *C. trachomatis*-negative. Of the samples evaluated, 55 were *C. trachomatis*-positive, and 31 were *C. trachomatis*-negative. Based on the fluorescence values obtained by the CRISPR-Cas13a method for 30 *C. trachomatis*-negative samples, the fluorescence threshold was set as three times the standard deviation above the mean value (1250; Figure 6A). Thus, samples with fluorescence values of 1250 or higher were defined as *C. trachomatis*-positive, and those with fluorescence values less than 1250 were defined as *C. trachomatis*-negative. Based on this cutoff, 54 samples were *C. trachomatis*-positive, and 32 samples were *C. trachomatis*-negative (Figure 6B). Therefore, of the 86 cervical swabs that were evaluated by the two methods, the results of only one sample were inconsistent (Table 2)

Figure 7

This sample was further evaluated using a commercial nucleic acid detection kit (DAAN GENE), and it was confirmed that it was *C. trachomatis*-positive, as indicated by qPCR. Comparing the results of the two detection methods, the Chi-squared test showed that the two methods had good correlation ($\chi^2 = 81.798$; $P < 0.001$), and this finding was confirmed by a Cohen's kappa test ($\kappa = 0.975$; $P < 0.001$). The area under the receiver operating characteristic curve was 0.991 (95% confidence interval: 0.970–1.000; $P < 0.001$), the sensitivity was 0.982 (95% confidence interval: 0.890–0.999), and the specificity was 1.000 (95% confidence interval: 0.863–1.000) (Figure 7B).