Article

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Development of Rapid Detection Technology for HPV16 Based on CRISPR-Cas12a

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Objective: Cervical cancer is one of the most common malignant tumors affecting women. It is estimated that 99.6% of cervical cancer patients are caused by persistent infection with human papillomavirus (HPV). The aim of this study was to combine the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/associated proteins (Cas12a) detection system with colloidal gold rapid diagnosis technology to develop a new rapid diagnosis technology for HPV16 nucleic acid detection.

Method: Based on the advantages of CRISPR-Cas12a and recombinase polymerase amplification (RPA) technology, this experiment intended to establish an innovative technology suitable for rapid clinical detection of the *L1* gene for HPV16 and develop a rapid nucleic acid lysis solution. The RPA technology was developed through primer verification to synthesize a large number of templates for the Cas12a-crRNA cleavage system swiftly. RPA technology was combined with lateral flow test strips to complete the interpretation of the results. The lysate contained 1.5 M guanidine hydrochloride, 50 mM Tris (pH = 8.0), 100 nM NaCl, 5 mM Ethylene Diamine Tetraacetic Acid (EDTA), and 1% Tween-20.

Result: In this study, according to the concentration of the template and the results of agarose gel electrophoresis without dragging phenomenon, a third solution was finally determined as the optimal cleavage scheme: 1.5 M guanidine hydrochloride, 50 mM Tris (pH = 8.0), 100 nM NaCl, 5 mM EDTA, 1% Tween-20. After optimization, the optimal concentration of the probe was finally determined as 0.02 μM.

Conclusions: The current study verified the rapid detetion capacity of CRISPR/Cas12a combined with RPA technology, hoping to provide a new technical method for HPV16 detection.

Keywords: HPV; CRISPR-Cas12a; recombinase polymerase amplification technology; lateral flow test strip

Introduction

Cervical cancer is one of the most common malignant tumors affecting women specially under the age of 45. Cervical cancer causes serious physical and psychological harm to patients. Almost 99.6% of the cases are caused by persistent infection of human papillomavirus (HPV) [1], being the most common types HPV16 and HPV18 [2]. Early screening, timely detection, appropriate treatment, and regular return visits are effective strategies to prevent cervical cancer and reduce its mortality. Nowadays, research on HPV16 detection has increased at home and abroad, in topics such as liquid-based cell thin layer technology (TCT) [3], Papanicolaou (PAP)-Smear [4] and Raman spectroscopy [5]. Unfortunately, the detection time, specificity, and sensitivity of the described techniques need to be improved. Lin Haiying *et al.* [6] have found that if PAP-Smears is not fixed in time during operation, it results in low film quality in the later period, affecting subsequent film reading thereby impairing accuracy. Similarly, studies have found that the high cost of Raman spectroscopy technology is the main reason why it is difficult to be popularized, and it is suitable for *in vivo* detection needs to be studied and determined [7]. Based on the advantages of CRISPR-Cas12a and recombinant enzyme polymerase amplification (RPA) technology, this experiment intends to establish a new technology suitable for the rapid detection of the HPV16 gene in the clinic. Hong Xinxin *et al.* [8] used the combination of recombinant polymerase amplification (RPA) technology and CRISPR-Cas12a technology to detect influenza A virus with strong specificity and sensitivity up to the 6 orders of magnitude, but this technology has not

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been widely used in the diagnosis of HPV.

Isothermal amplification technology of nucleic acid has been gradually developed and refined to replace the traditional polymerase chain reaction. Common isothermal technologies include Recombinase Polymerase Amplification (RPA) [9], strand displacement amplification [10], helicase-dependent amplification [11], nucleic acid sequence-based amplification [12] and loop-mediated isothermal amplification [13]. These techniques integrate well into microsystems or portable devices. They can be used to improve nucleic acid-based field assays while ensuring high sensitivity and minimal manipulation. Among them, the development of heavy RPA technology is more advanced. Its enzyme system can maintain good activity in a temperature range of 25~40 °C which makes it applicable and suitable for rapid detection without equipment [14].

RPA technology relies on three enzymes: Recombinase (binding primer) that can bind single-stranded nucleic acid, single-stranded deoxyribonucleic acid (ssDNA) binding protein, and strand displacement DNA polymerase. The protein-DNA complex formed by the combination of the recombinase and the primer can search for homologous sequences in double-stranded DNA. Firstly, Primers locate homologous sequences strand exchange reaction occurs, secondly, DNA synthesis begins and the target region is amplified, and finally, the replaced DNA strand binds to the single-stranded DNA-binding protein (SSB), preventing further replacement [15]. Compared to ordinary polymerase chain reaction (PCR) methods, the entire RPA process occurs rapidly. Generally, a detectable amount of amplification products can be obtained within 10 min, which requires less experimental operation and instrumentation. By adding different enzymes and probes to this basic system, a variety of RPA applications can be performed. Piepenburg *et al.* (2006) [9] used lateral flow-recombinase polymerase amplification (LF-RPA) technology to detect Staphylococcus aureus with a sensitivity of 10 copies/reaction. Zhang *et al.* (2014) [16] used LF-RPA technology to detect the Plum pox virus (PPV) within 20 min, and the reaction sensitivity reached 1 fg/μL. Ahmed *et al.* (2014) [17] used the RPA-Exo probe method to detect Leptospira, and the detection was completed within 25 min with a sensitivity that reached 10 fg/μL. However, since constant temperature PCR technology is difficult to avoid, non-specific amplification can cause false positive detection, which limits the application of this technology in diagnosis [18]. The emergence of CRISPR technology provides new solutions to these specific technical problems. CRISPR enables the immune system to recognize and break down foreign nucleic acids in bacterial genomes, guided by sequence-specific Ribonucleic Acid (RNA) molecules [19]. Thus, its value as a novel gene editing tool has been rapidly exploited [20]. Among the different varieties of CRISPR, CRISPR/Cas12a enzymes belong to the class 2 system in CRISPR-cas [21]. It recognizes spacer adjacent motif (PAM) that is rich in thymine nucleotides. Matured self-lead CRISPR-RNA (crRNA) can catalyze the generation of 5′ and 3′ staggered distal PAM double-stranded DNA (dsDNA) breaks. Furthermore, it hass been reported that once Cas12a form a ternary complex with crRNA and target DNA, the complex produce a strong “random cleavage activity” that allows to cut any single-stranded DNA (namely trans cut) [22,23].

The aim of this study was to combine CRISPR/Cas12a detection system with colloidal gold rapid diagnostic technology to develop a new rapid nucleic acid diagnostic technology for HPV16. It is hypothesized that his technology, used under the guidance of crRNA Cas12a enzyme specificity target and cleavage dsDNA, will nonspecificly cut signal single-stranded DNA molecular markers to release the signal molecules. Additionally, if combined with the corresponding antibody on the gold-labeled test strip after the principle of color, it may be used in disease detection and subtype classification to distinguish and confirm resistance gene and gene typing. Compared with the existing methods, the technique is more specific and sensitive. Moreover, it is convenient to operate and user-friendly without the requirement of professionals, providing a reliable and convenient technical means for the early diagnosis and rapid detection of HPV16.

Material and Method

Sample General Information

Inclusion criteria of cervical cancer included: (1) Age ≥18 years; (2) Pathological, imaging and biochemical tests confirmed the diagnosis of primary cervical cancer, which was in accordance with the diagnostic standards of Cervical Cancer Diagnosis and Treatment (2018 Edition) issued by the National Health Commission of the People’s Republic of China; (3) Able to complete questionnaire independently; (4) Informed consent of this investigation was provided. Exclusion criteria of cervical cancer included: (1) Patients with severe cardiovascular and cerebrovascular diseases, liver and kidney dysfunction, hematological diseases or other malignant tumors other than cervical cancer; (2) Patients with mental illness, cognitive impairment, intellectual impairment or dyslexia; (3) In the terminal stage of cancer care; Or (4) a history of psychoactive medications, drug use, or alcohol abuse within the previous three months.

HPV16 negative samples (A, B, and C), and HPV16 positive samples (I, II, and III) were obtained from cervical cancer (Chongqing Precision Medicine Industry Technology Research Institute Sample Bank).

Reagents

The reagents included the following: LbCas12a (cpf1) (Leshang Bio, Wuxi, China), lateral flow test strip (Murray Bio, Shanghai, China), isothermal nucleic acid am-

Table 1. Primer sequences.

Primer		Primer type	Length	Sequence (5′-3′)
Primer pair 1	HPV16-F1	upstream primer	31	TTGTTGGGGTAACCAACTATTTGTTACTGTT
	HPV16-R1	downstream primer	29	CCTCCCCATGTCGTAGGTACTCCTTAAAG
Primer pair 2	HPV16-F2	upstream primer	30	CTACACCTAGTGGTTCTATGGTTACCTCTG
	HPV16-R2	downstream primer	31	CGTCTGCAGTTAAGGTTATTTTGCACAGTTG
	Positive crRNA	upstream primer	24	TTTGGTTTAACGAGAACGTGAACC
	Reverse crRNA	downstream primer	24	ATTACAGCTGTAATGGACCTCAAA
	DNAprobe		8	(FITC) TTATT (Bio)

plification kit (Leshang Bio, Wuxi, China), Tris (pH = 8.0), NaCl, polyvinylpyrrolidone (PVP), Ethylenediaminetetraacetic acid (EDTA), Guanidine Hydrochloride (Qinke Bio, Beijing, China), TritonX100 (Sigma, USA), Tween-20 (ThermoFisher, Shanghai, China). HPV16 plasmids were constructed by the Chongqing Precision Medical Industry Technology Research Institute.

Primer Design and Synthesis

National Center for Biotechnology Information (NCBI) was used to query and obtain the main capsid protein L1 (GenBank: AY098925.1) sequence of HPV16, using a sequence analysis software to perform multiple sequence alignment. Primer 5 software (Premier Canada Inc., Charlotte, NC, USA) was used to design primers according to the alignment results. Two sets of primer pairs (Table 1) were obtained and amplified using the RPA amplification kit. The primers shown in the Table 1 were synthesized by Qingke Biological Company.

Construction of the Detection Reaction System

Solution of Rapid Nucleic Acid Lysis Solution.

Three lysis buffers were used: ① 50 mM Tris (pH = 8.0), 150 mM NaCl, 2% PVP, 1% Tween-20; ② 800 nM guanidine hydrochloride, 50 mM Tris (pH = 8.0), 0.5% Triton-X100, 1% Tween-20; And ③ 1.5 M guanidine hydrochloride, 50 mM Tris (pH = 8.0), 100 nM NaCl, 5 mM EDTA, 1% Tween-20 [24]. 100 μL of lysate as described above was added to three tubes. The samples were repeatedly scrubbed with a cotton swab, and shaken up and down for 10 s to facilitate sample lysis. The nucleic acid concentration in the three tubes was determined by Nanodrop and detected subsequently using agarose gel electrophoresis. The optimal solution was selected based on the nucleic acid concentration and band results.

Primer Verification

HPV16 negative samples (A, B, and C), and HPV16 positive samples (I, II, and III) were amplified using RPA with the primer pairs in Table 1 (F1 and R1, F2 and R2).

The RPA reaction system (50 μL) was added to 25 μL Buffer, 2 μL of 10 μM primer pair 1 (F1 and R1) and primer pair 2 (F2 and R2), 1 μL sample template, and 17.5 μL DEPC-treated water. To start the reaction, 2.5 μL magnesium acetate was added to the previously mentioned mixed, followed by fully dissolving the recombinase complex dry powder in the RPA reaction system. The whole reaction system was reversed, homogenized, instantaneously separated and then reacted at 37 °C for 30 min. After the reaction, the product was recovered using the gel recovery kit. Because there were diverse proteins during the reaction, direct electrophoresis may lead to a tailing phenomenon, so it was necessary to carry out a gel running test after protein purification.

Probe-Free Cleavage

The detection system (20 μL) was added to 2 μL of 10× Cas12a buffer, 1.5 μL of LbCas12a protein, 1.2 μL of gRNA (5 μM), 0.5 μL of RPA product and 14.8 μL DEPC-treated water. Agarose gel electrophoresis was performed after cleavage for 5 min and 30 min.

Probe Cleavage and System Optimization

The detection system before optimization was performed as described in section 2.4.3. Because Cas12a-crRNA recognizes, bound to the PAM sequence, and cleaves randomly, the probe was cut. Thus, it could not form a multiplex complex that could bind to the detection line. If the detection line was positive, only the control line had a color change, and if it was negative, both the control line and the detection line had color changes. The DNA probe concentration (0, 0.002, 0.02, 0.04, and 0.08 μM) and the cleavage time (5, 15 min) in the reaction were optimized.

Results

Determination of the Nucleic Acid Lysis Solution

HPV16 positive samples (I, II, and III) were lysed with the described three lysis solutions respectively, and the concentrations determined by Nanodrop are shown in Table 2. The results of agarose electrophoresis are shown in Fig. 1. Solutions 1 and 3 had higher extraction concentrations than solution 2, and there was less dragging in solution 3 in the electrophoretic diagram. Therefore, according to concentration and electrophoresis, solution 3 was regarded as the best one.

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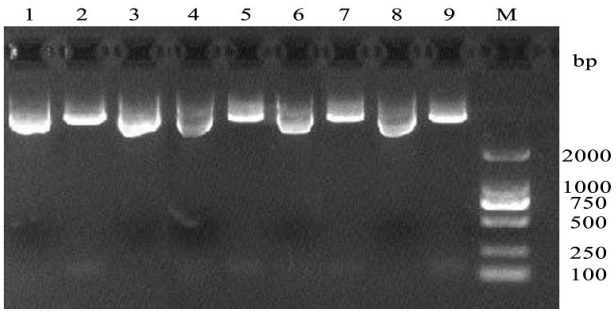

Fig. 1. Electrophoresis of different lysates. M: 2000 bp marker; 1, 2, 3: Solution 1 split HPV16 (I, II, and III) samples; 4, 5, 6: Solution 2 split HPV16 (I, II, and III) samples; 7, 8, 9: Solution 3 split HPV16 (I, II, and III) samples.

Table 2. Concentrations of HPV16 samples lysed using three lysis solutions.

Lysate type	Types of HPV16-positive samples	Concentration (ng/μL)
Solution 1	Sample I	58.0
	Sample II	55.7
	Sample III	59.2
Solution 2	Sample I	40.7
	Sample II	56.8
	Sample III	50.9
Solution 3	Sample I	56.9
	Sample II	58.7
	Sample III	60.2

Primer Verification

HPV16 negative samples (A, B, and C), positive samples (I, II, and III), and positive quality control were used as templates. RPA reaction was performed on F1 and R1, F2, and R2 primer pairs. After the reaction was completed, 2% of agarose gel electrophoresis was used to detect the results. The optimal primer pair was determined according to the band condition. Electrophoresis results showed that the negative template with F2 and R2 primer pairs had a false positive phenomenon (Fig. 2A). Therefore, in the follow-up experiments, we used the primer pairs F1 and R1 and the materials addressed in section 2.1 (HPV16 negative samples (A, B, and C), positive samples (I, II, and III)). The results are reported in Fig. 2B.

Probe-Free Cleavage

HPV16 RPA product was used as a template. LbCas12a-crRNA digestion system was added, and electrophoresis was performed for 5 and 30 min (80 v). The results showed that within 10–30 min, the longer the cleavage time, the higher the binding rate of the enzyme digestion system, and the more obvious the cleavage effect (Fig. 3A,B).

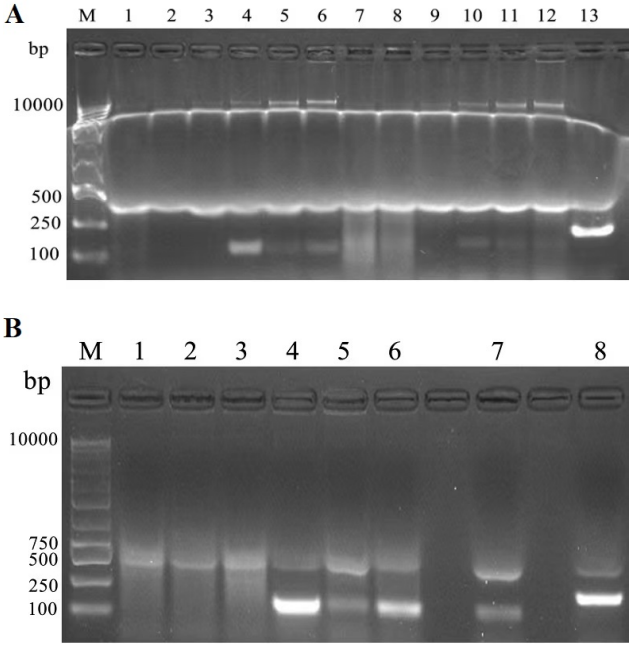

Fig. 2. (A) F1 and R1, F2 and R2 primer pair screening. M: Marker (100–10000 bp); 1, 2, 3: Negative samples with F1 and R1 as primers; 4, 5, 6: Positive samples with F1 and R1 as primers; 7, 8, 9: Negative samples with F2 and R2 as primers; 10, 11, 12: Positive sample with F2 and R2 as primers; 13: Positive quality control (230 bp). (B) F1 and R1 primer pair electrophoresis results. M: Marker (100–10000 bp); 1, 2, 3: Negative samples with F1 and R1 as primers; 4, 5, 6: Positive samples with F1 and R1 as primers; 7: HPV16 plasmid; 8: Positive quality control (230 bp).

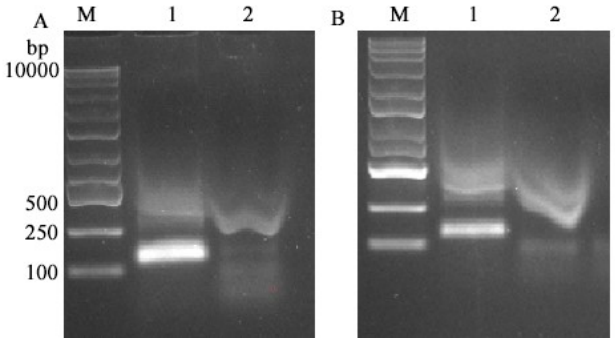

Fig. 3. Cut without a probe. (A) M: Marker (100–10000 bp); 1: Uncut control group; 2: Cut for 5 min. (B) M: Marker (100–10000 bp); 1: Uncut control group; 2: Cut for 30 min.

Plus Probe Cleavage and System Optimization

Cleavage with Probes

Based on the system described in Section 2.3, 0.1 μM probe was added and cleavage was performed for 30 min. After cleavage, the results of positive samples II were substantially different from those of the negative control group. The test results showed that only the control line of the pos-

Table 3. Determination of optimal probe concentration.

Probe concentration (OD)	0.002	0.02	0.04	0.08	0.1	Positive control
Test strip	+	++	++	++	+	-

"+/-" indicates the discoloration degree of bands; The more number of "+" indicates brighter the color; "-" indicates no color development.

Table 4. Discoloration degree of optimized cleavage time.

Sequence number	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
Test strip	++	-	+	-	++	++	++	-	+	-	++	++

"+/-" indicates the discoloration degree of the bands; The number of more "+" indicate brighter color; "-" indicates no color development.

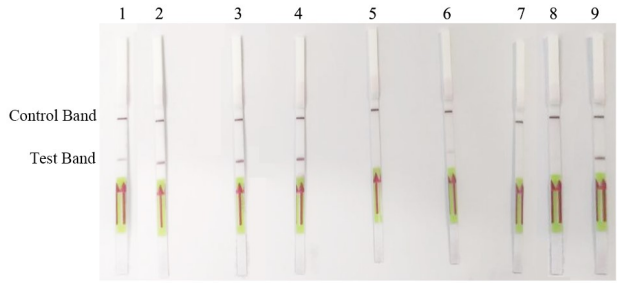

Fig. 4. Preliminary exploration of adding cut probe. 1: Water; 2, 3, 4: Negative samples (A, B, C); 5, 6, 7: Positive samples (I, II, III); 8: HPV16 plasmid; 9: Positive quality control (230 bp).

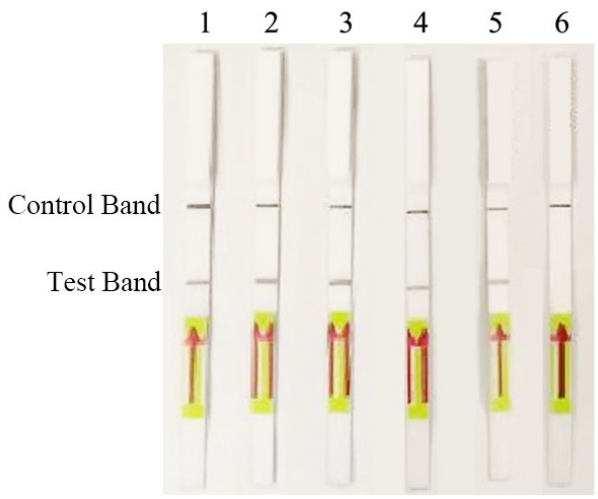

Fig. 5. Determination of probe concentration. The probe concentration is 1: 0.002 μM; 2: 0.02 μM; 3: 0.04 μM; 4: 0.08 μM; 5: 0.1 μM; 6: Positive control.

itive sample produced color, while both the control line and the detection line of the negative sample produced color (Fig. 4). However, the detection line of positive sample II, still showed a light color.

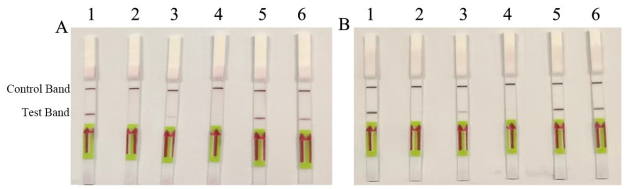

Fig. 6. Cleavage time optimization. (A) Depicting the results of cleavage for 5 min. (B) Depicting the results of cleavage for 15 min. Among them (1–6 templates are the same) 1: Negative sample A; 2, 3: Positive sample II, III; 4: HPV16 plasmid; 5: Positive quality control; 6: DEPC-treated water.

Probe Concentration Optimization

To determine the optimum concentration of the probe, the color degree of the detector line must be determined. The negative sample 1 was used as a template, and the concentration of the DNA probe was changed to 0, 0.002, 0.02, 0.04, and 0.08 μM. After the reaction, the test strip was inserted into 50 μL of probe buffer. The degrees of discoloration are repoted as +/- in Table 3, and results are shown in Fig. 5. Among them, the 0.02~0.04 μM control line and the detection line were the brightest. Based on the principle of saving agents, the optimal concentration was finally determined to be 0.02 μM.

Cleaverage Time Optimization

When the cleavage time was changed from 5 min to 15 min, the detection line of the test strip changed substantially, as shown in Table 4 and Fig. 6A,B. However, after 15 min of cleavage, the color of the positive sample III was still lighter than that of groups 1, 5, and 6, as shown in Fig. 6.

Discussion

Cervical cancer patients have no obvious symptoms at an early stage. Vaginal bleeding, genital, and anal warts, and other clear symptoms develop in the later stage [25]. High-risk HPV infection can lead to cervical intraepithelial neoplasia [2], extremely harmful to health. In 2009, the international agency for research on cancer defined HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 as class I

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carcinogens [26]. Among them, HPV16 and 18 infections are the most closely associated with cervical cancer, and type 16 or 18 infections can be detected in the cervical tissues of 80% cervical cancer patients [27–29]. Therefore, it is crucial to study the detection technology of HPV16 to aid preventing and treating cervical cancer. Thanks to the development of screening technology, the chances of preventing the disease has considerably increased. PAP-Smears [4] and thinprep cytology test (TCT) [3] have been widely used in clinical testing to detect HPV16. Despite PAP-smear detection can realize detection of disease at an early stage to reduce mortality, its sensitivity is not satisfactory and the rate of missed detection can not be underestimated [30]. It has been pointed out that the false positive rate of this method range between 50% to 90% [31]. Compared to the smear method, the liquid-based cell thin layer technique retains almost all specimens with single smear background, which improves clarity and detection rate [32]. It shows higher sensitivity than PAP-Smear in the diagnosis of cervical cancer, and effectively reduce the misdiagnosis rate [33]. However, the cost of this detection method is high, making it not suitable forless developed areas less developed areas, which restricts its extensive application in large-scale screening [34]. Raman spectroscopy provides a unique biochemical fingerprint that can identify and characterize molecular, cellular, and tissue structures. It is a promising biochemical tool for cervical cancer detection [5]. Being a promising biochemical tool for cervical cancer detection, Raman spectroscopy provides a unique biochemical fingerprint capable of identifying and characterizing the structure of a molecular, a cell and a tissue. However, Shen Zuowei *et al.* [7] have summarized the literature related to the diagnosis of cervical cancer by Raman spectroscopy, indicating that most of the studies focus on histology, predominantly *in vitro* studies, and the related studies of cytology and serology need further development. Ramos also points out that more comprehensive *in vivo* and *in vitro* studies are essential to verify the advantage of this technique in cervical cancer diagnosis [5].

The combination of CRISPR/Cas12a and RPA (RPA-CRISPR/Cas12a) has been widely used in clinical testing. Luan Tian *et al.* (2020) [35] have applied this technology to detect Actinobacter pneumonia. It took 30 min to be completed and showed negative results for control strains including Haemophilus parasuis (HPS), Streptococcus suis (SS), and Salmonella (SE) with high specificity. The sensitivity of this method can reach 10 copies/μL, 1000 times as high as that of an ordinary PCR method. Lei Rong *et al.* (2022) [36] have detected Phytophthora syringae and amplified them at 37 °C for 40 min, which succeeded in detecting Phytophthora syringe with a sensitivity of 133 fg/μL, implying that this detection method has high specificity and high sensitivity. Nevertheless, this method has been rarely applied in HPV16 detection. At present, the clinical diagnosis of cervical cancer mainly includes TCT detection and PAP-Smears and some scholars apply the combination of TCT and real-time quantitative polymerase chain reaction (qPCR) detection. Li Haiping and Gao Bowen *et al.* (2016) [37] have adopted the combination of TCT and qPCR to detect HPV16a with a positive detection rate of 94.44%, which is higher than that of TCT (59.09%) or qPCR (64.0%) alone. Yang Xiaolin *et al.* (2022) [38] have used TCT and immunohisto chemistry (IHC) to detect HPV and p16 proteins. The sensitivity, specificity, and accuracy of HPV, TCT, and P16 combined detection are higher than those of HPV detection, TCT detection, and P16 detection alone. However, both TCT-qPCR and IHC methods need long detection time, high cost, and show poor specificity and sensitivity. Taken together, the present study attempted to find a more specific detection method. The use of RPA technology improved the sensitivity of the reaction. The results were shown by an immunochromatographic strip making the test intuitive and reliable. This method could effectively avoid the problems of subjective judgment or difficult smear production. The test provides a new technology for clinical monitoring of HPV16. It is expected to be popularized in both developed and developing areas, with a new technique for clinical diagnosis.

The innovations of this paper are as follows: (1) Different lysate formulations were collected, and the optimal formulations were obtained through comparative analyses: 1.5 M guanidine hydrochloride, 50 mM Tris (pH = 8.0), 100 nM NaCl, 5 mM EDTA, 1% Tween-20; (2) For the first time, RPA-CRISPR-Cas12a technology was applied in the detection of HPV16. Compared with TCT, Pap smear and Raman spectroscopy, RPA-CrisPR-Cas12A technology did not require special equipment and is easy to operate. Because HPV16 specific genes and probes were selected for the experiment, the specificity and sensitivity were higher. Meanwhile, studies have shown that the entire detection process can be completed within 40 min [36], which can be used as an effective tool for the rapid diagnosis of cervical cancer; (3) The combination of RPA-CRISPR-Cas12a technology and the test strip detection method enhances the visualization of the results, and the operation is user-friendly and convenient. It is very suitable for rapid field detection and has the value of clinical promotion. The design of DNA probes during CRISPR/Cas12a reaction and results observation with an immunofluorescence dipstick might effectively avoid subjective judgment problems. Finally, the probe concentration and cleavage time were optimized to obtain a technique that could be inter-operator reliable. After optimization, the optimal concentration of the probe was finally determined as 0.02 μM.

There are some limitations in this study that need to be solved in the future. The optimal cleavage time is not clear, a large number of clinical samples are needed to be verified. Additionally, it is uncertain whether this technology affects the detection of people who have been inoculated with HPV vaccines.

Conclusions

In conclusion, this study combined the rapid recognition ability of CRISPR/Cas12a with RPA technology. This technique has low requirements on detection equipment, making it accessible for resource-limited areas or field studies.

Availability of Data and Materials

The data used to support the findings of this study are included in the article.

Author Contributions

XT and YXY—designed research, performed research, formal analysis, writing original draft; JX and ZL—performed research, formal analysis; NX and ZW—provided help and advice on the experiments; SC and XY—analyzed the data; YS—designed research, formal analysis, writing review & editing, supervision. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was conducted according to the principles of Declaration of Helsinki. The study was approved by the Ethics Committee of the Chongqing Precision Medicine Industry Technology Research Institute (JZYL2020-2). All participants provided written informed consent for sample collection and subsequent analyses.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Yang HP. Epidemiological investigation and analysis of related risk factors in 118 cases of cervical lesions infected with human papillomavirus. Chinese Remedies & Clinics. 2021; 21: 758–760.

[2] Xu YH. Clinical significance of high-risk HPV typing in screening for cervical cancer and precancerous lesions. Soochow University. 2018.

[3] Liu Y, Zhang L, Zhao G, Che L, Zhang H, Fang J. The clinical research of Thinprep Cytology Test (TCT) combined with HPV-DNA detection in screening cervical cancer. Cellular and molecular biology (Noisy-le-Grand, France). 2017; 63: 92–95.

[4] Bengtsson E, Malm P. Screening for Cervical Cancer Using Automated Analysis of PAP-Smears. Computational and Mathematical Methods in Medicine. 2014; 2014: 1–12.

[5] Ramos IRM, Malkin A, Lyng FM. Current Advances in the Application of Raman Spectroscopy for Molecular Diagnosis of Cervical Cancer. BioMed Research International. 2015; 2015: 1–9.

[6] Lin MY, Li W, Cheng WD. Analysis of Diagnostic Efficacy of Liquid-Based Cytology Combined with Pap Smear in Screening Cervical Precancerous Lesions in Young and Middle-aged People. Chinese and Foreign Medical Research. 2021; 19: 58–60.

[7] Shen ZW, Li R, Wang Y. Research progress of Raman spectroscopy in cervical cancer in recent five years. Spectroscopy and Spectral Analysis. 2022; 42: 2213–2217.

[8] Hong XX. Establishment of Gene Editing Enzyme Cas12a-Mediated RPA Isothermal Amplification Nucleic Acid Detection System. Xiamen University. 2020.

[9] Piepenburg O, Williams CH, Stemple DL, Armes NA. DNA detection using recombination proteins. PLoS Biology. 2006; 4: e204.

[10] Walker GT, Little MC, Nadeau JG, Shank DD. Isothermal *in vitro* amplification of DNA by a restriction enzyme/DNA polymerase system. Proceedings of the National Academy of Sciences of the United States of America. 1992; 89: 392–396.

[11] Vincent M, Xu Y, Kong H. Helicase-dependent isothermal DNA amplification. EMBO Reports. 2004; 5: 795–800.

[12] Compton J. Nucleic acid sequence-based amplification. Nature. 1991; 350: 91–92.

[13] Notomi T. Loop-mediated isothermal amplification of DNA. Nucleic Acids Research. 2000; 28: E63.

[14] Lillis L, Siverson J, Lee A, Cantera J, Parker M, Piepenburg O, *et al.* Factors influencing Recombinase polymerase amplification (RPA) assay outcomes at the point of care. Molecular and Cellular Probes. 2016; 30: 74–78.

[15] Jing ZG, Dong H, Di DD, T IAN, Fan WX. Research progress on recombinase ploymerase amplification. Biotechnology Bulletin. 2016; 32: 47–53.

[16] Zhang S, Ravelonandro M, Russell P, McOwen N, Briard P, Bohannon S, *et al*. Rapid diagnostic detection of plum pox virus in Prunus plants by isothermal AmplifyRP® using reverse transcription-recombinase polymerase amplification. Journal of Virological Methods. 2014; 207: 114–120.

[17] Ahmed A, van der Linden H, Hartskeerl RA. Development of a recombinase polymerase amplification assay for the detection of pathogenic Leptospira. International Journal of Environmental Research and Public Health. 2014; 11: 4953–4964.

[18] Shi Y, Xu CP, Yu BB, Lu YY, Mei LL. Research progress in recombinase ploymerase amplification (RPA). Chinese Journal of Virology. 2020: 36: 522–532.

[19] Swarts DC, Jinek M. Mechanistic Insights into the cis- and trans-Acting DNase Activities of Cas12a. Molecular Cell. 2019; 73: 589–600.e4.

[20] Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, Saunders SJ, *et al*. An updated evolutionary classification of CRISPR–Cas systems. Nature Reviews Microbiology. 2015; 13: 722–736.

[21] Shmakov S, Smargon A, Scott D, Cox D, Pyzocha N, Yan W, *et al*. Diversity and evolution of class 2 CRISPR-Cas systems. Nature Reviews Microbiology. 2017; 15: 169–182.

[22] Li S, Cheng Q, Wang J, Li X, Zhang Z, Gao S, *et al*. CRISPR-Cas12a-assisted nucleic acid detection. Cell Discovery. 2018; 4: 20.

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[23] Cofsky JC, Karandur D, Huang CJ, Witte LP, Kuriyan J, Doudna JA. CRISPR-Cas12a exploits R-loop asymmetry to form double-strand breaks. Elife. 2020; 9: e55143.
[24] Qiao YG. Characteristic and Treatment Progress of Cervical Cancer. Smaet Healthcare. 2022; 8: 37–39.
[25] Yiping Z, Michael GM, Yuling W, Eugene W, Conny T, Patrick JB, *et al.* Correction: Nucleic acid purification from plants, animals and microbes in under 30 seconds. PLoS Biology. 2018; 16: e1002630.
[26] Arbyn M, Verdoodt F, Snijders PJF, Verhoef VMJ, Suonio E, Dillner L, *et al*. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. The Lancet Oncology. 2014; 15: 172–183.
[27] Torres-Ibarra L, Lorincz AT, Wheeler CM, Cuzick J, Hernández-López R, Spiegelman D, *et al*. Adjunctive testing by cytology, p16/Ki-67 dual-stained cytology or HPV16/18 E6 oncoprotein for the management of HPV16/18 screen-positive women. International Journal of Cancer. 2021; 148: 2264–2273.
[28] Devine C, Viswanathan C, Faria S, Marcal L, Sagebiel TL. Imaging and Staging of Cervical Cancer. Seminars in Ultrasound, CT and MRI. 2019; 40: 280–286.
[29] Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, *et al*. The Elevated 10-Year Risk of Cervical Precancer and Cancer in Women with Human Papillomavirus (HPV) Type 16 or 18 and the Possible Utility of Type-Specific HPV Testing in Clinical Practice. JNCI: Journal of the National Cancer Institute. 2005; 97: 1072–1079.
[30] Yu XQ. Application of liquid-based thin-layer cytology in the screening of cervical cancer. Journal of Clinical Transfusion and Laboratory Medicine. 2011; 13: 254–257.
[31] Lang JH. Cervical cancer and its screening. Progress in Obstetrics and Gynecology. 1994; 3: 275–278.
[32] Zhou L. Comparative study of liquid-based thin-layer cytology detection and traditional Pap smear in the screening of cervical cancer. Qinghai Medical Journal. 2011; 41: 67–68.
[33] Xu DL. Analysis of diagnostic value of TCT and Pap cytology in cervical cancer screening. Chinese Medical Journal of Metallurgical Industry. 2018; 35: 165–166.
[34] Zhao ZS. Comparative study of liquid-based cytology and Pap smear in the screening of cervical cancer. Journal of Wenzhou Medical College. 2013; 43: 125–127.
[35] Luan T, Luan H, Liu W, Yang Q, Zhu Y, Wang C, *et al.* Establishment of a visual method for detection of Actinobacillus pleuropneumoniae based on CRISPR-Cas12a. Chinese Journal of Preventive Veterinary Medicine. 2020; 43: 843–847.
[36] Lei R, Sun XW, Jiang L. Development of rapid detection for Phytophthora syringae based on RPA/CRISPR-Cas12a. Plant Quarantine. 2022; 36: 31–38.
[37] Bowen LHG. Nucleic acid real-time fluorescence PCR detecting high risk HPV (type 16 and 18) combined with TCT to screen cervical cancer. Journal of Chinese High Altitude Medicine and Biology. 2016; 37: 268–272.
[38] Yang XL. Value of Liquid Based Thin Layer Cytology Combined with HPV and P16 Protein Immunohistochemical Staining in Screening Cervical Cancer. Guide of China Medicine. 2022; 20: 104–107.