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ORIGINAL ARTICLE

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CRISPR-based detection of *Helicobacter pylori* in stool samples

Enming Qiu[1] | Shaoqin Jin[2] | Zhuo Xiao[3] | Qianyun Chen[1] | Qiaohui Wang[4] | Huayong Liu[3] | Chanfang Xie[3] | Chong Chen[3] | Zhou Li[1] | Shuai Han[1]

[1]General Surgery Center, Department of Gastrointestinal Surgery, Zhujiang Hospital, Southern Medical University, Guangzhou, China

[2]Department of Gastroenterology, Zhujiang Hospital, Southern Medical University, Guangzhou, China

[3]Guangzhou Pluslife Technology Co, Ltd, Guangzhou, China

[4]The Second School of Clinical Medicine, Southern Medical University, Guangzhou, China

Correspondence
Zhou Li and Shuai Han, General Surgery Center, Department of Gastrointestinal Surgery, Zhujiang Hospital, Southern Medical University, No. 253. Gongye Middle Avenue, Haizhu District, Guangzhou, Guangdong 510280, China.
Emails: gzlizhou@smu.edu.cn; gzhanbo0624@smu.edu.cn

Chong Chen, Guangzhou Pluslife Technology Co., Ltd., Room 406, A01, No. 78. Luntou Road, Haizhu District, Guangzhou, China.
Email: noah.chen@pluslife.com

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Abstract

Background: Noninvasive detection of *Helicobacter pylori* plays an important role in clinical practice. However, few noninvasive methods have been applied in epidemiological studies due to the requirement for expensive equipment and complicated processes. The aim of this study was to establish a reliable, fast, and inexpensive noninvasive method based on CRISPR-Cas12a technology for the detection of *Helicobacter pylori* in stool specimens.

Method: A novel detection method based on CRISPR-Cas12a technology was established and validated with 41 stool specimens collected from Zhujiang Hospital and compared with reliable *Helicobacter pylori* detection assays, such as the rapid urease test and urea breath test.

Result: A CRISPR-Cas12a system-based method was established, and its sensitivity and specificity were evaluated. Utilizing a lateral flow biosensor, the limit of detection was 5 copies/μl, and our method could successfully distinguish *Helicobacter pylori* from other pathogens, suggesting no cross-reactivity with other pathogens. Furthermore, lateral flow biosensor strips were utilized to test stool specimens, which could display the detection results in an accessible way.

Conclusion: Our CRISPR-Cas12a system-based method successfully detected *Helicobacter pylori* in stool specimens. It is a rapid, simple, and inexpensive method for the detection and screening of *Helicobacter pylori*, which makes it a very promising supplemental test. However, its sensitivity and specificity compared with those of the gold standard test still need to be examined.

KEYWORDS
Helicobacter pylori, CRISPR technology, stool, diagnostic methods

1 | INTRODUCTION

Helicobacter pylori (*H. pylori*) is a helical, gram-negative, microaerophilic bacterium that is responsible for several gastroduodenal diseases, such as chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue (MALT), and gastric adenocarcinoma.[1] It is estimated that more than 50% of the global population is infected with *H. pylori*, and it could be worse in developing countries given that the prevalence of *H. pylori* may be correlated with socioeconomic status.[2,3]

It is widely known that *H. pylori* infection can be diagnosed by invasive and noninvasive methods. Noninvasive methods, such as the urea breath test and serological test, are widely applied in clinical practice. However, almost all the commonly used noninvasive

Qiu, Jin and Xiao should be considered joint first authors.

Han, Li and Chen should be considered joint corresponding authors.

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methods have limitations. The urea breath test can be interfered with by *H. pylori* in the oral environment; moreover, insufficient carbon dioxide production in children also limits the application of this test.[4,5] Antibodies against *H. pylori* can exist in the circulatory system for a long time even after eradication, which may cause false positive results, leading to unnecessary treatments.[6,7] Some laboratories utilize PCR to detect nucleic acids of *H. pylori* in stool samples, but it is challenging to obtain well-preserved stool samples with intact nucleic acids. In addition, certain substances from samples may inhibit PCR assays.[8] More importantly, none of these noninvasive methods can be widely applied for epidemiological studies, especially large-scale screening for infected patients. Therefore, novel rapid, accurate, simple, and portable techniques are urgently needed for point-of-care testing. A lateral flow biosensor (LFB) based on gold nanoparticles (AuNPs) or other nanoparticles is a suitable reporter for point-of-care testing. In the presence of analyte, colorimetric change occurs as nanoparticles aggregate, and the signal can be observed with by eye without using complicated instruments.[9] Although there are reports on the use of LFBs for nucleic acid detection,[10,11] LFBs are most likely used for antigen or antibody detection in current in vitro diagnostic (IVD) products.

To achieve this goal, we established a new detection assay based on CRISPR-Cas12a technology to detect *H. pylori* in stool specimens. Clustered regularly interspaced short palindromic repeats (CRISPR) designates a family of DNA sequences found in prokaryotic organisms, and the CRISPR-associated (Cas) immune system has been widely used in the field of molecular biology studies. The Cas immune system targets and cleaves specific nucleic acid sequences that can be used in genome editing.[12] In this study, we established a novel tool based on CRISPR-Cas12a technology to detect *H. pylori* in stool specimens, and the performance of this novel assay was evaluated.

2 | METHODS AND MATERIALS

2.1 | Patients' demographic information and Clinical specimens' collection

Forty-one patients with gastric symptoms who visited Zhujiang Hospital for endoscopic examination were recruited during the study period. The study was approved by the Institutional Review Board (IRB) of the ZhuJiang Hospital of Southern Medical University, and the study conformed to the Declaration of Helsinki. Informed consent was obtained from all the patients involved in this study. Patient demographic information is listed in Table 1. Stool samples (~5 g) produced by normal evacuation were collected from each patient before the endoscopic procedure. Stool samples were placed in flasks containing 3 ml of RNALater (Ambion, AM7020, Thermo Fisher Scientific) and stored in a deep freezer (−80°C) until analysis.

TABLE 1 Patients' demographic information

No.	Patient ID	Age	Sex	*Helicobacter pylori* status
1	2838312	65	Female	Positive
2	2944472	65	Male	Positive
3	2978734	50	Female	Positive
4	2978912	60	Male	Positive
5	2979265	67	Male	Positive
6	2980140	44	Male	Positive
7	2981009	29	Female	Positive
8	3058994	41	Male	Positive
9	2611005	70	Female	Positive
10	760505	44	Male	Positive
11	649973	54	Female	Positive
12	2967597	44	Male	Positive
13	4015221	59	Male	Positive
14	1825948	62	Male	Positive
15	286777	55	Male	Positive
16	1785547	48	Female	Positive
17	1281662	43	Male	Positive
18	1963689	60	Female	Positive
19	2895829	62	Female	Positive
20	4088048	57	Female	Positive
21	2398227	58	Female	Positive
22	609691	68	Female	negative
23	3002456	23	Male	positive
24	2072962	67	Female	positive
25	2057818	55	Male	negative
26	2516138	53	Female	negative
27	368098	71	Male	negative
28	4250177	50	Female	negative
29	1695616	43	Male	positive
30	645044	46	Female	negative
31	4245913	62	Male	negative
32	5012929	68	Female	positive
33	2057818	55	Male	negative
34	4186286	66	Female	negative
35	4084450	56	Female	negative
36	1005575	83	Male	positive
37	1663552	55	Female	negative
38	1222545	73	Male	negative
39	2516542	48	Male	negative
40	1299626	86	Female	negative
41	5011897	66	Male	negative

Note: In total, 41 patients were enrolled in our study. Twenty-six patients were *H. pylori* positive, and fifteen patients were *H. pylori* negative. *H. pylori* status of these patients was determined by rapid urease test or urea breath test.

2.2 | DNA extraction

For clinical samples, a TIANamp Stool DNA Kit (DP328-02, TIANGEN Biotech) was used. DNA was extracted following the manufacturer's instruction. Briefly, 200 µl was used for extraction, and the DNA was eluted in 50 µl.

Bacterial genomic DNA was extracted using a TIANamp Micro DNA Kit (DP316, TIANGEN Biotech) following the manufacturer's protocol. Briefly, 200 µl was used for extraction, and the DNA was eluted in 50 µl.

2.3 | Recombinase Polymerase Amplification

Primers for recombinase polymerase amplification (RPA) with amplicon sizes between 100 and 140 bp, primer melting temperatures between 54°C and 67°C, and primer sizes between 30 and 35 nt were designed using NCBI Primer-BLAST. The primers used in the RPA were the forward primer 5′-GAAATYTTATTGTGTTTA GTGGCTTTATCG-3′ and reverse primer 5′-GGATNGGCTTTCAA GTGGGCTATAARCAATT-3′. The RPA reaction was run following the instructions of TwistAmp Basic (TwistDx). The lyophilized RPA pellet was resuspended in 29.5 µl of rehydration buffer, followed by the addition of primers and 2 µl of the target DNA template, whose concentration was not measured. For comparison, another 2 µl of the target DNA template was used in qPCR detection. The volume was then brought up to 47.5 µl with dH_2O, followed by the addition of 2.5 µl of 280 mM magnesium acetate (MgOAc) to yield a 50-µl mixture containing 0.48 µM forward and reverse primers and 14 mM MgOAc. After briefly mixing, the mixture was incubated at an optimized temperature (37°C) for 30 min. The RPA product was visualized using agarose gel electrophoresis. The protocol of agarose gel electrophoresis was described in a previous study.[13]

2.4 | CRISPR Cas12a reaction

After the RPA reaction, 1 µl of the RPA product was added to the CRISPR-Cas12a reaction system, a 50-µl mixture containing 10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, 20 nM Cas12a (Magigen), and 1 ng/µl guide RNA (gRNA). For the Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay, a 10 nM fluorescence probe (FAM-5′-AAAAAAA-3′-Dabcyl) and 20 nM LFB probe (digoxin-5′-AAAAAAA-3′-biotin) were included, respectively. For the Fluorescence-CRISPR-HP assay, the reaction was incubated at 37°C for 20 min, and real-time or end-point fluorescence detection was performed using a SLAN-96S Real-Time PCR system (Hongshi Medical Technology Co., Ltd.). For the LFB-CRISPR-HP assay, the reaction mixture was loaded onto a LFB after a 20-min incubation at 37°C.

2.5 | Lateral flow biosensor detection

To prepare AuNP-anti-digoxin antibody conjugates, 10 µg of monoclonal anti-digoxin antibodies and 8 µl of 0.1 M K_2CO_3 were added to 1 ml of AuNP solution, and the mixture was shaken gently at room temperature for 1 h. Then, one hundred microliters of 10% BSA was added to the anti-digoxin antibody-coated AuNPs. The solution was incubated at room temperature for 1 h, followed by centrifugation (9.425×10^3 g, 20 min) and three rinses with rinsing buffer (20 mM Na_3PO_4, 5% BSA, 0.25% Tween-20, 10% sucrose, and 0.1% NaN_3) to remove any unbound monoclonal anti-digoxin antibodies. The red pellet was resuspended in 300 µl of rinsing buffer and then stored in a refrigerator at 4°C until use.

For LFB construction, 30 µl of 1 mg/ml streptavidin (SA) and 30 µl of 2 mg/ml goat anti-mouse antibodies were dispensed simultaneously onto a nitrocellulose membrane (25 mm × 30 cm, capillary rate: 140 ± 40 s, thickness: 145 ± 20 µm) with a lateral flow dispenser (Shanghai Kinbio) to form a test zone and a control zone. The membrane was then dried at room temperature for 12 h and stored at 4°C until use. Strips of fiberglass (27 mm in width) were used as sample pads after being soaked in sample pad buffer (1% Triton, 1% BSA, 2% glucose, 50 mM boric acid, pH 8.0). The sample pads were dried and stored in a low-humidity drawer at room temperature. The AuNP-anti-digoxin antibody conjugate was sprayed on the sample pad at a speed of 1 µl/cm. Absorbent pads were strips of thick absorbent paper 17 mm in width. A strip of sample pad, nitrocellulose membrane, and absorbent pad were attached along the long axis of an adhesive plate with an overlap of 2–3 mm. The plate was then cut into 0.22-cm wide strips using a paper cutter (programmed high-speed cutter, Shanghai Kinbio). The strips were stored in a low-humidity drawer at room temperature until use.

For LFB detection, all 50-µl CRISPR-Cas12a reaction products were loaded onto the sample pads, and the results were recorded 10 min later.

2.6 | Sensitivity of the CRISPR-HP assay

To determine the detection limits of the CRISPR-HP assay, *H. pylori* (ATCC 43504) was serially diluted, and genomic DNA was extracted using a TIANamp Micro DNA Kit (DP316, TIANGEN Biotech, Beijing, China). The extract was tested by both the Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay.

2.7 | Specificity of the CRISPR-HP assay

Enterococcus faecalis (ATCC 29212), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 43300), *Staphylococcus epidermidis*, *Salmonella enterica*, *Shigella flexneri*, *Shigella sonnet*, and *Vibrio parahaemolyticus* were used for specificity determination. Genomic DNA

was extracted using a TIANamp Micro DNA Kit (DP316, TIANGEN Biotech). The extracts were tested by the Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay.

3 | RESULTS

3.1 | Establishment of RPA and CRISPR-HP system

Based on the genomic sequence of *H. pylori*, 5 sets of RPA primers and corresponding CRISPR gRNAs were designed and screened (Table 2). The conservation of candidate primers was evaluated by alignment of sequences of six *H. pylori* strains. The performance of the five primer sets was evaluated with genomic DNA extracted from *H. pylori* ATCC 43504 using a TwistAmp Basic Kit. Amplification was carried out at 37°C for 30 min. The RPA products were separated in agarose gels and visualized under ultraviolet light. As shown in Figure 1A, among the five primer sets, the F4/R4 set generated the most products with the expected size, while no nonspecific products were observed in the negative control. Therefore, primer set F4/R4 was chosen and employed in the following RPA assay.

The optimized gRNA for the CRISPR-Cas12a system was also evaluated by fluorescence signal detection. To perform fluorescence detection, 1 μl of RPA product was added to the CRISPR-Cas12a system, and the reaction system was incubated at 37°C for 20 min (20 cycles, 1 min each cycle). The results revealed that gRNA1 generated the most fluorescence signal. Thus, gRNA1 was chosen and applied for the CRISPR-Cas12a system (Figure 1B).

3.2 | LFB-CRISPR-HP assay

Although the Fluorescence-CRISPR-HP assay is a simple method for *H. pylori* detection, fluorescence excitation sources and fluorescence readers are still required. To make the assay simpler and more feasible, for example, not relying on sophisticated instruments, we applied the LFB method, the result of which can be read by eye. The principle is shown in Figure 2A. The target sequence of *H. pylori* was first amplified by RPA and then supplied to the CRISPR-HP system. The presence of the target sequence activated Cas12a, leading to the degradation of the probe. The CRISPR system was similar in the Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay except for the probe used. In the LFB-CRISPR-HP assay, a dual-labeled probe labeled with both digoxin and biotin was used. Finally, the product of the CRISPR-HP system was loaded onto the LFB. The LFB contained anti-digoxin antibodies labeled with gold nanoparticles on the sample pad and two lines on a nitrocellulose membrane. SA and a goat anti-mouse antibody were bound on the first and second lines, respectively. In the absence of target DNA, the probe stayed intact, and AuNPs were captured by the first line through the AuNP-probe-SA link; consequently, a red line appeared at the first line. The remaining AuNPs could also be captured by the second line, and a red line could be observed at the second line. When target DNA exists, the probe is degraded by nonspecific cleavage by Cas12a, and the AuNPs could not be captured by the first line; instead, they were captured by the second line; therefore, a red line appeared at only the second line.

The reaction mixture, which did not use a fluorescence probe but rather a biotin-digoxin probe, was also incubated at 37°C for 20 min for LFB testing, and the sample pad of the strips was inserted into the reaction solution for 10 min. The LFB results were completely consistent with the fluorescence detection results (Figure 2B).

3.3 | Sensitivity and specificity of CRISPR-HP detection assay

After the establishment of the Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay, the sensitivity and specificity of the CRISPR-HP detection assay were evaluated. The genomic nucleic acid of *H. pylori* (ATCC 43504) was serially diluted, and the lowest concentration that CRISPR-HP could detect was considered the limit of detection (LoD). The RPA system was established as previously described and then placed in an isothermal bath at 37°C for 30 min. Then, 1 μl of the RPA product was added into the CRISPR-Cas12a reaction system, and the mixture was placed into

TABLE 2 Sequences of primer and probe used in the article

F1/R1	CGCCCTAAACGATGGATGCTAGTTGTTGGA
	CCACATGCTCCACCGCTTGTGCGGGTCCC
F2/R2	GGCTTTATCGTTGATGAAATTCACTAACAA
	GCTAATCGCYGCTTCTACCACCAAYAACGG
F3/R3	TAGYACTTGGTTTGCAAGYAACAATCTCAC
	TATATGTATAGTTAAGATAAACGCTCACCA
F4/R4	GAAATYTTATTGTGTTTAGTGGCTTTATCG
	GGATNGGCTTTCAAGTGGGCTATAARCAATT
F5/R5	GTAGTCCACGCCCTAAACGATGGATGCTAG
	CGGATTCTCTCAATGTCAAGCCTAGGTAAG
HP-F4-gRNA1	TAATTTCTACTAAGTGTAGATTGGATTACAACCACACCTAT
HP-F4-gRNA2	TAATTTCTACTAAGTGTAGATTTATAGGTGTGGTTGTAATC

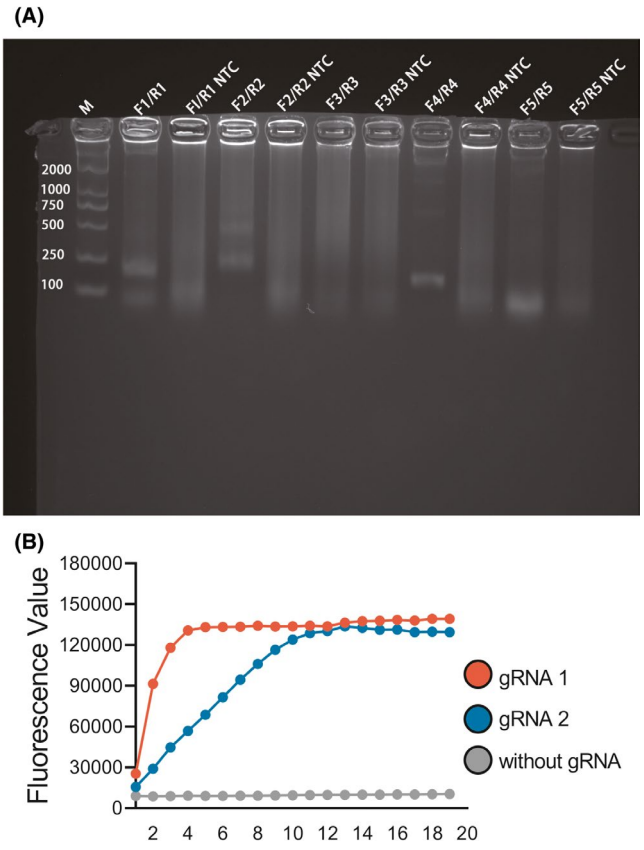


FIGURE 1 Establishment of RPA and CRISPR-HP system. (A) Agarose gel electrophoresis results of all the primer sets. All the primer sets (F1/R1, F2/R2, F3/R3, F4/R4) were separately added into RPA reaction with or without *Helicobacter pylori* DNA, and RPA reaction products were analyzed with electrophoresis. (B) Real-time fluorescence signal of two gRNA designed for CRISPR-HP assay; red, gRNA 1; blue, gRNA 2; gray, without gRNA

an isothermal bath at 37°C for 20 min. Sterile water was set as the non-template control (NTC). The product of the CRISPR-HP assay was tested by both the Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay. As shown in Figure 3A, although the end-point fluorescence intensity of 1 copy/μl was not as high as that with higher *H. pylori* DNA concentrations, it was significantly higher than that of the NTC. The detection limit of the Fluorescence-CRISPR-HP assay was 1 copy/μl. For the LFB-CRISPR-HP assay, the test lines appeared when testing *H. pylori* DNA at a concentration above 5 copies/μl. Thus, the LoD of the LFB-CRISPR-HP assay was 5 copies/μl (Figure 3B).

Then, the specificity of the CRISPR-HP assay was also evaluated. Several common gut pathogens, such as *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *S. aureus* (ATCC 43300), *S. epidermidis*, *S. enterica*, *S. flexneri*, *S. sonnet*, and *V. parahaemolyticus*, were used to evaluate the discrimination of the CRISPR-HP assay. The genomic DNA of all the pathogens, including *H. pylori*, was extracted according to the procedure described above. We established an RPA system in an isothermal bath at 37°C for 20 min. The products were tested by both the Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay (Figure 4).

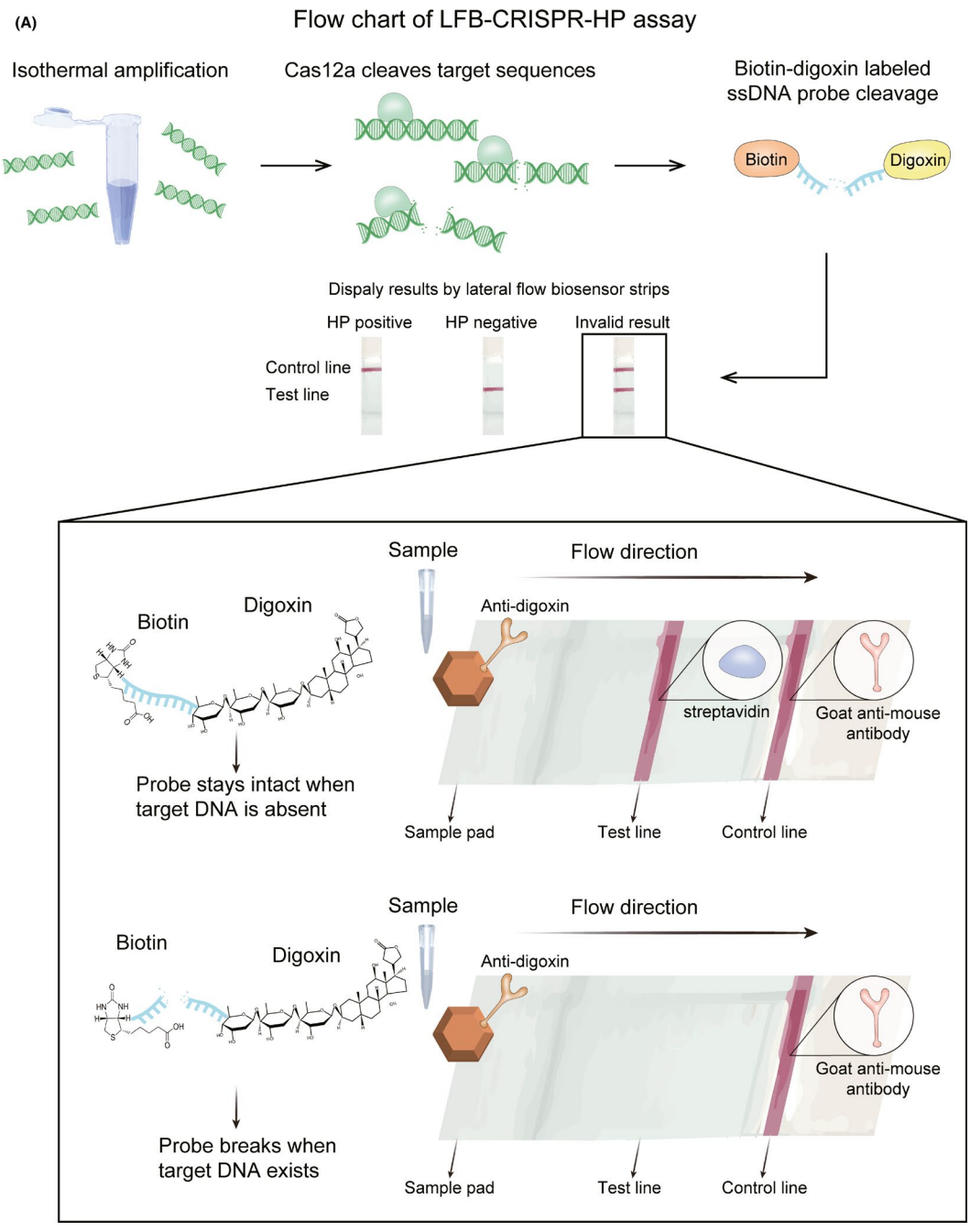
3.4 | RPA system effectively amplify Helicobacter pylori DNA from stool sample

After establishment of the CRISPR-HP assay and evaluation of the performance of the assay, novel testing was applied to detect *H. pylori* DNA in clinical stool specimens. Stool specimens from 41 patients who were diagnosed by either rapid urease test or urea breath test were collected. Since the sampling sites of both methods are different from stool, the diagnosis result may not truly reflect whether there is *H. pylori* DNA in stool specimens. Thus, we set up a qPCR method to detect *H. pylori* DNA in stool specimens as the gold standard. Total DNA was extracted from stool samples and tested by qPCR and the CRISPR-HP assay. Comparison of the clinical diagnosis and qPCR is shown in Table 3. Three samples in first batch which were clinically positive were negative according to the qPCR result. The results of the CRISPR-HP assay are shown in Figure 5. All results for the 41 samples were consistent among qPCR, the Fluorescence-CRISPR-HP assay, and the LFB-CRISPR-HP assay, showing the excellent performance for clinical stool sample analysis of both the Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay.

4 | DISCUSSION

Although the prevalence of *H. pylori* has tended to decrease, millions of people are still infected worldwide. Due to the importance of eradication of *H. pylori* infection and the majority of people becoming infected in childhood, novel rapid, and accurate noninvasive methods are urgently needed for epidemiological studies and large-scale screening tests. Therefore, we established a novel detection method based on CRISPR-Cas12a technology to achieve this goal.

CRISPR technology has recently been widely used in infectious disease detection. Pardee et al. combined isothermal amplification of DNA and CRISPR-Cas9 technology and successfully distinguished different types of Zika virus strains at single-base resolution both in vitro and in a macaque model.[14] After establishing the specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) system based on CRISPR-Cas13 technology, Gootenberg et al. applied it in the identification of *E. coli* and *Pseudomonas aeruginosa* as well as in resistant genotype differentiation of *Klebsiella pneumoniae*.[15] Myhrvold et al. improved the SHERLOCK system by creating a new system named heating unextracted diagnostic samples to obliterate nuclease (HUDSON). HUDSON is a system that can lysis virus vesicles and inactivate the high concentration of RNase present in body fluids through chemical and thermal techniques. By combining those two systems, they directly detected dengue virus strains from blood, serum, and saliva samples within 2 h with high sensitivity and without DNA extraction. In addition to pathogen detection, CRISPR technology has also been used in the study of antibiotic resistance. Several researchers have reported applying CRISPR technology for the identification of antibiotic resistance mutations, providing useful information for antibiotic resistance-related sequence and resistant bacterium detection.[16-19]


(A)
Flow chart of LFB-CRISPR-HP assay
Isothermal amplification
Cas12a cleaves target sequences
Biotin-digoxin labeled ssDNA probe cleavage
Biotin
Digoxin
Dispaly results by lateral flow biosensor strips
HP positive
HP negative
Invalid result
Control line
Test line
Sample
Flow direction
Anti-digoxin
streptavidin
Goat anti-mouse antibody
Probe stays intact when target DNA is absent
Sample pad
Test line
Control line
Probe breaks when target DNA exists

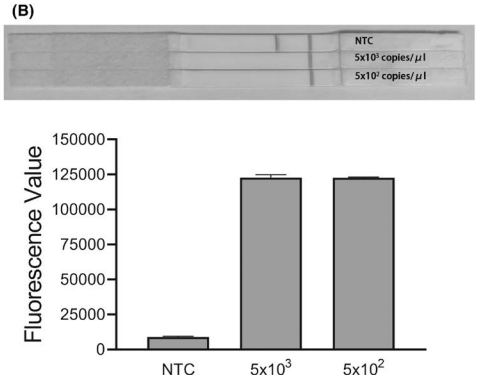

(B)
NTC
5x10³ copies/μl
5x10² copies/μl
Fluorescence Value
150000
125000
100000
75000
50000
25000
0
NTC
5x10³
5x10²

FIGURE 2 Principle and establishment of LFB-CRISPR-HP assay. (A) Illustration of the principle of LFB-CRISPR-HP assay. Target sequence is amplified by RPA and added into the CRISPR. Cas proteins is activated upon specific binding of gRNA to target sequence, which causes the degradation of ssDNA probe. Finally, the integrity of ssDNA probe is detected by LFB. Broken of ssDNA probe leads to the disappearance of test line. (B) Results of LFB-CRISPR-HP assay and Fluorescence-CRISPR-HP assay. NTC, non-template control. Error bars represent ±SD, where n = 3 independent experiments. In CRISPR reaction, 10 mM Tris-HCl, 10 mM MgCl2, 50 mM NaCl, 1 mM DTT, 20 nM Cas12a, and 1 ng/μl gRNA were used

TABLE 3 Result comparison of clinical diagnosis and qPCR

	Clinical diagnosis	
	Positive	Negative
qPCR Positive	23	0
qPCR Negative	3	15

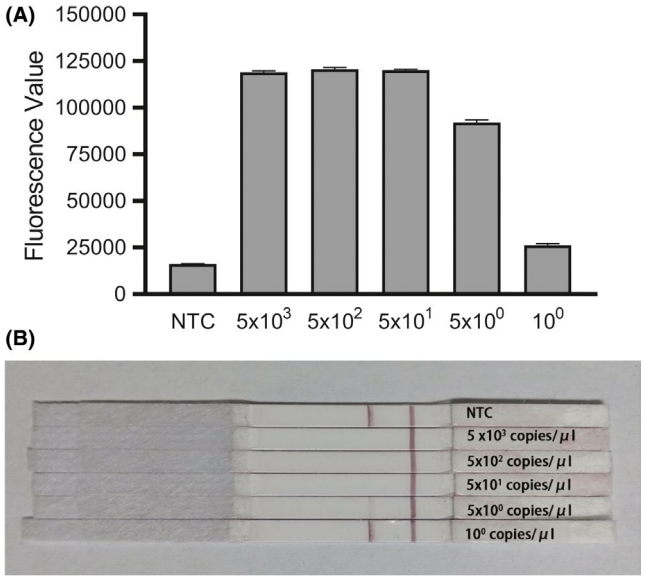


FIGURE 3 Sensitivity of CRISPR-HP assay. (A) Sensitivity of Fluorescence-CRISPR-HP assay. *Helicobacter pylori* DNA with concentration ranging from 1 to 5000 copies/μl was tested. NTC, non-template control. Error bars represent ±SD, where n = 3 independent experiments. (B) Sensitivity of LFB-CRISPR-HP assay. *Helicobacter pylori* DNA with concentration ranging from 1 to 5000 copies/μl was tested. NTC, non-template control

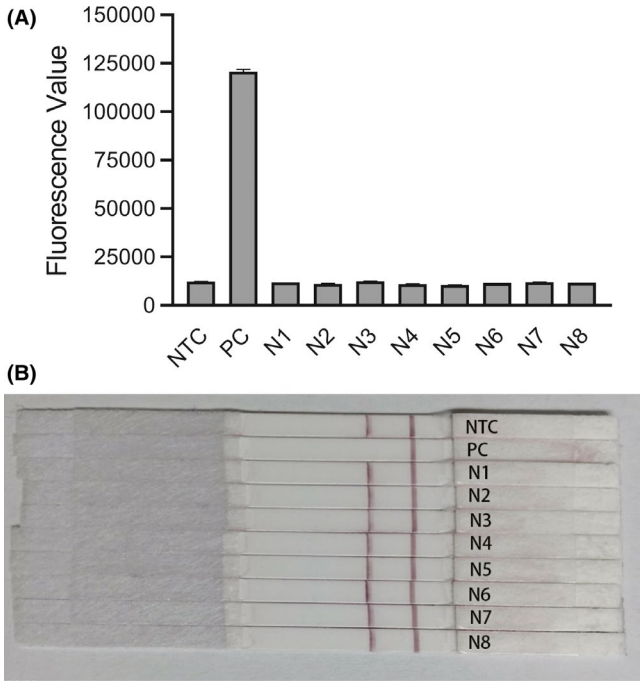


FIGURE 4 Specificity of CRISPR-HP assays. NTC: non-template control; PC: *Helicobacter pylori*; N1: Enterococcus faecalis; N2: Escherichia coli; N3: Staphylococcus aureus; N4: Staphylococcus epidermidis; N5: Salmonella enterica; N6: Shigella flexneri; N7: Shigella sonnet; N8: Vibrio parahaemolyticus. (A) Results of Fluorescence-CRISPR-HP assay. Error bars represent ±SD, where n = 3 independent experiments. (B) Results of LFB-CRISPR-HP assay

In this study, CRISPR-Cas12a technology was applied for the detection of *H. pylori* infection. First, RPA, which can isothermally amplify targeted DNA,[20] was combined with CRISPR-Cas12a technology to build a novel testing method. Primer optimization for RPA was conducted by agarose gel electrophoresis, and the best gRNA for the Cas12a reaction was selected according to the fluorescence signal. After establishment of the new method, the detection limit and discrimination were evaluated. The detection limit was 5 copies/μl, and the new method could accurately identify *H. pylori* in stool specimens without cross-reactivity to other common gut pathogens. Furthermore, our method was utilized to detect *H. pylori* from stool specimens of 41 patients, and the results revealed that our CRISPR-Cas12a method achieved a good diagnostic performance.

Unlike other researchers who usually use CRISPR-Cas13a or CRISPR-Cas9 technology, we applied CRISPR-Cas12a here for several reasons. Cas13a is an RNase that can be guided and activated by RNA. Although Cas13a has great activity in CRISPR RNA (crRNA) processing and RNA cleavage targeting,[21,22] the process may need complicated pretreatment for targeted RNA, which may restrict its application in clinical practice. In contrast to Cas13a, Cas12a is a DNA endonuclease, and its requirements for detection are much lower than those of Cas13a (no complicated preparations are needed), thus enabling Cas12a to be applied in different settings. The structures of Cas12a and Cas9 are similar, but they have different mechanisms of target sequence recognition. For example, Cas9 needs one crRNA and one trans-activating CRISPR RNA (tracrRNA) to target the DNA sequence, while Cas12a needs only one crRNA, which makes Cas12a a simpler system than Cas9.[23] Therefore, the CRISPR-Cas12a method is easier to establish and apply. Moreover, a LFB was utilized to display the result of the whole detection. The LFB has a pretreated strip containing reagents that could react with the sample applied, allowing direct reading of the results by eye.[24] Thus, the LFB helps to reduce the requirement of expertise for

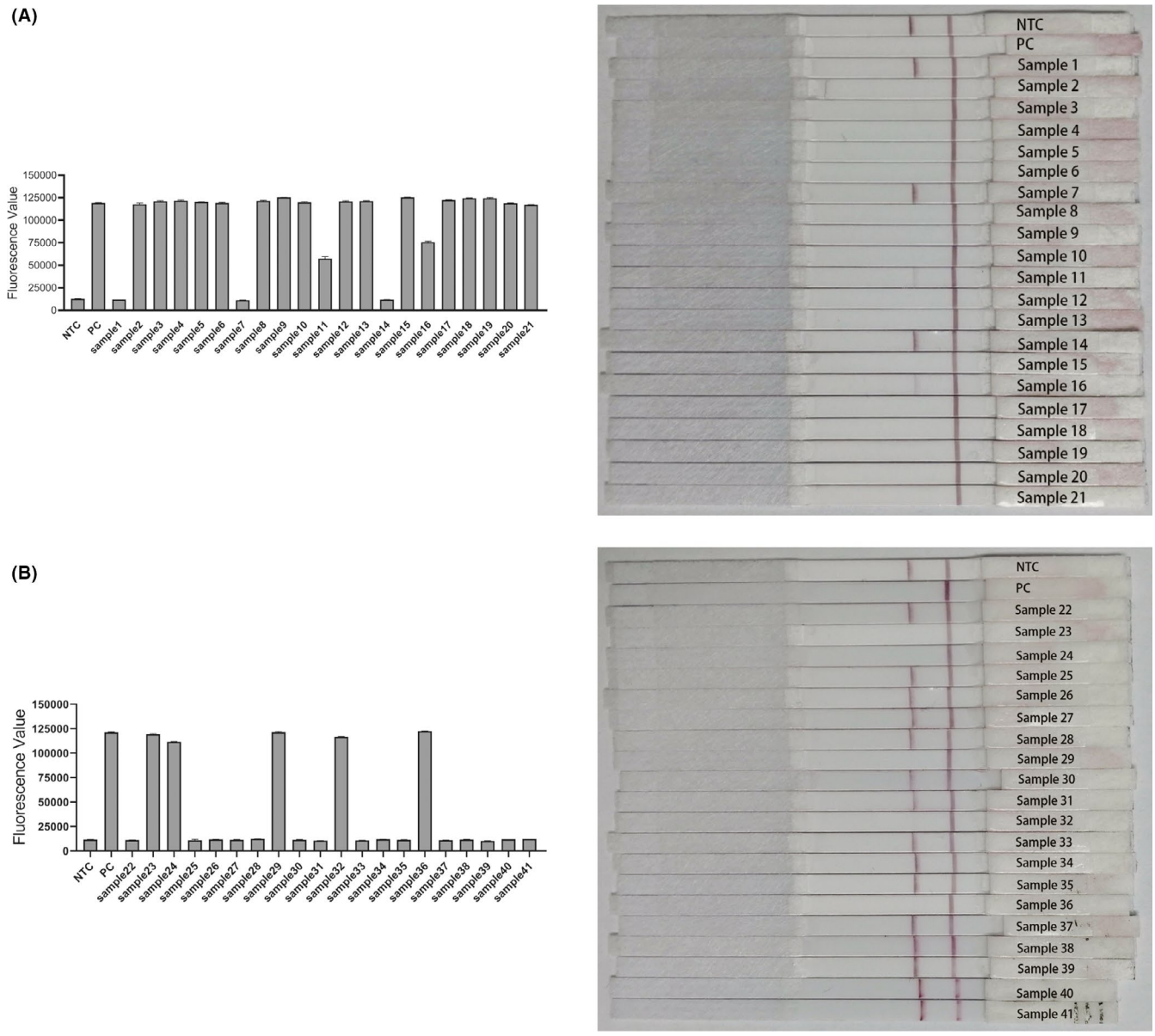


FIGURE 5 Results of CRISPR-HP assay analyzing 41 clinical stool samples. NTC: non-template control; PC: *Helicobacter pylori*; Sample 1 ~ 41: clinical samples. (A) Results of first batch tested by Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay. The first batch contained 21 clinical samples, and all these samples are clinically positive. Three samples were negative according to both Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay. Error bars represent ±SD, where $n = 3$ independent experiments. (B) Results of second batch tested by Fluorescence-CRISPR-HP assay and LFB-CRISPR-HP assay. The second batch contained 20 clinical samples with 15 clinical negative samples and 5 clinical positive samples. All results of these 20 samples are consistent among clinical diagnosis, Fluorescence-CRISPR-HP assay, and LFB-CRISPR-HP assay

interpreting the results, which can broaden its application to various scenarios.

This research has several limitations. First, the sample size was small, namely, 41 patients were enrolled in our study, and their stool specimens were collected. The conclusions might be exaggerated due to the small sample size. Second, the discrepancy in detection results between stool sample-based testing and urea breath tests proposed by other researchers remains unclear. One probable reason that the differences exist might be related to the concentration of *H. pylori* in stool samples, which may be associated with the way that *H. pylori* enters the digestive tract. Barbora Šeligová et al reported that *H. pylori* occurrence is at least 5 times lower in stool specimens than in biopsy samples.[25] In the near future, additional patients will be enrolled for stool specimen collection to evaluate the sensitivity and specificity of our novel testing strategy.

5 | CONCLUSION

We established a novel testing approach based on CRISPR-Cas12a technology, and its rapidity and accuracy make it a promising tool for *H. pylori* detection. Displaying the detection result with a LFB makes

it a possible point-of-care test for epidemiological and large-scale screening studies. However, additional stool specimens are needed for the evaluation of its sensitivity and specificity in clinical trials.

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Not available.

CONFLICT OF INTEREST

The authors declare no conflicts of interest here.

AUTHOR CONTRIBUTION

Enming Qiu and Zhuo Xiao drafted the manuscript. Shaoqin Jin analyzed the clinical data. Qianyun Chen and Qiaohui Wang collected and transported the samples. Huayong Liu, Chanfang Xie, and Chong Chen finished the experiments. Zhou Li and Shuai Han designed the whole project and revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Zhou Li https://orcid.org/0000-0002-4785-261X

REFERENCES

1. Camilo V, Sugiyama T, Touati E. Pathogenesis ofHelicobacter pyloriinfection. *Helicobacter*. 2017;22:e12405. http://dx.doi.org/10.1111/hel.12405
2. Mitchell H, Katelaris P. Epidemiology, clinical impacts and current clinical management of *Helicobacter pylori* infection. *Med J Aust*. 2016;204(10):376-380.
3. Ke H, Li J, Lu B, et al. The appropriate cutoff gastric pH value for *Helicobacter pylori* eradication with bismuth-based quadruple therapy. *Helicobacter*. 2021;26(1):e12768.
4. Khalilpour A, Kazemzadeh-Narbat M, Tamayol A, Oklu R, Khademhosseini A. Biomarkers and diagnostic tools for detection of *Helicobacter pylori*. *Appl Microbiol Biotechnol*. 2016;100(11):4723-4734.
5. Yee JK. *Helicobacter pylori* colonization of the oral cavity: A milestone discovery. *World J Gastroenterol*. 2016;22(2):641-648.
6. Kawai S, Arai K, Lin Y, et al. Comparison of the detection of *Helicobacter pylori* infection by commercially available serological testing kits and the (13)C-urea breath test. *J Infect Chemother*. 2019;25(10):769-773.
7. Kosunen TU, Seppälä K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA, and IgM antibody titres after eradication of *Helicobacter pylori*. *Lancet*. 1992;339(8798):893-895.
8. Beer-Davidson G, Hindiyeh M, Muhsen K. Detection ofHelicobacter pyloriin stool samples of young children using real-time polymerase chain reaction. *Helicobacter*. 2018;23(1):e12450. http://dx.doi.org/10.1111/hel.12450
9. Peña-Rodríguez M, Viera-Segura O, García-Chagollán M, et al. Performance evaluation of a lateral flow assay for nasopharyngeal antigen detection for SARS-CoV-2 diagnosis. *J Clin Lab Anal*. 2021:35(5);e23745.
10. Xiao Z, Lie P, Fang Z, et al. A lateral flow biosensor for detection of single nucleotide polymorphism by circular strand displacement reaction. *Chem Commun (Camb)*. 2012;48(68):8547-8549.
11. Zhang J, Cao J, Zhu M, Xu M, Shi F. Loop-mediated isothermal amplification-lateral-flow dipstick (LAMP-LFD) to detect Mycoplasma ovipneumoniae. *World J Microbiol Biotechnol*. 2019;35(2):31.
12. Tsou JH, Leng Q, Jiang F. A CRISPR test for detection of circulating nuclei acids. *Transl Oncol*. 2019;12(12):1566-1573.
13. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. *J Vis Exp*. 2012;62:3923.
14. Pardee K, Green AA, Takahashi MK, et al. Rapid, low-cost detection of zika virus using programmable biomolecular components. *Cell*. 2016;165(5):1255-1266.
15. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science*. 2018;360(6387):439-444.
16. Müller V, Rajer F, Frykholm K, et al. Direct identification of antibiotic resistance genes on single plasmid molecules using CRISPR/Cas9 in combination with optical DNA mapping. *Sci Rep*. 2016;6:37938.
17. Guk K, Keem JO, Hwang SG, et al. A facile, rapid and sensitive detection of MRSA using a CRISPR-mediated DNA FISH method, antibody-like dCas9/sgRNA complex. *Biosens Bioelectron*. 2017;95:67-71.
18. Aydin S, Personne Y, Newire E, et al. Presence of type I-F CRISPR/Cas systems is associated with antimicrobial susceptibility in *Escherichia coli*. *J Antimicrob Chemother*. 2017;72(8):2213-2218.
19. Price VJ, Huo W, Sharifi A, Palmer KL. CRISPR-Cas and restriction-modification act additively against conjugative antibiotic resistance plasmid transfer in Enterococcus faecalis. *mSphere*. 2016;1(3):e00064-16.
20. Daher RK, Stewart G, Boissinot M, Bergeron MG. Recombinase polymerase amplification for diagnostic applications. *Clin Chem*. 2016;62(7):947-958.
21. Liu L, Li X, Wang J, et al. Two distant catalytic sites are responsible for C2c2 RNase activities. *Cell*. 2017;168(1–2):121-134.e112.
22. Liu L, Li X, Ma J, et al. The molecular architecture for RNA-guided RNA cleavage by Cas13a. *Cell*. 2017;170(4):714-726.e710.
23. Zetsche B, Gootenberg JS, Abudayyeh OO, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*. 2015;163(3):759-771.
24. Yrad FM, Castañares JM, Alocilja EC. Visual detection of Dengue-1 RNA using gold nanoparticle-based lateral flow biosensor. *Diagnostics*. 2019;9(3):74.
25. Šeligová B, Lukáč Ľ, Bábelová M, Vávrová S, Sulo P. Diagnostic reliability of nested PCR depends on the primer design and threshold abundance of *Helicobacter pylori* in biopsy, stool, and saliva samples. *Helicobacter*. 2020;25(2):e12680.

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