One-tube SARS-CoV-2 detection platform based on RT-RPA and CRISPR/Cas12a

Yangyang Sun [a, b, c, d,†], Lei Yu [a, b, c, d,†], Chengxi Liu [e,†], Wei Chen [a, b, c, d], Dechang Li [f,*] and Weiren Huang [a, b, c, d,*]

[a] Department of Urology, Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, International Cancer Center, Shenzhen University School of Medicine, Shenzhen 518039, China

[b] International Cancer Center, Shenzhen University School of Medicine, Shenzhen 518060, China

[c] The First Affiliated Hospital of Shantou University, Shantou 515041, China

[d] Guangdong Key Laboratory of Systems Biology and Synthetic Biology for Urogenital Tumors, Shenzhen 518035, China

[e] Shanghai Center for Systems Biomedicine, Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Jiao Tong University, Shanghai 200240, China

[f] Yuebei Second People's Hospital, Shaoguan, Guangdong 512000, China

[*] Corresponding author: lidechangsg@sina.com and pony8980@163.com

[†] These authors contributed equally to this work.

Abstract

Background: COVID-19 has spread rapidly around the world, affecting almost every person. When lifting certain mandatory measures for an economic restart, robust surveillance must be established and implemented, with nucleic acid detection for SARS-CoV-2 as an essential component.

Methods: We designed RT-RPA (Reverse Transcription and Recombinase Polymerase Isothermal Amplification) primers of RdRp gene and N gene according to the SARS-CoV-2 gene sequence. We optimized the components in the reaction so that the detection process could be carried out in one tube. The specificity was demonstrated through detecting nucleic acid samples from seven human coronaviruses. Clinical samples were used to validate the platform and all results were compared to rRT-PCR. RNA standards diluted by different gradients were used to demonstrate the limit of detection. Furthermore, we have developed a lateral flow assay based on OR-DETECTR for the detection of COVID-19.

Results: We have developed a one-tube detection platform based on RT-RPA and DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) technology, termed OR-DETECTR, to detect SARS-CoV-2. The detection process is completed in one tube, and the time is 50min. The method can specifically detect SARS-CoV-2 from seven human coronaviruses with a low detection limit of 2.5 copies/μl input. Results from six SARS-CoV-2 patient samples, eight samples from patients with fever but no SARS-CoV-2 infection, and one mixed sample from 40 negative controls showed that OR-DETECTR is 100% consistent with rRT-PCR. Furthermore, we have developed a lateral flow assay based on OR-DETECTR for the detection of COVID-19.

Conclusions: OR-DETECTR detection platform is rapid, accurate, tube closed, easy-to-operate, and free of large instruments for COVID-19 detection.

Keywords COVID-19; SARS-CoV-2 assay; RT-RPA; CRISPR/Cas

Background

The outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has attracted the attention of people all over the world. By August 1, 2020, there were 17,396,943 cases and 675,060 deaths worldwide *(1)*. In China, for example,

although the spread of the epidemic has potentially been contained, there are still sporadic cases of cluster infection or imported coronavirus disease (COVID-19). Coexistence with the novel coronavirus will become a new normality. To ensure orderly production, life, work and study, large-scale, rapid, and accurate viral detection is essential *(2)*.

For SARS-CoV-2, laboratories around the world have developed a variety of viral nucleic acid detection platforms, such as sequencing *(3, 4)*, digital PCR *(5)*, and the widely used real-time reverse transcription PCR (rRT-PCR) recommended by China, CDC, and the WHO *(6, 7)*. Generally, the sensitivity of rRT-PCR assays ranges from 3.8 to 10 RNA copies per reaction, with high specificities. These technical platforms require expensive equipment and professional technicians. Additionally, the testing processes take more than 1.5 hours *(8)*.

In vitro nucleic acid detection technologies based on clustered regularly interspaced short palindromic repeats (CRISPR)/Cas targeted recognition and promiscuous cleavage activities have been widely reported *(9-12)*. At present, the method of combining loop-mediated isothermal amplification (LAMP) with CRISPR/Cas12 *(13)* or the combination of recombinase polymerase amplification (RPA) and CRISPR/Cas13 *(14)* has been used for the detection of SARS-CoV-2. Relatively complex primers need to be designed in LAMP amplification, and more components need to be added in the CRISPR/Cas13a testing mix. Since the reactions of isothermal amplification and CRISPR/Cas interfere with each other, the isothermal amplification step is performed first and then a small amount of amplification product is transferred to the CRISPR/Cas detection system. That might easily produce aerosol contamination in the lab. These rapid platforms have low cost and low equipment requirements. The highest sensitivity of these assays ranges from 1 to 4.5 RNA copies per μl *(8)*.

In this study, OR-DETECTR platform was used to detect SARS-CoV-2. This platform might shorten detection time, reduce equipment cost and not produce aerosol contamination.

Methods

Oligonucleotides and crRNA preparation. Primers with a T7 promoter for RPA amplification are compatible with CRISPR/Cas12a or CRISPR/Cas13a detection system. The primers for RdRp gene amplification were forward primer with T7 promoter 5'-GAAATTAATACGACTCACTATAGGGctaatgagtgtgctcaagtattgagtgaaat-3' and reverse primer 5'-caaatgttaaaaacactattagcataagcagt-3'. The primers for N gene amplification were forward primer 5'-GAAATTAATACGACTCACTATAGGGcagcagtaggggaacttctcctgctagaatgg-3' and reverse primer 5'-tggcctttaccagacattttgctctcaagctg-3'. The crRNA for RdRp gene (5'-GGGAAUUUCUACUGUUGUAGAU acauauagugaaccgccacaca-3') and the crRNA for N gene (5'-GGGAAUUUCUACUGUUGUAGAU cugcugcuugacagauuga-3') were from synthetic oligo fragments. Each oligo fragment contained a T7 promoter which was used as the template for *in vitro* transcription at 37℃ for 16 h using HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB). RNA was purified using Agencourt RNAClean XP (Beckman Coulter). RNA was quantified by Nanodrop and diluted in nuclease-free water to working concentrations. The crRNAs and ssDNA reporter (5'-6-FAM-TTATTATT-BHQ1-3' and 5'-6-FAM-TTATTATT-Biotin-3') were used for DETECTR detection. The crRNAs (5'-GATTTAGACTACCCCAAAAACGAAGGGGACTAAAACgcagcagcaaagcaagagcagcatcac-3') targeting N gene and ssRNA reporter (5'-6-FAM-UUUUUC-BHQ1-3') were used for SHERLOCK detection. All these oligonucleotides were synthesized by General Biosystems, Ltd. (Anhui).

Artificial sample preparation. Genomes of seven coronaviruses that infect humans were aligned using Clustalw. Seven 300 bp genomic fragments of RdRp gene encompassing 88 bp upstream and 90 bp downstream of the RPA amplification site were synthesized and inserted into pUC57, respectively. Seven 300 bp genomic fragments of N gene encompassing 93 bp

upstream and 90 bp downstream of the RPA amplification site were synthesized and individually inserted into pUC57. These plasmids were used to assess the specificity of the primers and crRNAs. All these oligonucleotides were synthesized by General Biosystems, Ltd. (Anhui).

COVID-19 RNA reference material for LoD determination was purchased from the Chinese Academy of Metrology. ORF1ab gene segment (14911-15910, GenBank No.NC_045512) was 1.1×10^6 copies/μl and N gene was 8.38×10^5 copies/μl. The RNA standard of the RdRp gene was diluted with TE buffer to 6 concentrations of 100 copies/μl, 50 copies/μl, 25 copies/μl, 12.5 copies/μl, 10 copies/μl, 5 copies/μl and extra 0 copies/μl (blank control). The RNA standard of the N gene was diluted with TE buffer to 7 concentrations of 80 copies/μl, 40 copies/μl, 20 copies/μl, 10 copies/μl, 5 copies/μl, 2.5 copies/μl, 1.25 copies/μl and extra 0 copies/μl (blank control).

Human clinical sample collection and preparation. Pharyngeal swab samples were collected from 14 fever patients in Shenzhen Second Peoples' Hospital by the Clinic Diagnosis Laboratory and the nucleic acids of each sample was extracted with the pre-packaged nucleic acid extraction kit (Da'An Gene., Ltd.), according to the manufacturer's instructions, resulting in 55 μl extracts for each sample.

An additional 40 pharyngeal swab samples from 40 fever patients in Shenzhen Second Peoples' Hospital were also collected and extracted. rRT-PCR assay employing 5 μl of extract from each sample, were all negative. The remaining 50 μl extract of each sample was combined and 5 μl of the mixture was once again analyzed by rRT-PCR and confirmed to be SARS-CoV-2 negative. The RNA mix was named N50B.

OR-DETECTR assays. The OR-DETECTR platform combines recombinase polymerase amplification (RPA) and CRISPR/Cas12a detection. One RPA pellet per tube was

resuspended with 29.5 μl rehydration buffer in the TwistAmp basic RPA kit. RT-RPA reactions containing 5 μl of sample extract, 14.75 μl resuspended RPA solution, 1.2 μl of each primer (10 mM), 0.75 μl RNA reverse transcriptase (100 U/ml), 0.85 μl nuclease-free water and 1.25 μl MgAc (280 mM), with the total volume of 25 μl. The multiple RT-RPA reaction contains four primers, each of which is 0.6 μl (10 mM). After being gently mixed and centrifuged, the mixture was placed at the bottom of a centrifuge tube. The CRISPR/Cas12a reaction mix consisted of 2 μl NEB Buffer 3.1 (10×), 2 μl LbCas12a (100 ng/μl), 2 μl crRNA (200 nM), 2 μl ssDNA reporter, and 12 μl nuclease-free water. This 20 μl CRISPR/Cas12a mix was added to the lid of the centrifuge tube before sealing. Tube was placed in a thermocycler or water bath at 42 ℃ and incubated for 30 min. The CRISPR/Cas12a mixture in the cap was combined with the RPA reaction product by short spin. If ssDNA reporter was 5'-6-FAM-TTATTATT-BHQ1-3', the centrifuge tube was then placed in the PE microplate reader at 42 ℃, with the fluorescence signal collected every min for 20 min total. If ssDNA reporter was 5'-6-FAM-TTATTATT-Biotin-3', the centrifuge tube was then placed in a thermocycler or water bath at 42°C. The reaction product (3 μl) was diluted in 400 μl water, after which 80 μl was added to the sample hole of the test card (GenDx Suzhou).

OR-SHERLOCK assay. The OR-SHERLOCK platform combines recombinase polymerase amplification (RPA) and CRISPR/Cas13a detection. RPA amplification step is same as OR-DETECTR. The CRISPR/Cas13a reaction mix consisted of 2 μl Buffer (400 mM Tris, pH 7.4), 2 μl LwCas13a (20 ng/μl), 1 μl crRNA (10 nM), 2 μl ssRNA reporter (4 μM), 0.5 μl RNAse inhibitor (40 U/μl), 0.1 μl T7 Polymerase (100 U/μl), 0.8 μl rNTP (100 mM each), 1 μl $MgCl_2$ (120 mM), 10.6 μl nuclease-free water. The centrifuge tube was incubated in a PCR machine or water bath at 42 ℃ for 30 min. The detection process of OR-SHERLOCK was the same as OR-DETECTR.

rRT-PCR assays. Real time RT-PCR reaction kit was supplied by Biogerm, which uses the

primers recommended by the China CDC. Reactions were conducted in a 25 μl volume following the kit instructions. Reaction cycle parameters were set as reverse transcription at 50℃ for 10 min, denaturation at 95℃ for 5 min, followed by 40 cycles of amplification, 95℃ for 10 s and 55℃ for 40 s.

Statistical Analysis. Statistical analyses were performed using GraphPad Prism. $P<0.001$ was considered to indicate a statistically significant difference.

Results

Here OR-DETECTR was used for the detection of RNA extracted from pharyngeal swabs of COVID-19 patients. The detection process of the OR-DETECTR platform is shown in Figure 1a. The RNA sample extracted from the pharyngeal swab is added to the RT-RPA mix at the bottom of the centrifuge tube while the DETECTR mix is located on the transparent tube lid. To reduce the use of the equipment, the reaction temperature for reverse transcription, amplification and detection was set at 42℃. Reverse transcription of viral RNA and high-sensitivity amplification of specific sequences can be completed simultaneously in 30 min at 42℃. RT-RPA and DETECTR components are physically separated during this amplification process. After the amplification, the DETECTR mix on the lid is mixed with amplification products by centrifugation. The amplicon can be recognized by specific CRISPR RNA (crRNA) segments and the reaction activates the cis- and trans-nuclease activities of Cas12a. Cas12a nonspecifically cleaves the reporter sequence (quenched fluorescent ssDNA) and resulting fluorescence generated by the reaction system is recorded within 20 minutes at 42℃. There is no need to open the lid during the entire detection process and the turnaround time is only 50 min. A Cas13a-based molecular detection platform is named Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) *(12)*, and we have validated that the one-tube detection platform based on RT-RPA and SHERLOCK, termed OR-SHERLOCK, also works. Preliminary comparison of

OR-DETECTR and OR-SHERLOCK revealed that the detection effect was basically the same, but the components of the SHERLOCK mix are more complex (Figure S1, S2).

SARS-CoV-2 is an enveloped virus that contains a positive-sense single-stranded RNA genome of 29,903 nucleotides *(15)*. We designed primers targeting the RdRp (RNA-dependent RNA polymerase) and N (nucleoprotein) genes of SARS-CoV-2 (Fig. 1b). There is a partial overlap within the RdRp gene amplification region between OR-DETECTR assay and the World Health Organization (WHO) assay *(16)*, and this RdRp gene of pan beta-CoV was designed as a reference assay *(2, 17)*. The majority of our N gene amplification region overlaps the China-CDC assay (N gene assay) *(6)*. Including the required protospacer adjacent motif sites (PAM), we designed Cas12a crRNAs to detect RdRp and N gene amplicons. In addition, we added a T7 promoter to the 5' end of the forward primers, so the amplification products could also be detected by the OR-SHERLOCK platform (Figure S2).

There are seven coronaviruses that are established that infect humans. Four groups are endemic worldwide, namely alpha coronaviruses 229E and NL63, and beta coronaviruses OC43 and HKU1, all causing the common cold. Novel human pathogens can evolve from animal coronaviruses zoonotically jumping into humans. Three recent examples are the beta coronaviruses SARS-Cov-2 (causes COVID-19), SARS-CoV (causes severe acute respiratory syndrome, or SARS), and MERS-CoV (causes Middle East Respiratory Syndrome, or MERS) *(4)*. Partial RdRp and N gene sequences of all seven coronaviruses were aligned to the corresponding sequences of amplicons by ClustalW and GeneDoc (Fig.1c, 1e). It can be seen from the sequence similarity that the RdRp gene sequence of SARS and MERS virus has the highest similarity with SARS-COV-2, and the N gene sequence of SARS virus has the highest similarity with SARS-COV-2. Using artificially synthesized plasmids carrying the N gene and RdRp gene of the seven coronaviruses respectively (Table S1), OR-DETECTR can

distinguish SARS-CoV-2 with no cross-reactivity for the other six coronavirus strains using the N gene target, and it can distinguish SARS-CoV-2, SARS-CoV, MERS-CoV with expected cross-reactivity for the other three coronavirus strains with the RdRp gene target (Figure 1d, 1f). Including one positive control (PC: 80 copies per μl input) and one negative control (NC: no template) per trial, we recorded the fluorescence signal value every min for 19 minutes, defined the fluorescence signal value at 0 min as B, and defined the fluorescence signal value at 19 min as F. In each test, the background subtracted fluorescence was normalized by the negative control, and the fold change values (FC) were generated as FC = (F (PC) - B (PC)) / (F (NC) - B (NC)).

Next, we used the OR-DETECTR platform to test 14 RNA samples extracted from pharyngeal swabs, including six rRT-PCR-positive COVID-19 patients and eight rRT-PCR-negative patients with fever. N50B was a mixture of 40 negative samples, which was included as one sample and also tested via OR-DETECTR. Cycle Threshold (CT) values of N and Orf1ab genes were assessed using the SARS-CoV-2 test kit from BioGerm (Table S2). From the results, 4001N was a positive sample (FC-RdRp is 1.9 and FC-N is 4.6) by OR-DETECTR, and CT values of 4001N were 38.9 (Orf1ab) and 36.8 (N gene), which meant this clinical sample with very low viral loads. Although RT-PCR can be used for quantitative analysis and OR-DETECTR cannot, the judgment of negative and positive results between the two platforms was consistent comparing CT and FC values (Figure 2a, 2b, S3, Table S2). The negative samples yielded a maximal FC value of 0.8 with the RdRp gene and 1.3 with the N gene, whereas positive samples had a minimal FC value of 1.9 on RdRp and 4.6 on the N gene. Cut-offs (1.6 on RdRp gene and 2.6 on N gene) were set at two times the maximal FC value of negative samples.

We next evaluated the limit of detection (LoD) of the OR-DETECTR system with SARS-CoV-2 (Figure. 2c, 2d). A commercial RNA standard containing RdRp transcripts

(1.1×10^6 copies/μl) and N gene transcripts (8.38×10^5 copies/μl) was serially diluted in TE buffer, with seven replicates at each concentration, with a no RNA negative control of buffer alone. The estimated LoD for the OR-DETECTR is 10 copies per μl input with RdRp, as FC values of all seven replicates were greater than 1.6. The LoD with the N gene transcript target is 2.5 copies per μl input, because FC values of all seven replicates were greater than 2.6. The N gene target has superior sensitivity and specificity to RdRp, so RdRp gene was designated as a reference assay.

To avoid using large equipment, the OR-DETECTR assay can be visualized by a test card with a lateral flow strip. Activated-Cas12a cleaves FAM-biotin reporter and generates a signal at the first detection line (test line), while uncleaved-reporters are captured at the second detection line (that is, the control line). The OR-DETECTR assay is considered positive if there are two lines, is considered negative if there is one control line and is meaningless if there is no control line. There were two replicates per dilution, and both 2.5 copies per μl input were classified as positive, but only one of 1.25 copies per μl input was positive. The LoD of the lateral flow strip is 2.5 copies per μl input, which is the same as the fluorescence plate reader (Figure 2e). Additionally, the visual signal was achieved within 4 min by test card. Based on the lateral flow strip, we tried a multiple RT-RPA reaction. The RdRp gene and N gene were amplified in a RT-RPA mixture, after which the amplified product was detected in two CRISPR mixtures containing the crRNA specifically targeting the RdRp or N gene. The results were shown through two lateral flow strips. Results indicated that multiple RT-RPA detection was feasible and that when the sample concentration was 10 copies per μl input, both RdRp and N genes were positive (Figure S4).

Discussion

Effective SARS-CoV-2 diagnosis is an important prerequisite for controlling the COVID-19 pandemic. The WHO proposed the ASSURED criteria (Affordable, Sensitive,

Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users) to determine whether the diagnostic methods being used meet the needs of epidemic control *(18)*. However, there are very few diagnostic methods that meet all standards. For example, rRT-PCR testing is not fast enough, and many kits require qualified laboratories, expensive equipment and professional testing personnel.

Although rRT-PCR is currently the most commonly used diagnostic method for COVID-19, the LOD of those kits developed by different companies ranges from 100 copies/ml to 1000 copies/ml *(8)*. A real-time PCR system is a relatively complex and expensive instrument which not all hospitals could configure in their laboratory departments. This means that samples need to be sent to the local CDC for testing, which increases testing time as well as various other risks. The detection method based on rRT-PCR is not flexible, since one instrument detects one 96-well plate at a time and the turnaround time is greater than 1.5h. In contrast, the detection method based on isothermal amplification is flexible, which greatly improves detection efficiency as the number of samples ranges from 1 to 384 *(13)*, and the fluorescence detection time is less than 30 minutes at a time.

The detection methods based on CRISPR/Cas are widely used for the detection of COVID-19, using crRNA combined with Cas12 or Cas13 to specifically target DNA or RNA to stimulate the trans-cleavage function of the Cas protein and so amplify the fluorescent signal of the reporter group to detect SARS-CoV-2 nucleic acid amplification products *(14, 19, 20)*. The amplification of viral nucleic acid is the key to whether it can be specifically detected. Comparative analysis of several isothermal amplification methods indicated that the primer design of the LAMP method is relatively complicated and that it is hard to perform multiple LAMP amplifications *(21)*. Abbott ID Now, which is based on NEAR (Nicking Enzyme Amplification Reaction), only requires 5 min to give a positive result. However, issues with false negativity have been raised due to its relatively high LOD. RT-RPA

amplification has high sensitivity and its primers are easily designed. Therefore, in our OR-DETECTR assay, we used RT-RPA as a potential means of template amplification. In this way, multiple RT-RPA can be performed.

We have developed two detection platforms, OR-DETECTR and OR-SHERLOCK. Through comparison, we found that: 1) the turnaround time, sensitivity, and specificity of OR-DETECTR based on Cas12a and OR-SHERLOCK based on Cas13a are approximately the same; 2) The design of crRNA is very simple in the OR-SHELOCK platform because the PAM region (Protospacer Adjacent Motif) is not needed, but since Cas13a targets RNA, the detection mixture needs to be added to the components necessary for in vitro transcription, increasing the cost; 3) The crRNA used by OR-DETECTR requires a specific PAM region. As the genome of the SARS-CoV-2 virus is 29,903 nucleotides, it is not hard to find suitable PAM regions. Additionally, OR-DETECTR targets DNA, meaning no further components are required.

According to current reports, most COVID-19 detection methods based on isothermal amplification and CRISPR/Cas require a two-step reaction. First, the reverse transcription and isothermal amplification are performed, after which a small amount of amplification product is taken for CRISPR/Cas detection. This is because isothermal amplification and the CRISPR detection interfere with each other. The transfer process requires opening the amplification reaction tube, which will cause aerosol pollution *(13, 14)*. In clinical laboratories, if aerosol contamination occurs, false-positive results will be produced. Our OR-DETECTR test uses a one-tube detection method. We put the CRISPR mixture in the lid of the tube, and RT-RPA mixture in the bottom of the tube. After the RT-RPA reaction is completed, the amplification product is mixed with the CRISPR mixture by transient centrifugation, after which the fluorescence signal is detected. This reduces the operation of

pipetting, shortens the time and reduces the waste of consumables. Importantly, it can effectively avoid the unavailability of clinical laboratories caused by aerosol contamination.

Lateral flow paper has been applied to CRISPR-based detection methods for signal output. For single-sample detection, this is a good solution as it does not require any equipment to read the results, and it is promising for COVID-19 detection at home. According to current reports, its LOD is much lower than fluorescence-based detection methods *(13)*. Our OR-DETECTR assay can be easily read by a fluorescence plate reader to achieve sensitive and high-throughput SARS-CoV-2 detection. Additionally, the sensitivity of our lateral flow paper detection is the same as the fluorescence signal; that is, 2.5 copies per μl input. Finally, our OR-DETECTR test results are consistent with the rRT-PCR test results in the clinic, even for samples with very low viral loads. OR-DETECTR is suitable for clinical laboratories, point-of-care settings with the appropriate equipment, and perhaps for household purposes. Our OR-DETECTR is designed to provide a clear positive or negative result, rather than a quantitative viral load. Considering that the diagnosis of COVID-19 requires a fast, specific, and sensitive method, we do not think this is a disadvantage. Further, our OR-DETECTR method continues to use samples after nucleic acid extraction. Since it is currently difficult to obtain clinical samples, we have no way to continue developing methods for direct nucleic acid amplification from nasopharyngeal swabs.

Conclusion

Expensive equipment and longer detection time are limitations of the rRT-PCR platform. The currently available CRISPR/Cas-based detection platform requires separation of the amplification and detection steps, increasing the complexity of the operation and the risk of aerosol contamination. Here we combined RT-RPA with CRISPR/Cas12a DETECTR technology to develop a one-tube rapid (50 min) test for detection of SARS-CoV-2 in clinical samples, called OR-DETECTR. It can specifically detect SARS-CoV-2 from seven human

coronaviruses with LoD of 2.5 copies/μl input. Results of OR-DETECTR are 100% consistent with rRT-PCR. The enzymes and detection reagents used in the OR-DETECTR detection platform are different from rRT-PCR, which can be used as a complementary detection method to avoid the testing debacle caused by an insufficient supply of reagents in the rRT-PCR platform. With the number of COVID-19 tests required continuing to climbing, a high-throughput nucleic acid testing platform is urgently needed. OR-DETECTR is not limited by high-end equipment. It can be used for high-throughput clinical testing or combined with lateral flow strips for individual testing. After the current epidemic situation is controlled, this type of detection platform, with minimal equipment and a short turnaround time, can be widely used in local emergency departments, clinics, airports, stations and other field locations.

Abbreviations

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

DETECTR: DNA Endonuclease-Targeted CRISPR Trans Reporter

OR-DETECTR: One-tube detection platform based on RT-RPA and DETECTR

COVID-19: Corona virus disease 2019

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

RT-RPA: Reverse transcription and recombinase polymerase isothermal amplification

LAMP: Loop-mediated isothermal amplification

RdRp: RNA-dependent RNA polymerase

N: Nucleoprotein

ORF1ab: Open reading frame 1ab

SHERLOCK: Specific High-Sensitivity Enzymatic Reporter UnLOCKing

OR-SHERLOCK: One-tube detection platform based on RT-RPA and SHERLOCK

crRNA: CRISPR RNA

PAM: Protospacer adjacent motif

rRT-PCR: Real-time reverse transcription polymerase chain reaction

CDC: Centers for disease control and prevention

WHO: World Health Organization

LoD: Limit of detection

CT: Cycle threshold

FC: Fold change

Declarations

Ethics approval and consent to participate

Pharyngeal swab samples were collected from residual samples those had been obtained for clinical respiratory virus detection. All procedures complied with the Measures for the Ethical Review of Biomedical Research Involving Human Subjects issued by the National Health and Family Planning Commission of The People's Republic of China. All protocols were approved by the Ethics Committees of Shenzhen Second People's Hospital (#202003009005). All procedures were performed in biosafety level 2 facilities.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

Conceptualization, Weiren Huang and Dechang Li; Funding acquisition, Weiren Huang; Methodology, Yangyang Sun and Chengxi Liu; Validation, Yangyang Sun, Lei Yu and Chengxi Liu; Writing – original draft, Yangyang Sun, Chengxi Liu and Lei Yu; Writing – review & editing, Wei Chen, Dechang Li and Weiren Huang. All authors have read and agreed to the published version of the manuscript.

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Figure 1. An OR-DETECTR platform for detection of SARS-CoV-2. a, Schematic of OR-DETECTR testing SARS-CoV-2 workflow. RNA extraction from pharyngeal swab can be used as an input to OR-DETECTR (One-tube detection platform combined RT-RPA based preamplification and CRISPR/Cas12a based DETECTR), which is visualized by a fluorescent reader. The RT-RPA mix is located at the bottom of the centrifuge tube, while the DETECTR mix is located in the transparent tube lid. b, Genome map showing primers and crRNAs. RT–RPA primers are indicated by black arrows, those partial overlap with rRT-PCR primers of RdRp gene (WHO) or N gene (China CDC). Two crRNAs are indicated by light blue curves. c-d, crRNA specificity. The crRNA targeting RdRp gene is designed to detect SARS-CoV-2, SARS-CoV and MERS-CoV, which causes severe respiratory infection presented as fever, cough and dyspnea. e-f, The crRNA targeting N gene was specific for SARS-CoV-2. A positive result requires detection of SARS-CoV-2 viral N gene target. Dots represent identical nucleotides compared to sequence of RPA amplicon, ("--") means sequence gaps not covered by oligonucleotides. Black shadows represent primers binding sequences and blue shadows represent crRNAs recognition sequences. Fold change value was used to normalize the fluorescence signal values. Fold change (FC) = (F (PC)-B (PC)) / (F (NC)-B (NC)), F= fluorescence signal value of 19 min, B = fluorescence signal value of 0 min, PC= Positive control, NC=negative control. The result was shown as mean ± SD, N=3. *** $P<0.01$.

Figure 2. OR-DETECTR assay for SARS-CoV-2 in standard RNA and clinical oropharyngeal swab samples. a, Fold change data (OR-DETECTR) on RdRp gene and CT values (rRT-PCR) on Orf1ab of patient samples. There were 14 RNA samples from 14 suspected COVID-19 patients. N50S was one RNA mixed sample from 40 fever patients, and the CT value of each RNA sample was larger than 40 and it was undetected. The blue pentagrams **(★)** represented the CT values of the samples on Orf1ab gene, and the blue pentagrams exceeded the coordinate axis represented the CT values of the samples on Orf1ab gene greater than 40. The black columns represented the FC values of samples on RdRp gene, and the cutoff was 1.6. b, Fold change data (OR-DETECTR) and CT values (rRT-PCR) on N gene of suspected COVID-19 patient samples. The samples were same as a. The red pentagrams **(★)** represented the CT values of the samples on N gene, and the red pentagrams exceeded the coordinate axis represented the CT values of the samples on N gene greater than 40. The black columns represented the FC values samples on N gene, and the cutoff of N gene was 2.6. c-d, LoD for OR-DETECTR assay. SARS-CoV-2 RdRp and N gene standard RNA mix was used as the samples for evaluating LoD of OR-DETECTR assay. Standard RNA mix is serially diluted, and there were 7 replicates per dilution. e, Visual OR-DETECTR assay by test card with lateral flow strip. Standard RNA mix is serially diluted, and there were 2 replicates per dilution.

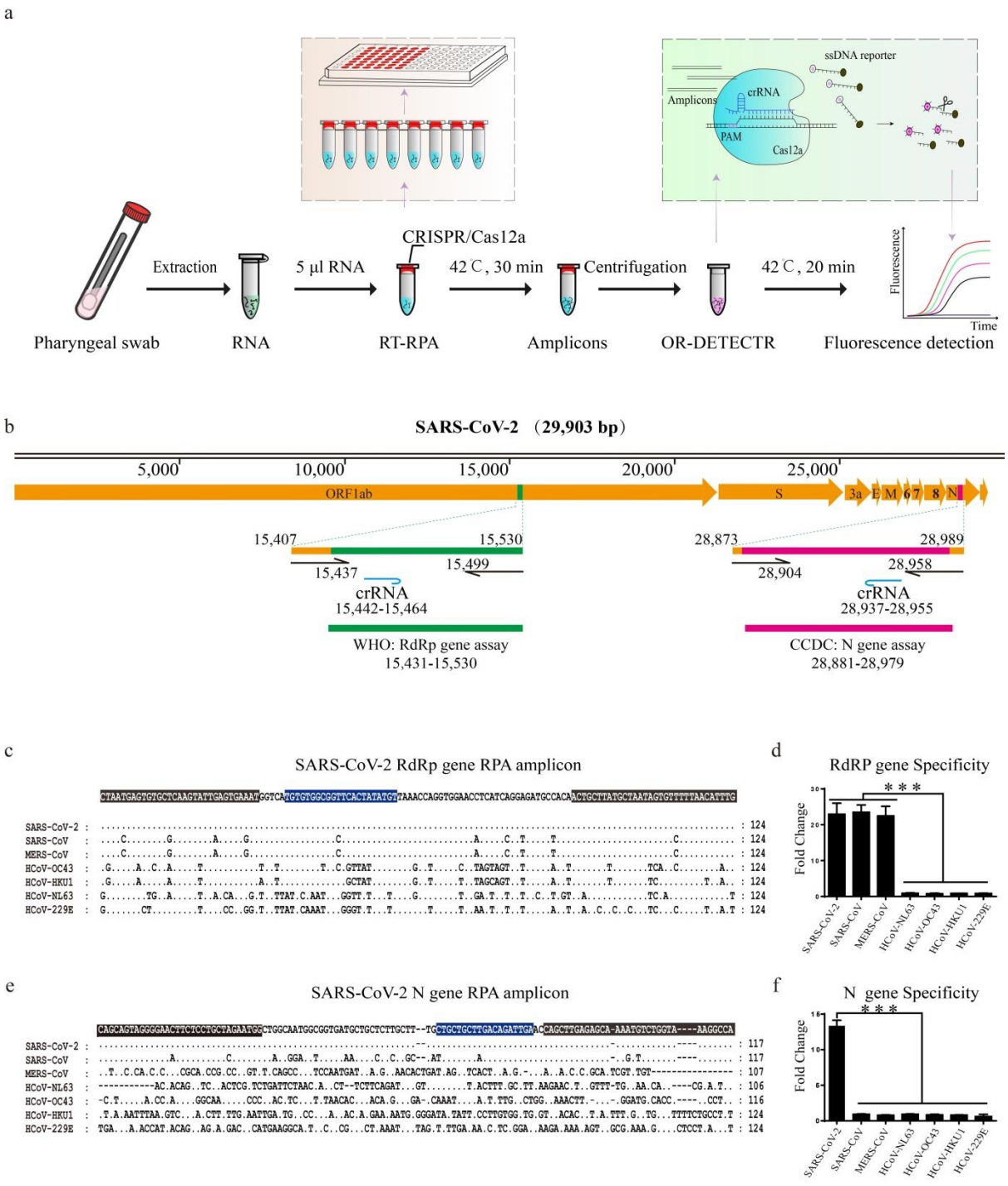
a
ssDNA reporter
Amplicons
crRNA
PAM
Cas12a
CRISPR/Cas12a
Extraction
5 μl RNA
42 ℃, 30 min
Centrifugation
42 ℃, 20 min
Fluorescence
Time
Pharyngeal swab
RNA
RT-RPA
Amplicons
OR-DETECTR
Fluorescence detection
b
SARS-CoV-2 （29,903 bp）
5,000
10,000
15,000
20,000
25,000
ORF1ab
S
3a
E
M
6
7
8
N
15,407
15,530
15,437
15,499
crRNA
15,442-15,464
WHO: RdRp gene assay
15,431-15,530
28,873
28,989
28,904
28,958
crRNA
28,937-28,955
CCDC: N gene assay
28,881-28,979
c
SARS-CoV-2 RdRp gene RPA amplicon
d
RdRP gene Specificity
Fold Change
SARS-CoV-2
SARS-CoV
MERS-CoV
HCoV-NL63
HCoV-OC43
HCoV-HKU1
HCoV-229E
e
SARS-CoV-2 N gene RPA amplicon
f
N gene Specificity
Fold Change

Figure 1

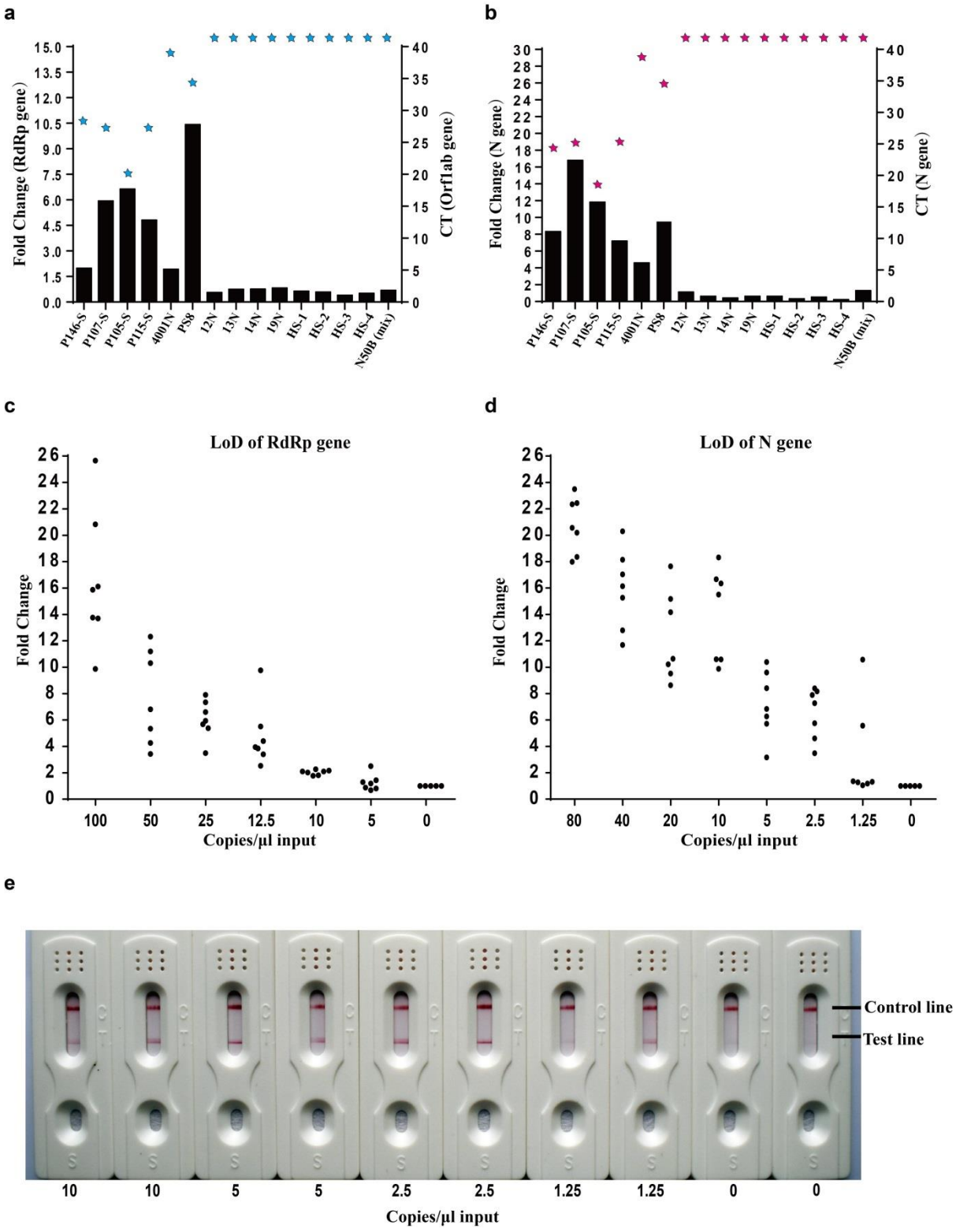
a
Fold Change (RdRp gene)
CT (Orf1ab gene)
P146-S
P107-S
P105-S
P115-S
4001N
P58
12N
13N
14N
19N
HS-1
HS-2
HS-3
HS-4
N50B (mix)
b
Fold Change (N gene)
CT (N gene)
c
LoD of RdRp gene
Fold Change
Copies/μl input
d
LoD of N gene
Fold Change
Copies/μl input
e
Control line
Test line
10
10
5
5
2.5
2.5
1.25
1.25
0
0
Copies/μl input

Figure 2