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Analytica Chimica Acta 1212 (2022) 339937

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

# VIR-CRISPR: Visual in-one-tube ultrafast RT-PCR and CRISPR method for instant SARS-CoV-2 detection

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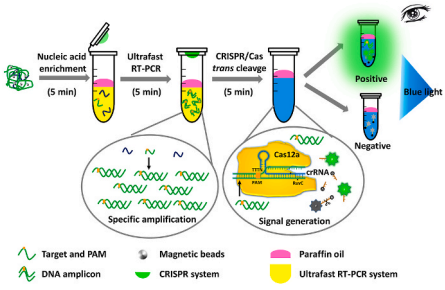
## HIGHLIGHTS

- 1.An instant, ultrasensitive and visual method was proposed for SARS-CoV-2 detection.
- 2.Ultrafast RT-PCR accomplished in only a few minutes was reported for the first time.
- 3.It integrated all components in-one-tube and realized sampling-to-result detection.
- 4.It is highly specific and could detect SARS-CoV-2 at nearly single molecule level.
- It showed 100% positive predictive agreement during clinical samples detection.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Keywords:*
CRISPR
SARS-CoV-2
Viral
Integration
Visual
Molecular diagnosis

## ABSTRACT

Until now, corona virus disease 2019 (COVID-19) remained to be an enormous threat for global health. As one viral illness induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), versatile, rapid and sensitive method for SARS-CoV-2 detection in early stage is urgently needed. Here, we reported an ultrasensitive and visual in-one-tube detection method which could be accomplished within half an hour from sampling-to-result. By integrating all reactions in one tube, liquid handling steps were omitted and amplicon contamination could be totally avoided. Magnetic beads were employed to achieve the fast extraction of viral nucleic acid and increase the sensitivity. Using portable thermocycler and blue light, the fluorescent results could be directly observed by naked eyes. The proposed method is of higher specificity and sensitivity, nearly at single molecule

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https://doi.org/10.1016/j.aca.2022.339937
Received 14 March 2022; Received in revised form 28 April 2022; Accepted 10 May 2022
Available online 13 May 2022

level. More important, results demonstrated 100% positive detection rate for 40 clinical samples, which was consistent with standard RT-PCR. Thus, our method is considerably simple, rapid, sensitive and accurate, holding great promise for the instant detecting of viruses including SARS-CoV-2 and the next generation of molecular diagnosis.

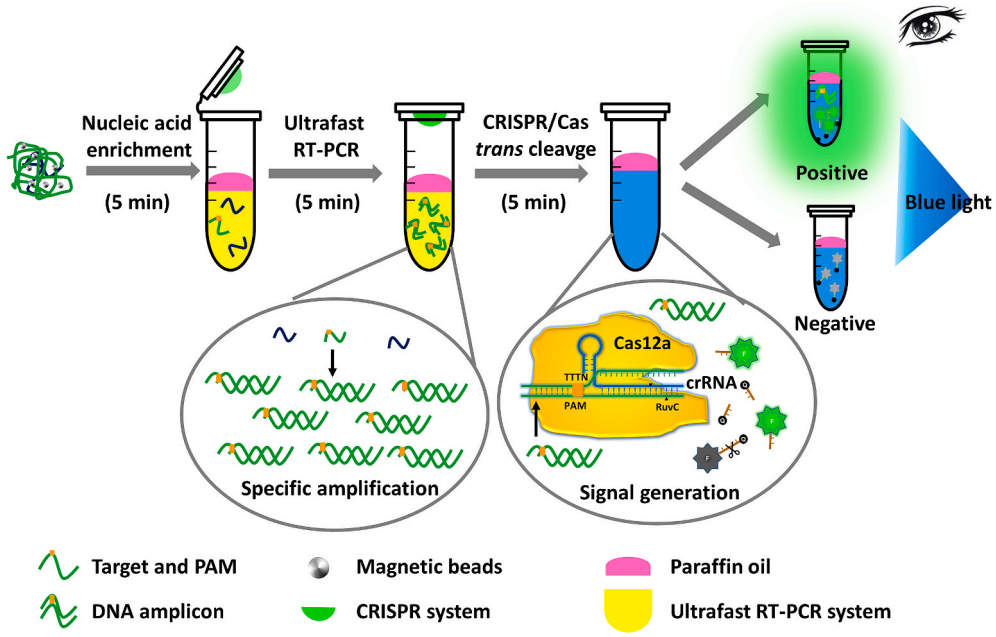
## 1. Introduction

As one global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the coronavirus disease 2019 (COVID-19) had led 455,886,145 infected cases with 6,061,495 deaths up to March 13th, 2022 (World Health Organization Coronavirus Dashboard). To top all this, SARS-CoV-2 mutants emerged in many places and made people to be more susceptible [1]. Hence, there is an urgent need for rapid and sensitive SARS-CoV-2 detection especially in its early stage to reduce its spread risk. Concurrently, such methods may also be useful guideline for other potential virus. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) is the gold standard in the detection of SARS-CoV-2 [2]. But the detection time is $>2$ h and the total turnaround time is $>24$ h by considering the need of shipping samples to centralized laboratories [3]. An ideal method with rapid, sensitive and easy-to-use properties is essential to adequately combat the COVID-19 pandemic especially in resource limited area.

At present, several isothermal amplification methods including rolling circle amplification (RCA), crossing priming amplification (CPA) and loop-mediated isothermal amplification (LAMP) appeared in many practical applications [4–6]. To an extent, isothermal amplification methods simplified operation process greatly. But long incubation time (>30min) is still required, nonspecific amplification signals may appeared and the detection sensitivity is limited [4,7–9]. Considering these defects, rapid PCR seems to be an ideal alternative with its higher sensitivity and shorter amplification time [10]. However, the secondary structure of primers used for rapid PCR would bring great interference during visual results judgment [11,12].

Despite this issues, nucleic acid detection methods based on nucleases associated with clustered regularly interspaced short palindromic repeats (CRISPR) held higher specificity due to the collateral cleavage activity of CRISPR-Cas enzymes [13,14]. During the detection process, once the combined CRISPR RNA (crRNA) complementarily pairing with targets, the Cas enzyme will be activated and indiscriminately cleavage single strand nucleic acid reporters nearby, thus producing detectable signals [15,16]. Researchers had combined nucleic acid amplification with CRISPR/Cas enzymes for SARS-CoV-2 detection [3,17–19]. However, most of them require multiple liquid-handling steps due to the incompatibility of pre-amplification and CRISPR detection, which are complex and increase the risk of cross-contamination. Moreover, they need an extra RNA extraction process, which makes the whole assay time-consuming. Hence, integrated, rapid and sensitive method is desperately needed for the instant determination of SARS-CoV-2.

Herein, we developed a sampling-to-result visual detection method termed as VIR-CRISPR (Visual in-one-tube ultrafast reverse transcription polymerase chain reaction (ultrafast RT-PCR) and CRISPR strategy for instant SARS-CoV-2 detection). So far as we know, this is the first report for methods to be established as ultrafast RT-PCR which could be accomplished within 5 min for RNA amplification. As illustrated in Scheme 1, the ultrafast RT-PCR and CRISPR system were integrated in one-tube. After pre-amplification, the two systems were mixed and incubated for another 5 min for Cas12a cleavage. Fluorescent result could be observed with our naked eyes under blue light. Moreover, the sampling duration was shortened without sacrificing detection sensitivity by employing magnetic beads for rapid RNA extraction and enrichment. Thus, the proposed method was greatly simplified and could be accomplished in half-an-hour from sampling-to-result, with the help of minimal equipment containing a portable thermocycler and blue light (Fig. S1).




**Scheme 1.** The scheme of VIR-CRISPR. The CRISPR system and ultrafast RT-PCR system were pre-added in the lid and bottom of the tube, respectively. The RT-PCR system was sealed with 20 μL paraffin oil to prevent its evaporation and potential inactivation of Cas12a enzyme. After pre-amplification, the CRISPR system was shaken into bottom and mixed thoroughly. Then the tube was placed at 37 °C for 5 min to perform Cas12a cleavage. Fluorescent result was observed with naked eye.

## 2. Experimental section

### 2.1. Materials and reagents

Sangon Biotech. (Shanghai, China) synthesized all primers, single stranded DNA (ssDNA) reporters and crRNAs. DNase I, EngenLba Cas12a and kits used for HiScribe T7 High Yield RNA Synthesis were bought from New England Biolabs (Ipswich, MA, USA). SpeedSTAR HS DNA Polymerase, PrimeScript RT Master Mix and RNase inhibitor were offered by Takara Bio Inc. (Dalian, China). QIAGEN OneStep RT-PCR kit was bought from Qiagen (Frederick, MD, USA). EvaGreen dye was purchased from Biutium (Shanghai, China). The ddPCRsupermix for probes (no dUTP) was purchased from Bio-Rad Lab. Inc. (CA, USA). Tiangen Biotechnology Co., Ltd. (Beijing, China) provided the DNA purification kit and RNA clean kits. Magnetic beads based nucleic acid extraction kit was kindly provided by Lingjun Biotechnology Co., Ltd. (Shanghai, China). BGI (Beijing, China) offered the pUC57 plasmids inserted the SARS-CoV-2 gene sequences. Sangon Biotech. (Shanghai, China) also sold us the plasmids inserted gene sequences from bat SARS-like coronavirus, SARS-CoV and one human coronaviruses (HKU1). The First People's Hospital of Yuhang district (Hangzhou, China) offered the 20 clinical samples with SARS-CoV-2 infection and 20 control samples. Scientific Research Ethics Review Committee in the hospital offered samples approved our study. Throat swap samples were collected with written or oral informed consents form patients.

### 2.2. The preparation of SARS-CoV-2 RNA templates in vitro

SARS-CoV-2 genome sequence in pUC57 plasmid were firstly transcribed (Fig. S2). Products were gel electrophoresed and purified to be used as *in vitro* transcribed templates. The products were also further sequenced and blasted (Fig. S3). Then, we purified the RNA templates with DNase I and RNA clean kit. Their concentration was measured by NanoDrop ND-1000 and finally they were stored at −80 °C.

### 2.3. Cleavage assays mediated by Cas12a

The cleavage system was built as 0.6 μM crRNA, 1 μM reporter, 0.2 μM EnGenLba Cas12a, 10 ng plasmid, 1 × NEB buffer 2.1, 4 U RNase inhibitor and finally add RNase-free sterile water to 40 μL for every reaction. We incubated the whole system at 37 °C for 20 min and then terminated by LightCycler 480 at 95 °C for 2 min. Last, all reaction tubes were observed under the blue light (LABGIC Inc., Beijing, China) and photographed with smart phone.

### 2.4. Standard RT-PCR assay for SARS-CoV-2 determination

QIAGEN OneStep RT-PCR kit was employed for standard RT-PCR amplification. We designed the primers targeting S gene in SARS-CoV-2 genome by primer premier 5.0. Each reaction system contained 1 × PCR buffer ($Mg2^+$ plus), 0.6 μM primers (forward and reverse), 400 μM dNTP (each), 1 μL enzyme mixture, 1 × EvaGreen Dye, 1 μL template and RNase-free water up to 25 μL. The PCR amplification process was reverse transcription at 50 °C for 30 min, hot-start at 95 °C for 15 min and 45 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min on LightCycler 480 system.

Droplet digital PCR (ddPCR) was employed for quantitative detection of SARS-CoV-2 gene. Each reaction system included 1 × ddPCR supermix, 1 μL primescript, 1 μL RNA template, 0.5 μM primers (forward and reverse), 1 × EvaGreen Dye and RNase-free sterile water up to 20 μL. After droplet generation on the QX200™ Droplet Generator (Bio-Rad, CA, USA), each reaction system was amplified on 96-well plate in the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, CA, USA). The amplification process was reverse transcription at 37 °C for 15 min, hot-start at 95 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 30 s and annealing/extension 60 °C for 1 min. All the droplets were read on the QX200™ Droplet Reader (Bio-Rad, CA, USA).

### 2.5. Ultrafast RT-PCR system build up for SARS-CoV-2 detection

SpeedSTAR HS DNA Polymerase was used for ultrafast RT-PCR amplification. During system build up, each reacting contained 1 μL RNA template, different volume of Primescript, 200 μM dNTP, 6.25 U SpeedSTAR HS DNA Polymerase, 1 × PCR buffer ($Mg2^+$ plus), 0.4 μM primers (forward and reverse), 1 × EvaGreen Dye and RNase-free sterile water up to 25 μL. The amplification process was reverse transcription at 37 °C for different time, hot-start at 95 °C for 30 s and 40 cycles of 95 °C for 2 s and 60 °C for 3 s on LightCycler 480 system.

### 2.6. Rapid nucleic acid extraction and enrichment with magnetic beads

Spiked saliva samples were prepared by adding 1 μL 10-fold gradually diluted purified SARS-CoV-2 RNA into 20 μL saliva. At the same time, 4 U RNase inhibitor was added into saliva to prevent RNA degradation. During rapid RNA extraction and enrichment, 1 μL of 100 mg $mL^{-1}$ magnetic beads with 30 μL lysate and binding solution was added into spiked samples and mixed thoroughly. Then, the mixture was maintained at room temperature for adequate absorption of magnetic beads. After the lysate and binding solution removed, RNA absorbed on the surface of magnetic beads was eluted into 10 μL sterile water as template for nucleic acid amplification and detection.

### 2.7. One-tube VIR-CRISPR detection of SARS-CoV-2

Ultrafast RT-PCR system and CRISPR system were separated in the bottom and the lid of the same tube. The ultrafast RT-PCR system contained 1 μL RNA template, 2 μL Primescript, 200 μM dNTP, 6.25 U SpeedSTAR HS DNA Polymerase, 1 × PCR buffer ($Mg2^+$ plus), 0.4 μM primers (forward and reverse) and then add RNase-free sterile water up to 25 μL. Last, the system was sealed with paraffin oil (20 μL). The CRISPR system was 25 μL reaction volume included 1 × NEB buffer 2.1, Cas12a 0.2 μM, RNase inhibitor 4 unit, crRNA 0.6 μM and ssDNA reporter 1 μM. Pre-amplification was set as reverse transcription at 37 °C for 1 min, hot-start at 95 °C for 30 s and 40 cycles of 95 °C for 2 s and 60 °C for 3 s on a portable thermocycler (Youning instrument Co. Ltd., Hangzhou, China). Then, the CRISPR system was shaken into ultrafast RT-PCR mixture and incubated at 37 °C for 5 min. The tube was placed on blue light immediately for naked eye observation and recording with accompanied smart phone.

## 3. Results

### 3.1. crRNA design for SARS-CoV-2 determination

During CRISPR reaction, crRNA plays a key role by complementary pairing with target sequences to activate the cleavage feature of Cas enzymes. The secondary structure and spacer sequence of crRNA determines targeting cleavage efficiency, thus affecting detection signals. In this assay, we designed a total of 10 crRNAs targeting orf1ab, E, N and S gene of SARS-Cov-2 (Fig. 1). Each crRNA was separately reacted with Cas12a, ssDNA reporter and plasmid template to evaluate their detection efficiency. Results showed that the fluorescence intensity of S-crRNA2 was the strongest, suggesting that it owns the highest work efficiency. Then, we further determined its detection specificity by comparing the sequence with 3 SARS-like coronaviruses including bat SARS-like coronavirus, SARS-CoV and SARS-CoV-2. One human coronaviruses (HKU1) was also selected in our study. Results demonstrated that single nucleotide polymorphisms (SNPs) in SARS-CoV-2 genome could be distinguished from other viruses (Fig. 1a). Moreover, for all the virus detection, only SARS-CoV-2 sample generated bright fluorescence, whereas others showed no fluorescence, demonstrating high specificity

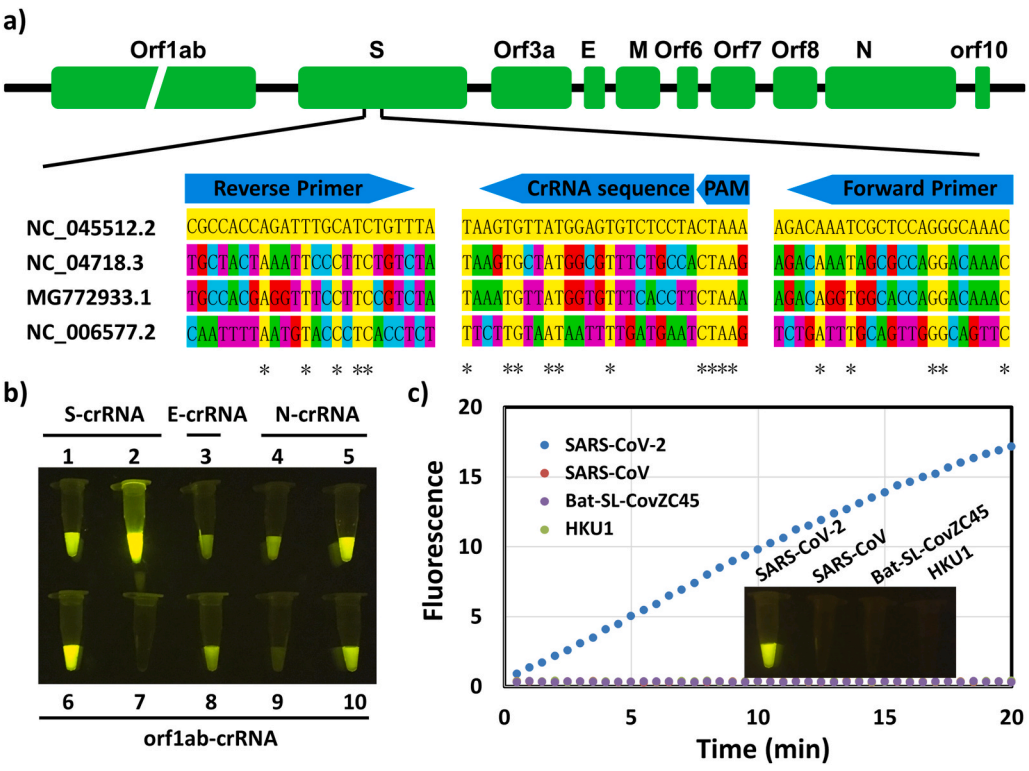



**Fig. 1.** a) The location of SARS-CoV-2 specific crRNA and primers on the genome of three SARS-like coronaviruses and one human coronaviruses. Sequences of different types of coronavirus and HKU1 were aligned by Clustal Omega. Positions with complete alignment to the primers or crRNA are labeled in yellow. b) Efficiency evaluation of designed crRNAs targeting different domains of SARS-CoV-2 gene. c) Specificity assessment of S-crRNA2 for four types of coronavirus determination. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

of selected S-crRNA2 for SARS-CoV-2 determination (Fig. 1c). Therefore, S-crRNA2 was employed for the following assay.

### 3.2. The establishment of ultrafast RT-PCR system

As one RNA virus, the first step for SARS-CoV-2 gene detection is reverse transcription. To avoid liquid handling process and greatly shorten the amplification time from hours to minutes, we attempted to establish a one-step ultrafast reverse transcription and amplification system (ultrafast RT-PCR) in one tube. In this assay, we chose SpeedSTAR™ polymerase for ultrafast nucleic acid amplification as its extension speed was 10 s/kb, which was much faster than normal polymerase. Firstly, the compatibility of reverse transcription and SpeedSTAR™ polymerase-based amplification system was studied with $5 \times 10^2$ copies of pure SARS-CoV-2 RNA as templates (Fig. 2). Results indicated that 2 μL of Primescript in the ultrafast RT-PCR system demonstrated optimal performance according to their minimum Ct values (Fig. 2b). Moreover, Ct values of different reverse transcription duration ranging from 1 min to 15 min were compared. Results demonstrated that for the same ultrafast RT-PCR system, the Ct values of different reverse transcription time showed no significant difference. In other words, 1 min was enough for reverse transcription of RNA to DNA format before amplification. Furthermore, the optimal system displayed comparable Ct values with that of standard RT-PCR, suggesting its good amplification efficiency (Figs. 2a, 2b, S4). Therefore, the ultrafast RT-PCR system was built up with reverse transcription at 37 °C for 1 min, hot-start at 95 °C (30 s), then 40 cycles of denaturation at 95 °C (2 s) and annealing/extension at 60 °C (3 s). Furthermore, to validate the accuracy of established ultrafast RT-PCR system, the amplicon was sequenced as illustrated in Fig. 2c. Results demonstrated 100% amplification accuracy though its reaction time shortened from hours to minutes. Therefore, the ultrafast RT-PCR system was employed in the following assay.

### 3.3. Integrated system of one-tube ultrafast PCR and CRISPR

We built one integrated system including ultrafast RT-PCR and CRISPR in one-tube to simplify operation and avoid amplicon contamination. To prevent Cas12a enzyme inactivation during thermal cycling, the CRISPR system was pre-added inside the lid while ultrafast RT-PCR system was placed inside the tube with 20 μL of paraffin oil on top. After ultrafast RT-PCR amplification, the CRISPR system was shaken into the bottom and mixed with amplified product thoroughly. Then the tube was incubated at 37 °C for Cas12a cleavage. To explore the fluorescence generation speed inside tube, we prepared a bunch of positive samples with $5 \times 10^2$ copies of pure RNA as templates while no template added as negative control. After shaking, all tubes were incubated at 37 °C. Each three positive tubes were taken out every 1 min and terminated immediately at 95 °C for 2 min to stop Cas12a cleavage. When all reactions finished, the tubes were placed on blue light with inside fluorescence recorded by smart phone. In parallel, upon mixing of ultrafast PCR and CRISPR/Cas12a, positive and negative tubes were placed on the Lightcycler 480 system for real-time detection with 1 min interval. Then, we aligned the fluorescent image of tubes with real-time fluorescence curve (Fig. 3). Results showed that distinguishable fluorescence could be observed after only 1 min of CRISPR reaction, demonstrating ultrahigh efficiency of Cas12a cleavage. Additionally, fluorescence intensity inside tube increased as time went on and became obvious in less than 5 min, whereas no fluorescence generated in negative tubes after 20 min reaction. Considering lower concentration of templates, 5 min of Cas12a cleavage was conducted for one-pot visual detection in the following assay.

### 3.4. Magnetic beads based rapid RNA extraction and enrichment

To simplify RNA extraction and boost detection sensitivity, we employed magnetic beads for rapid RNA extraction and enrichment. By adding 10-fold gradually diluted RNA templates of SARS-CoV-2 into saliva, we prepared a series of spiked samples. RNA templates of SARS-CoV-2 were concentrated by magnetic beads and eluted into sterile

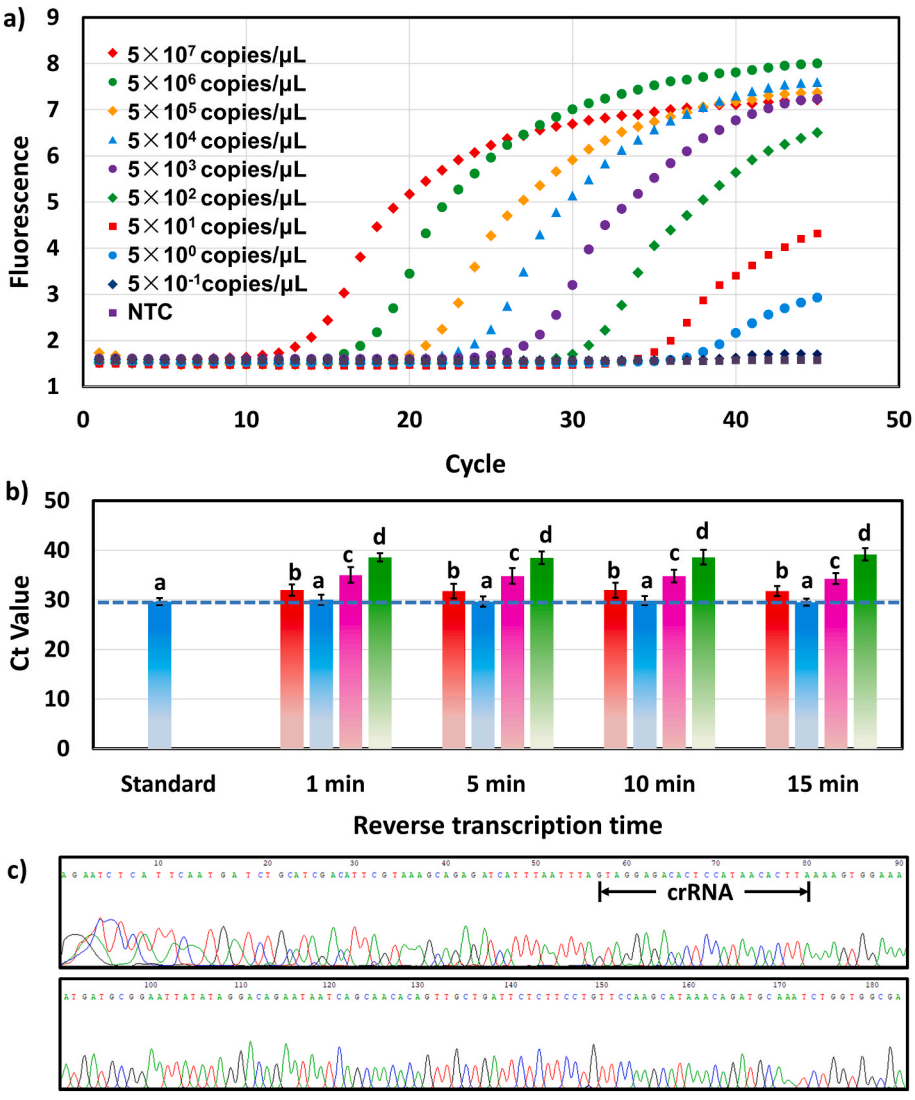



**Fig. 2.** a) The detection sensitivity for pure SARS-CoV-2 RNA determination with standard RT-PCR. b) Establishment of ultrafast RT-PCR system. In all reactions, 5 × $10^2$ copies of pure RNA of SARS-CoV-2 gene were as templates. 1 μL (red), 2 μL (blue), 4 μL (pink) and 6 μL (green) Primescript was added in ultrafast RT-PCR system, respectively. For each system, it was reverse transcribed at 37 °C for 1 min, 5 min, 10 min and 15 min, respectively. Standard RT-PCR was as control (light blue). Every experiment was repeated 3 times. Error bars showed ±SD. c) Sequencing result of ultrafast RT-PCR amplified product. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

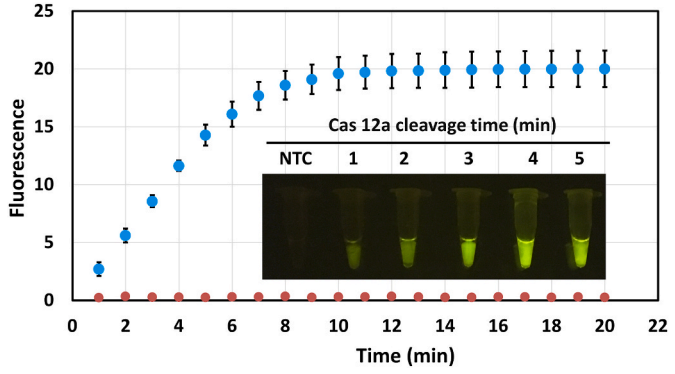



**Fig. 3.** Real-time fluorescence curves and images of Cas12a cleavage with ultrafast RT-PCR amplified products as targets. 5 × $10^2$ copies of SARS-CoV-2 RNA were as targets in positive samples and no template added samples were as negative controls. Three replicates were imaged for each time point with one of them presented here.

water with complex ethanol washing steps omitted. To shorten the RNA extraction time, we explored the enrichment duration of magnetic beads using 5 × $10^2$ copies of pure RNA of SARS-CoV-2 gene spiked samples. Results indicated that with time extension from 1 min to 5 min, the enrichment effect of magnetic beads enhanced, while it showed no significant difference from 5 min to 15 min incubation (Fig. S5). Therefore, 5 min was adequate for magnetic beads completing RNA enrichment from saliva samples. Furthermore, we studied the sensitivity of rapid RNA extraction coupled with ultrafast RT-PCR and CRISPR for spiked samples detection. In parallel, standard RT-PCR for pure SARS-CoV-2 RNA dilutions detection was as reference. Results displayed that the detection sensitivity of spiked samples with the proposed method was 5 copies per reaction, which was nearly at single molecule level and comparable with standard RT-PCR (Figs. 4a, S4, S6). Results in Fig. 4b showed that it would generate distinguishable fluorescence inside the tube even when the amplicon accumulated a little, demonstrating signal amplification through CRISPR/Cas *trans* cleavage. Therefore, it was feasible to employ magnetic beads for rapid RNA extraction with properties of simplified operation and satisfying sensitivity. Thus, we established a streamline for rapid SARS-CoV-2 detection from sampling-to-result, which contains rapid RNA extraction (~5 min), ultrafast RT-PCR (5 min) and CRISPR detection (5 min).

### 3.5. Clinical samples determination with VIR-CRISPR method

To further evaluate the practical feasibility of VIR-CRISPR method, 40 clinical samples were tested with standard RT-PCR as reference. Results indicated that all positive samples determined by standard RT-

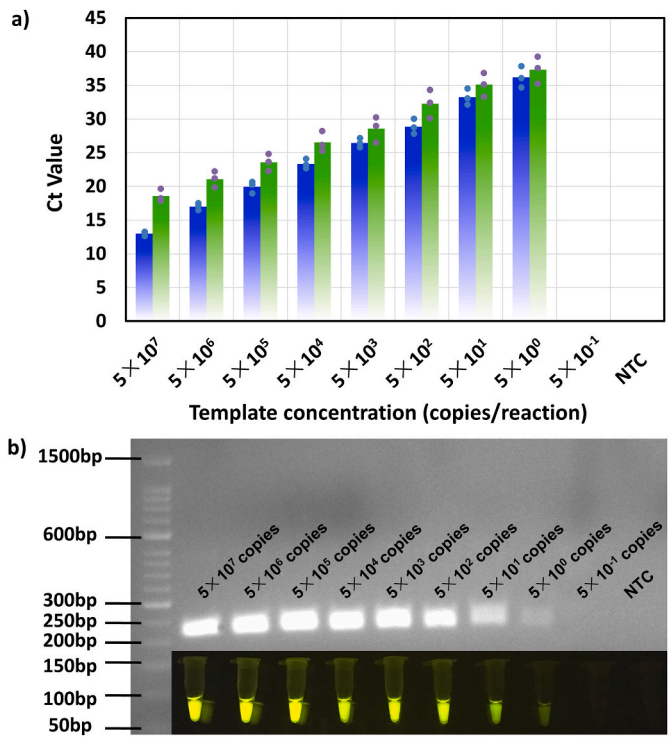



**Fig. 4.** a) The feasibility and detection sensitivity of magnetic bead-based rapid RNA extraction and enrichment from spiked saliva samples (Green column). Standard RT-PCR amplification for pure SARS-CoV-2 RNA detection was as control (Blue column). b) Gel image of ultrafast RT-PCR amplification results with magnetic bead extracted templates from spiked saliva samples. The inside are corresponded visual detection results. Three replicates were carried out for each reaction and one represented image was illustrated here. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PCR generated distinguishable fluorescence, which could be observed obviously with naked eye and recorded with smart phone (Fig. 5). All negative samples determined by standard RT-PCR generated no fluorescence in each tube (Fig. S7). Therefore, the positive detection rate of this proposed method was 100%, demonstrating good feasibility and reliability for rapid diagnostic field test of SARS-CoV-2.

## 4. Discussion

As is known, nucleic acid extraction has great impact on detection sensitivity. Most reported methods employed separated nucleic acid extraction before amplification and detection, which is time-consuming, labor-intensive and require professional personnel, thus unsuitable for rapid diagnostic field test of SARS-CoV-2 [20]. In this assay, we adapted a magnetic bead-based method to greatly simplify the RNA extraction and ensure detection sensitivity. The magnetic beads could complete the enrichment of SARS-CoV-2 RNA within only 5 min (Fig. S5). We also found that magnetic beads would significantly influence nucleic acid amplification not only for PCR amplification but also for isothermal thermal amplification due to the absorption of polymerase on the surface of magnetic beads (Fig. S8). Therefore, we eluted RNA from the surface of magnetic beads and used it as template for subsequent nucleic acid amplification and detection. As a result, the RNA extraction duration were reduced to 5 min with minimal hands-on time.

As the gold standard, RT-PCR was most commonly used for SARS-CoV-2 testing. However, drawbacks of long-time amplification and sophisticated fluorescence detection equipment prevent its application in point-of-care testing. We developed an ultrafast RT-PCR method which only needed several minutes. It only requires a portable thermocycler ($23 \times 18 \times 16\ cm^3$, 2.9 kg) with fluorescent result observed directly with naked eye. The ultrafast RT-PCR system was robust and could detect raw extracted RNA targets. Therefore, it has great potential to be used out of central laboratories, such as emergency department, airports and other locations.

Rapid, direct and readily applicable visual detection holds great promise for rapid diagnostic field test of SARS-CoV-2 [21]. Many researchers have developed visual SARS-CoV-2 detection methods. However, majority of them have low detection specificity for the reason that they treat targets as by-products rather than amplicon, i.e., $H^+$ [22], double stranded DNA secondary structure [23] and Magnesium pyrophosphate [24]. The emerging CRISPR method can ensure detection specificity during visual detection by crRNA specifically recognizing target sequence. However, most of the reported CRISPR-based methods need multi-step handling process and uncapping operation, which is complicated and will greatly increase the risk of aerosol contamination. Consequently, several one-step method based on CRISPR had been designed, such as STOPCovid.v2, AIOD-CRISPR and HOLMESv2 [17,21,25]. However, their incubation time varied from 60 to 120 min, and their background signal interference was high [17,26].

Our proposed method features remarkable advantages. Firstly, it possesses high specificity owing to ultrafast RT-PCR thermal cycling, which avoided primer dimers generation to some extent. The specificity was further improved by following CRISPR reaction and single base mutation could also be distinguished [13]. Secondly, it owns high detection sensitivity with distinguishable results. Its fluorescence signal was easier to be identified compared with colorimetric methods because the results judgment of the latter usually depends on color change from one to an adjacent one [22]. Moreover, compared with RPA coupled CRISPR detection, the proposed method demonstrated lower fluorescence background interference due to the preclusion of multiple enzymes [27]. Thirdly, this is a rapid, one-pot and easy-to-implement strategy. It omitted multiple liquid-transferring steps and uncapping operation, thus avoiding amplicon contamination from the source. It could be accomplished within half an hour from sampling-to-result with minimal equipment of a portable thermocycler and blue light.

## 5. Conclusion

We have developed an integrated visual, rapid and ultrasensitive strategy for SARS-CoV-2 detection. Ultrafast RT-PCR was initiatively established in this work. Coupled with CRISPR/Cas12a in one-pot, the operation was greatly simplified and amplicon contamination was totally avoided. Magnetic beads were employed to simplify SARS-CoV-2 gene extraction and boost detection sensitivity. The detection limit of the VIR-CRISPR method was nearly at single molecule level. We further validated this method by testing 40 clinical samples and the result was agreement with that of standard RT-PCR. Therefore, VIR-CRISPR holds great potential for field SARS-CoV-2 testing and other virus. Further studies should also be conducted to optimize the method for simultaneous detection of multiple viruses. Moreover, a mini-integrated device is to be established to perform the streamline of nucleic acid extraction, amplification and visual detection for molecular diagnosis.

## CRediT authorship contribution statement

**Rui Wang:** Methodology, Data curation, Validation, Writing – original draft. **Yongfang Li:** Data curation, Formal analysis, Validation, Writing – review & editing. **Yanan Pang:** Formal analysis, Validation, Writing – review & editing. **Fang Zhang:** Validation, Writing – review & editing. **Fuyou Li:** Validation, Writing – review & editing. **Shihua Luo:** Validation, Writing – review & editing, Supervision. **Chunyan Qian:** Resources, Writing – review & editing.

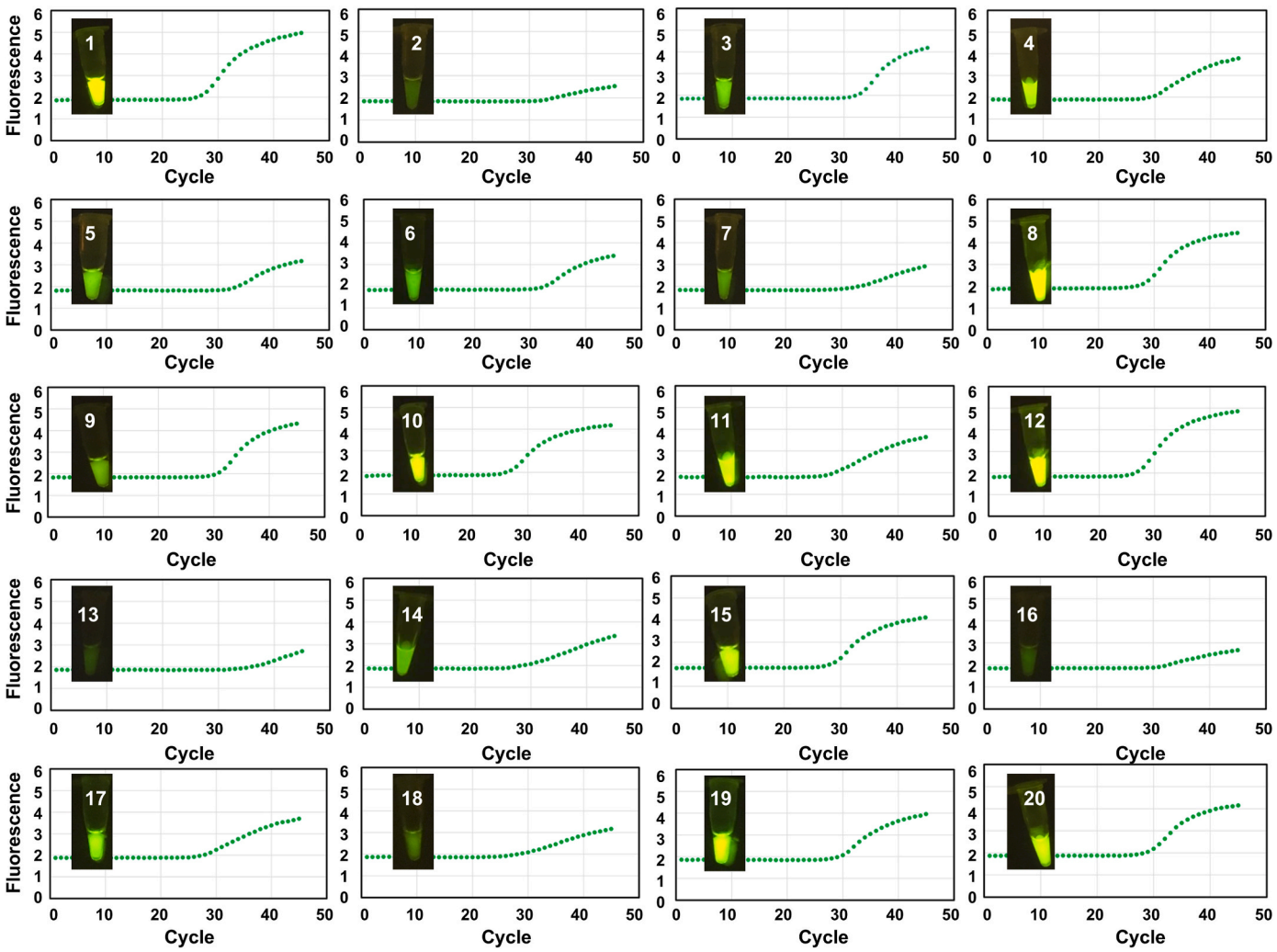



**Fig. 5.** Feasibility evaluation of proposed VIR-CRISPR method for 20 samples with SARS-CoV-2 infection. Standard RT-PCR amplification was as reference.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work was supported by National Key R&D Program of China (2017YFA0205100), Special project of China postdoctoral science foundation for prevention and control of novel coronavirus pneumonia (2020T130018ZX), the National Natural Science Foundation of China (32001786), the Research and Development Project for Novel Technology and Products in Zhejiang Provincial Health Commission (2021PY018).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2022.339937.

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