



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Rapid and highly sensitive one-tube colorimetric RT-LAMP assay for visual detection of SARS-CoV-2 RNA

Yugan He, Tie Xie, Yigang Tong*

Beijing Advanced Innovation Center for Soft Matter Science and Engineering, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, 100029, PR China

ARTICLE INFO

Keywords:
One-tube
SARS-CoV-2
LAMP
Point-of-care test
Visual readout

ABSTRACT

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is a highly contagious disease. To tame the continuously raging outbreak of COVID-19, developing a cheap, rapid and sensitive testing assay is absolutely imperative. Herein, we developed a one-tube colorimetric RT-LAMP assay for the visual detection of SARS-CoV-2 RNA. The assay integrates Si-OH magnetic beads (MBs)-based fast RNA extraction and rapid isothermal amplification in a single tube, thus bypassing the RNA elution step and directly amplifying on-beads RNA molecules with the visualized results. This one-tube assay has a limit of detection (LOD) as low as 200 copies/mL for sample input volumes of up to 600 μ L, and can be performed in less than 1 h from sample collection to result readout. This assay demonstrated a 100% concordance with the gold standard test RT-qPCR test by using 29 clinical specimens and showed high specificity. This one-tube colorimetric RT-LAMP assay can serve as an alternative platform for a rapid and sensitive diagnostic test for COVID-19 and is particularly suitable for use at community clinics or township hospitals.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread worldwide, bringing serious consequences for human life and the global economy (Wu, 2020; Hogan, 2020). Fortunately, the development and deployment of COVID-19 vaccines is the best hope for ending the pandemic (Koff, 2020; Poland, 2020). However, curbing the spread of infection is paramount at present. Testing is a key starting point to contain COVID-19 transmission, and accurate SARS-CoV-2 detection is an essential prerequisite for treatment, isolation of infected individuals, and contact tracing (Panpradist, 2021; Rivett, 2020; Guglielmi, 2020; Mina, 2020). The real-time quantitative polymerase chain reaction (RT-qPCR) method is considered the gold standard for the sensitive and specific detection of SARS-CoV-2 RNA (Vogels, 2020; Hu, 2020). However, performing an RT-qPCR assay typically requires a centralized laboratory, expensive and specialized instrumentation, and is always time-consuming. In the context, the urgency for the development of a simpler, faster, cheaper and accurate SARS-CoV-2 detection assay cannot be emphasized.

Nucleic acids, antigens and antibodies are the three key types of

biomaterials used for coronavirus detection. Nucleic acid-based tests detect viral genetic materials, whereas antigen-based tests detect the viral proteins. On the other hand, serology-based tests detect antibodies produced by immune system in response to the viruses. Both the antigen- and antibody-based tests can be performed in minutes even by non-experts; however, they invariably suffer from poor sensitivity (Kevadiya, 2021; Guglielmi, 2021; Crozier, 2021; Wise, 2020; BDJ Team.). In addition, antibody-based tests are not effective in the earliest phase of the infection, because the IgG and IgM antibodies are produced only after two weeks of exposure to the virus (Li, 2020; Sette, 2021; Baron, 2020). Due to these reasons, both antigen- and antibody-based tests are not the optimal approaches for SARS-CoV-2 detection.

Nucleic acid-based tests are playing a vital role in effectively containing SARS-CoV-2 transmission; particularly the RT-qPCR assay. In the context of the COVID-19 pandemic, to meet the ever-increasing testing demands, reduce the load on RT-qPCR testing, and for development of rapid nucleic acid detection platforms, nucleic acid-based isothermal amplification methods have been extensively deployed (Broughton, 2020; Klein, 2020; Rabea, 2020; Ganguli, 2020; Huang, 2020). The two fundamental isothermal amplification techniques are the recombinase polymerase amplification (RPA) and loop-mediated isothermal

* Corresponding author.

E-mail address: tongyigang@mail.buct.edu.cn (Y. Tong).

<https://doi.org/10.1016/j.bios.2021.113330>

Received 26 February 2021; Received in revised form 6 May 2021; Accepted 6 May 2021

Available online 13 May 2021

0956-5663/© 2021 Elsevier B.V. All rights reserved.

amplification (LAMP). While the RPA reaction can be performed at 42 °C, but it requires the introduction of additional magnesium acetate prior to amplification, which complicate the test protocol (Behrmann et al., 2020). Nevertheless, the RT-LAMP assay is straightforward. The typical requirements and procedure for such an assay are a solution comprising mainly the Bst DNA polymerase, reverse transcriptase, primer sets and reaction buffer. For example, Brian et al. developed a rapid and inexpensive RT-LAMP assay for SARS-CoV-2 detection (Rabea, 2020). This assay has achieved an LOD of 1 copy/μL through glass milk-based concentrating genomic RNA, and the results were available in 30 min. Anurup demonstrated a portable and point of care (POC) detection system for SARS-CoV-2, which has an LOD of 50 copies/μL and a test time of 30 min (Ganguli, 2020). The LAMP technique coupled with CRISPR Cas12 cleavage has also been utilized for SARS-CoV-2 diagnosis and showed better performance (Broughton, 2020). A few LAMP-based tests are available at present. Color SARS-CoV-2 RT-LAMP diagnostic assay developed by Color Genomics was demonstrated to have an LOD of 0.75 copy/μL (Color Genomics)(www.color.com). The colorimetric readout and high-throughput detection capacity are the highlights of this assay. However, it operates by a beads-based RNA extraction and elution, which limits rapid testing. The Lucira COVID-19 all-in-one test kit is another RT-LAMP-based assay and an at-home testing kit authorized for use in POC settings for individuals aged 14 and older (www.lucirahealth.com). While this kit exhibited higher analytical sensitivity, validation with more clinical samples is necessary.

The molecular test approach includes three main steps in the routine protocol; specimen collection, nucleic acid purification and detection. The latter two steps play an extremely important role in a highly sensitive nucleic acid testing, and thus we explored the development of a simple and rapid SARS-CoV-2 testing assay by integrating the RNA purification and detection in an all-in-one step approach. Herein, a one-tube colorimetric RT-LAMP assay was developed via the integration of Si-OH MBs-based fast RNA extraction and rapid isothermal amplification in one single tube. MBs demonstrate a strong ability to bind RNA molecules and are capable of realizing the automatic extraction of the nucleic acid. Upon capture by MBs, the RNA together with the MBs were directly used for the downstream RT-LAMP reaction, and thereby avoiding RNA elution. This one tube assay not only simplifies the testing pipeline, but can also concentrate RNA molecules, producing a rapid and highly sensitive test. The assay demonstrated an LOD down to 200 copies/mL in less than 1 h, and showed a 100% concordance with the gold standard RT-qPCR test by using 29 clinical specimens. This one-tube colorimetric RT-LAMP assay is a promising platform to support widespread testing for COVID-19 and is also an alternative for POC testing.

2. Materials and methods

2.1. Chemicals and reagents

The oligonucleotide used in this work was synthesized by Rui Biotech (Beijing, China). WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) and WarmStart® LAMP Kit (DNA & RNA) were purchased from New England Biolabs (New England, USA). QIAamp Viral RNA Mini Kit was obtained from QIAGEN (Germany). One Step TB Green™ PrimeScript™ RT-PCR Kit was bought from Takara (Dalian, China). Si-OH MBs was produced by PuriMag Biotech (Xiamen, China). 2019-nCoV RNA reference material was produced by National Institute of Metrology (Beijing, China). Nuclease-free water was obtained from Thermo Fisher Scientific (USA). Mineral oil was purchased from Sigma-Aldrich (St. Louis, USA). EDTA Na₂, TRIS and guanidine thiocyanate (GuSCN) were purchased from Solarbio Life Sciences (Beijing, China). Sodium hydroxide, sodium chloride, hydrochloric acid, and absolute ethanol were obtained from Macklin Biochemical (Shanghai, China). Phosphate buffered saline (PBS, pH7.4, without Calcium and Magnesium) was procured from BI biological industries (Beijing, China). The

lysis buffer was prepared by mixing 2.5 mM EDTA, 20 mM Tris, 4 M GuSCN, and 2 M NaCl in pH 6 aqueous solution. Ultrapure water (18.2 MΩ cm) was produced by an ultrapure water system (Milli-Q® reference). GX/P2V betacoronavirus was isolated by using Vero E6 cells.

2.2. Viral RNA concentration utilizing Si-OH magnetic beads

A facile and low-cost viral RNA concentration assay was developed, for which the corresponding protocol is as follows. (1) Vortex to thoroughly mix Si-OH MBs and the lysis buffer in a 1.5-mL microtube (Nonpyrogenic & DNase-/RNase-free); (2) Add certain volume of the sample to the tube; (3) Vortex or invert to mix, and incubate at room temperature for 5 min; (4) Place the tube on a magnetic rack, wait 15 s until the solution is clear; (5) Transfer the MBs to the bottom of the tube and remove the supernatant; (6) Add freshly-prepared 80% aq. ethanol, keep for 15 s and remove the supernatant; (7) Air dry the MBs at room temperature and ensure the removal of ethanol; (8) Cap and remove from the magnetic rack for downstream processing.

2.3. One-tube colorimetric RT-LAMP protocol

The MBs, which contain the captured viral RNA, was directly used for performing the colorimetric RT-LAMP without RNA elution in the same 1.5-mL tube. To 50 μL of the colorimetric RT-LAMP reaction mix, 25 μL 2 × WarmStart Colorimetric LAMP Master Mix, 5 μL 10 × primer mix including 16 μM FIP and BIP, 4 μM LF and LB, and 2 μM F3 and B3, 2 μL 0.2% phenol red, and 18 μL Nuclease-free water was added. In brief, a 50 μL colorimetric RT-LAMP reaction mix was directly added to the 1.5-mL tube, prepared according to the procedure in section 2.2. Next, an equal volume of 50 μL mineral oil was added, and the reaction was performed at 65 °C for 40 min in a heating block or water bath. Upon reaction completion, the reaction tubes were removed from heating block and cooled down to room temperature. The reaction tubes were then placed on a white cardboard and the results could be read either by the naked eyes or imaged using a smartphone with default setting. As LAMP suffers from aerosol/cross-contamination. *Never ever open LAMP reaction tube unless it is absolutely necessary.*

2.4. RT-qPCR reactions

RT-qPCR reactions were set up using the One Step TB Green™ PrimeScript™ RT-PCR Kit in a QuantStudio 1 thermal cycler. The RT-qPCR reaction using a SYBR Green method was performed as follows: 42 °C for 5 min, 95 °C for 10 s followed by 40 cycles consisting of 95 °C for 5s, 60 °C for 34 s and the collection of fluorescence signal in the final step. For the detection of the viral RNA extracted by MBs, RNA together with MBs was mixed thoroughly with 50 μL reaction buffer containing 25 μL 2 × One Step TB Green RT-PCR buffer, 1 μL Takara Ex TaqHS, 1 μL PrimeScript RT enzyme mix, 1 μL ROX Reference dye (50 ×), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), and 20 μL nuclease-free water. Then, the reaction buffer together with the MBs were transferred to 0.2-mL tubes to run the RT-qPCR reaction. For the detection of the viral RNA extracted using the QIAamp Viral RNA Mini Kit, 200 μL sample was input and eluted with 50 μL nuclease-free water, followed by 5 μL of the eluent to perform the RT-qPCR reaction according to the kit instruction.

2.5. Validation of the one-tube colorimetric RT-LAMP using COVID-19 specimen

Nine clinical specimens, which had been confirmed SARS-CoV-2 positive by RT-qPCR, were obtained from a Wuhan hospital in February 2020 (Table S1). The SARS-CoV-2 RNA was extracted from nasopharyngeal swabs using QIAamp Viral RNA Mini Kit and stored in a 1.5-mL RNastable tube at -70 °C. The SARS-CoV-2 RNA samples were first diluted 1000-fold using 1 × PBS. Then, 600 μL of the diluted sample

was concentrated using Si–OH MBs, followed by the performance of the one-tube colorimetric RT-LAMP reaction as described above. In addition, 20 oropharyngeal swab samples were acquired from healthy donors in the Tong Lab, and 600 μL of an initial sample was pipetted to run the one-tube assay. Negative control (NTC) was performed in every experiment by using nuclease-free water instead of the SARS-CoV-2 clinical samples.

2.6. Ethics statement

This study was carried out with the intention of developing a fast SARS-CoV-2 diagnostic assay for fighting COVID-19. Deidentified surplus SARS-CoV-2 samples that had been collected for SARS-CoV-2 diagnostic testing were used to establish and validate our developed protocol. Ethics approval was obtained from the Review Board of Beijing University of Chemical Technology, and all participants gave informed consent.

3. Results and discussion

3.1. Construction of the one-tube colorimetric RT-LAMP assay

A fast and highly sensitive assay for viral RNA detection was developed by the integration of Si–OH MBs-based nucleic acid extraction and loop-mediated isothermal amplification. As shown in Fig. 1, the lysis buffer, Si–OH MBs and the sample were mixed in a 1.5-mL tube followed by allowing the virion to lyse and release the viral RNA. The RNA molecules would be captured by MBs in the salt buffer of high concentration and lower pH, likely due to the formation of hydrogen bond or electrostatic interaction between the negatively charged RNA phosphate groups and the silicon hydroxyl (Si–OH) groups of the SiO₂ coated on the MBs. The MBs were then isolated from the lysis buffer using a magnet, and then the resulting supernatant was removed. A freshly-prepared 80% aqueous ethanol solution was used to wash the MBs to remove the residual guanidinium and some other inhibitors, followed by air drying at room temperature. Subsequently, the RT-LAMP reaction mixture was directly added to the tube. Notably, it is definitely unnecessary to elute RNA from MBs at the end of RNA extraction because an elution will lead to a dilution and the loss of target RNA, especially for

those samples with lower concentrations. Lastly, the RT-LAMP reaction was set up at 65 °C for 40 min by placing the microtube in a thermotank or water bath apparatus. Finally, the result was easy to read by the naked eyes upon introducing phenol red as the indicator to sensitively flag the pH change of the reaction buffer. In the initial stages of RT-LAMP reaction, the reaction solution is weakly alkaline showed by a red coloration of the solution. Once the reaction starts in the presence of the target genomic materials, thousands of millions of DNA molecules are amplified, which result in sample becoming acidic and causing a color change from red to yellow. Overall, a rapid and sensitive one-tube colorimetric RT-LAMP assay was constructed for the viral RNA detection.

3.2. Optimization of the one-tube colorimetric RT-LAMP assay

The one-tube colorimetric assay was optimized using a novel real coronavirus GX/P2V instead of SARS-CoV-2, as they have a high degree of similarity in their nucleotide sequence (Lam, 2020). Firstly, the effect of the size of the MBs on the nucleic acid extraction efficiency was explored. As shown in Fig. 2A, 200-nm MBs exhibited the best performance with the lowest Ct value, and the performance of 20-nm MBs was the lowest, likely due to the lower amount of SiO₂ coated on magnetic beads, and then which reduced their capacity to capture RNA. For 1000-nm MBs, the lower performance could be attributed to their large particle size with relatively smaller specific surface area, indicating a decreased capacity for RNA binding. To ensure complete lysis of the viral particles and the release of genomic materials, sufficient incubation time is necessary. The amount of captured RNA increased by 13% with the increase in the incubation time from 3 to 5 min. However, when incubation time was greater than 7 min, the amount of captured RNA did not increase further and decreased slightly (Fig. 2B). Accordingly, the optimal incubation time required for the MBs to capture free viral RNA from the lysis buffer was determined to be 5 min. Other crucial parameters related to MB-based viral RNA extraction were also investigated in detail in our previous work.

Considering that the MBs carrying viral RNA are directly used for downstream amplification, we explored the sensitivity improvement by increasing the volume of initial sample. As depicted in Fig. 2C, the amount of RNA captured by the MBs increased with increased sample

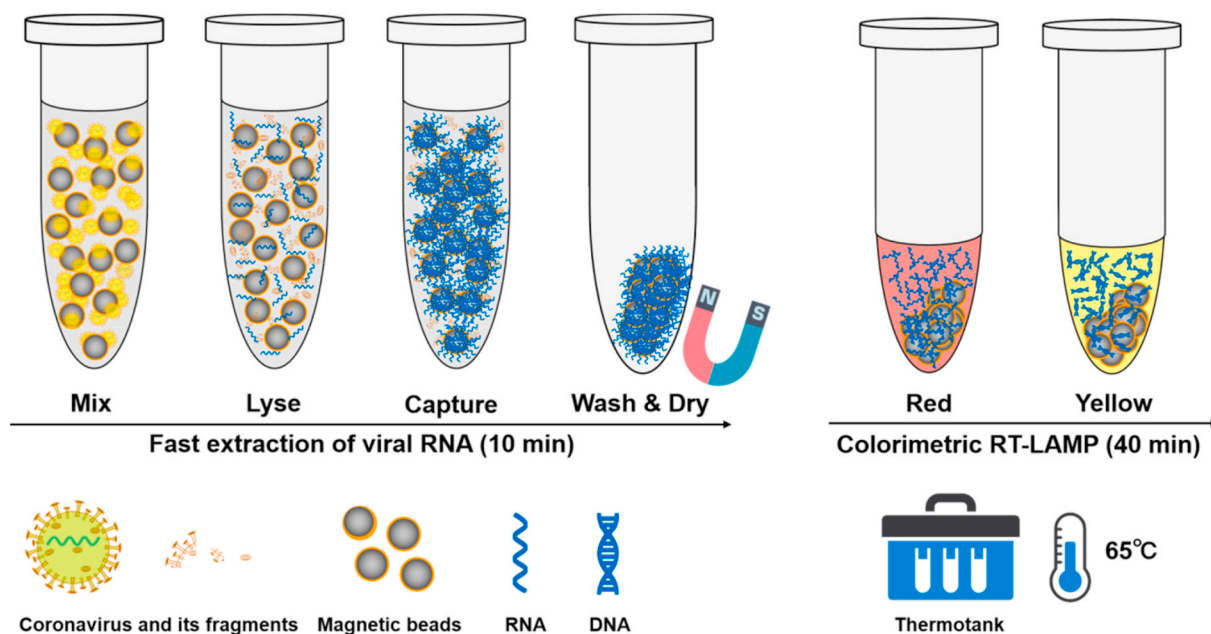


Fig. 1. Schematic of the one-tube colorimetric RT-LAMP assay for the visual detection of viral RNA. This assay is developed via the integration of Si–OH MB-based fast viral RNA extraction and rapid isothermal amplification in a single tube in less than 1 h.

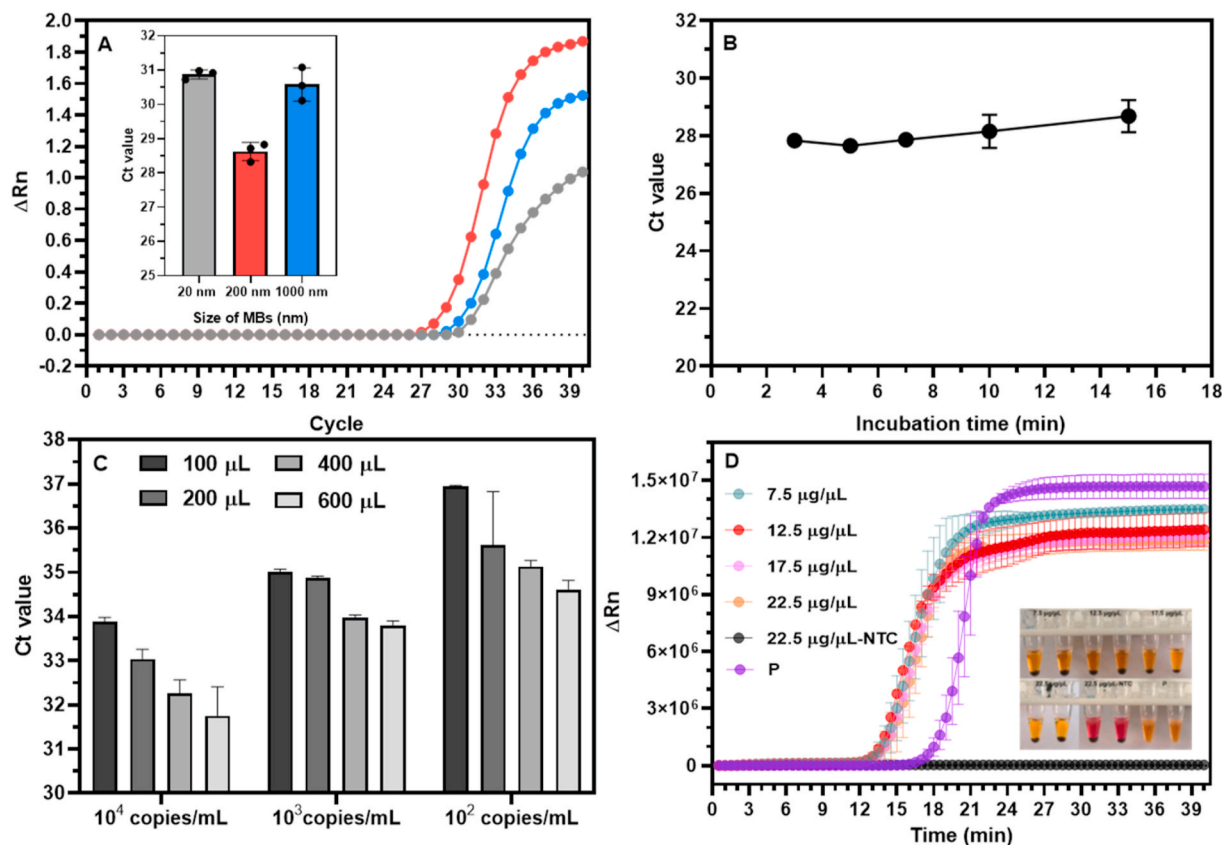


Fig. 2. Optimization and characterization of MBs-based nucleic acid extraction and RT-LAMP reaction tolerance to MBs. (A) Effect of MBs size on RNA extraction efficiency, (B) Effect of incubation time on RNA extraction efficiency, (C) Characterization of the extraction yield of MBs using various concentrations of GX/P2V virus particles under different sample volumes, (D) Assessment of RT-LAMP reaction tolerance to MBs by using GX/P2V RNA, the inset picture is corresponding colorimetric RT-LAMP result, P represents positive control using plasmid DNA as template.

input. For 10^4 copies/mL of GX/P2V virions, the amount of captured RNA increased about 4.35-fold after the total input sample volume was increased by 5 times. For 10^3 and 10^2 copies/mL of GX/P2V virions, when the total input sample volume increased by 5-fold, the amount of captured RNA increased by 2.31-fold and 5.03-fold, respectively, which revealed that an increase in sample volume increases the amount of captured RNA. Furthermore, a comparison of RNA extraction yield from the MB-based method and that with QIAamp Viral RNA Mini Kit using RT-qPCR technique was carried out, and six ten-fold serial dilution GX/P2V virions were used to study RNA extraction efficiency. The results showed the priority of our MB-based method in comparison to QIAamp Viral RNA Mini Kit (Fig. S1A). More importantly, our method displayed a strong ability to concentrate low concentration RNA samples (10^2 copies/mL) by increasing sample input, and showed an improvement of one order of magnitude compared to the QIAamp Viral RNA Mini Kit. In addition, spike recovery tests indicated greater than 94% recovery for both DNA and RNA samples (Figs. S1B and S1C).

In comparison with the conventional RT-LAMP assay, as our method involves the retention of MBs in the reaction solution, the additional introduction of MBs may inhibit DNA amplification. Therefore, the tolerance of the RT-LAMP reaction to MBs was studied. Varying amounts of MBs were added to 50 μ L RT-LAMP reaction mixture prior to running the amplification, followed by the real-time monitoring of the reaction process in a qPCR thermocycler. Interestingly, we observed that the MBs introduced in the RT-LAMP reaction system did not suppress DNA replication, but showed a certain degree of enhancement. As shown in Fig. 2D, the threshold time (T_t) of LAMP reaction with different concentrations of MBs (7.5–22.5 μ g/ μ L) was approximately 14 min, whereas that for positive control reaction without MBs was approximately 19 min, suggesting that a 5-min improvement was achieved.

Next, different concentrations of SARS-CoV-2 RNA or plasmid DNA were employed to investigate the enhancement of the RT-LAMP reaction by MBs. The results showed that MBs could indeed enhance nucleic acid amplification in LAMP reaction for both low-concentration and high-concentration samples (Figs. S1D and E). The enhancement of LAMP reaction by MBs can be attributed to: possible electrostatic interaction between the Si-OH MBs and LAMP components (Kambli, 2016); heat conduction by Si-OH MBs (Li, 2005); and the single-strand DNA binding protein-like property of Si-OH MBs (Li, 2016). To further enhance the visual readout, a 50 μ L LAMP mixture was modified by adding 2 μ L of 0.2% phenol red. As seen in Fig. S2, the color change was more distinguishable in the solution with modified reaction.

3.3. Sensitivity of the one-tube colorimetric RT-LAMP assay

The sensitivity of the method to the real coronavirus GX/P2V and SARS-CoV-2 RNA was explored next. GX/P2V RNA was extracted from 200 μ L of the cell supernatant using an equal volume of lysis buffer and 15 μ L of 25 mg/mL MBs, and then the obtained RNA/MBs composite was directly used as the template to amplify the target DNA. Test results of both 2×10^4 and 2×10^3 copies/mL samples were positive (Fig. 3A). However, only half of the tested 2×10^2 copies/mL sample indicated a positive result. Samples with 2×10^1 and 2×10^0 copies/mL, and NTC gave negative results. Accordingly, the sensitivity of the one-tube colorimetric RT-LAMP assay was about 2×10^3 copies/mL for GX/P2V for a 200 μ L sample input. To further improve the sensitivity, an enrichment protocol, by increasing sampling volume, was implemented. Upon increasing sample input by 2–3 times while holding the amount of MBs in constant, we observed half of the tests for a 200- μ L input, a quarter of the tests with a 400- μ L input, and three-fourths of the 600- μ L

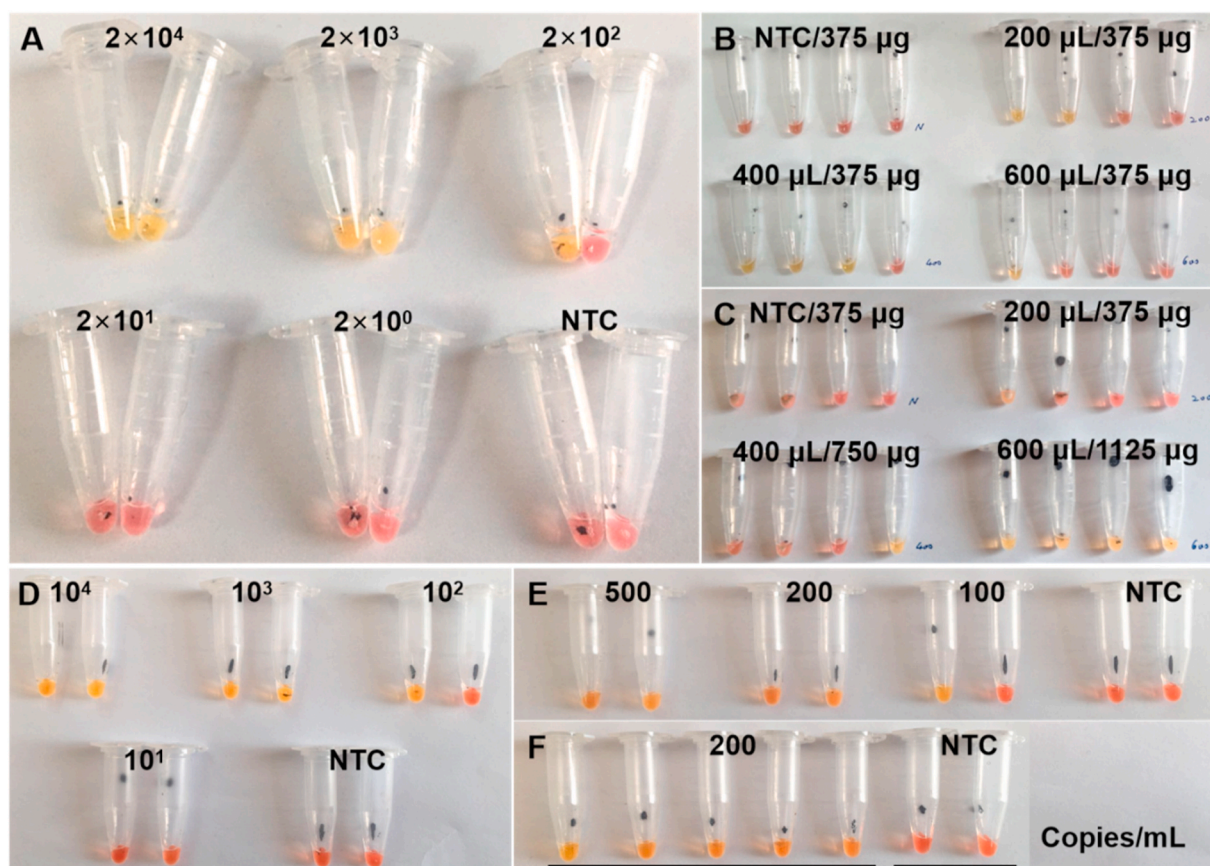


Fig. 3. Sensitivity of the one-tube colorimetric RT-LAMP assay for GX/P2V virions and SARS-CoV-2 RNA. (A) Sensitivity test using GX/P2V virus particles. (B) Reproducibility test using 200 copies/mL GX/P2V virus particles with various input volumes and a constant amount of MBs. (C) Reproducibility test using 200 copies/mL GX/P2V virus particles with various input volumes and the proportional increase of MBs amounts. (D and E) Sensitivity test for SARS-CoV-2 using RdRp primer sets with 600- μ L input volume. (F) Reproducibility test using 600 μ L of the 200 copies/mL SARS-CoV-2 RNA solution. In above tests, negative control (NTC) was performed using $1 \times$ PBS instead of samples.

input tests gave a false negative result (Fig. 3B). These results suggested that the insufficient concentration of MBs prevented the capture of the RNA molecules completely. Therefore, the amount of MBs was increased in proportion to the total sample input volume. The results shown in Fig. 3C indicated that three-fourths of the tests for both 200- and 400- μ L inputs showed a false negative, whereas for the sample input of 600- μ L, a 100% positive was observed from the four replicates, indicating a stable LOD of 200 copies/mL for the initial sample. By comparison with the conventional RT-LAMP and RT-qPCR assays, the LOD of the developed assay showed an improvement of two and one order of magnitude, respectively (Table S3).

Furthermore, SARS-CoV-2 RNA reference material was spiked into the viral transport medium solution and mixed with an equal volume of lysis buffer containing 1.125 mg of MBs before performing the one-tube assay. As shown in Fig. 3D, E, and F, the LOD for the one-tube assay using RdRp primer sets was 200 copies/mL, which was comparable to that of the RT-qPCR and revealed an improvement of two orders of magnitude compared with the conventional RT-LAMP assay (Fig. S3 and Table S4). Moreover, two plasmid vectors comprising NSP2 and RdRp regions of SARS-CoV-2, respectively, were used as templates to further characterize the one-tube assay. The results showed the assay was robustly stable with an LOD of 10^2 copies/mL, which is two orders of magnitude higher than that of the conventional RT-LAMP and the same as that of the RT-qPCR (Figs. S4 and S5; Tables S5 and S6). It is known that SARS-CoV-2 viral load is widely distributed, ranging from 10^3 – 10^{10} copies/mL, and the median viral load in nasopharyngeal swab is around 10^5 copies/mL (Feng, 2020; Pan, 2020). Accordingly, the readout level for SARS-CoV-2 RNA using the one-tube assay is consistent with the viral

load in COVID-19-infected patients, thus supporting the use of this assay for SARS-CoV-2 testing.

3.4. Specificity and clinical validation of the one-tube colorimetric RT-LAMP assay

Because three primer sets are used to specifically hybridize with the eight different regions of the target molecule, the LAMP reaction has a very high specificity (Tsugunori, 2000). SARS-CoV-2 shares a lower nucleotide identity with SARS-CoV, MERS CoV, HCoV-229 E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1, and shares over 85.8% nucleotide sequence with GX/P2V (Fig. S6; Table S7). Therefore, GX/P2V was used to evaluate the specificity of one-tube assay. The purified GX/P2V RNA was spiked in the clinical sample of a healthy individual without COVID-19 infection, and was used as the template to run the RT-LAMP reaction. Two primer sets, which target NSP2 and RdRp regions of SARS-CoV-2, respectively, were designed to amplify the target molecules for COVID-19 diagnosis. According to the Tt values of fluorescence-based RT-LAMP amplification curves in Fig. 4A, we observed that 1) Tt value (13 min) for NSP2 primers was 10 min earlier than that (23 min) for the RdRp primers, indicating that NSP2 primer sets performed better than the RdRp primer sets; 2) both GX/P2V and NTC samples did not amplified using either NSP2 or RdRp primer sets, suggesting that there was no cross-reactivity between those two primer sets and GX/P2V or human genomic materials. In addition, we also further verified no cross-reaction between SARS-CoV-2 and other human coronaviruses (Fig. S7; Table S8). Moreover, we found that these two primer sets could robustly identify multiple emerging SARS-CoV-2

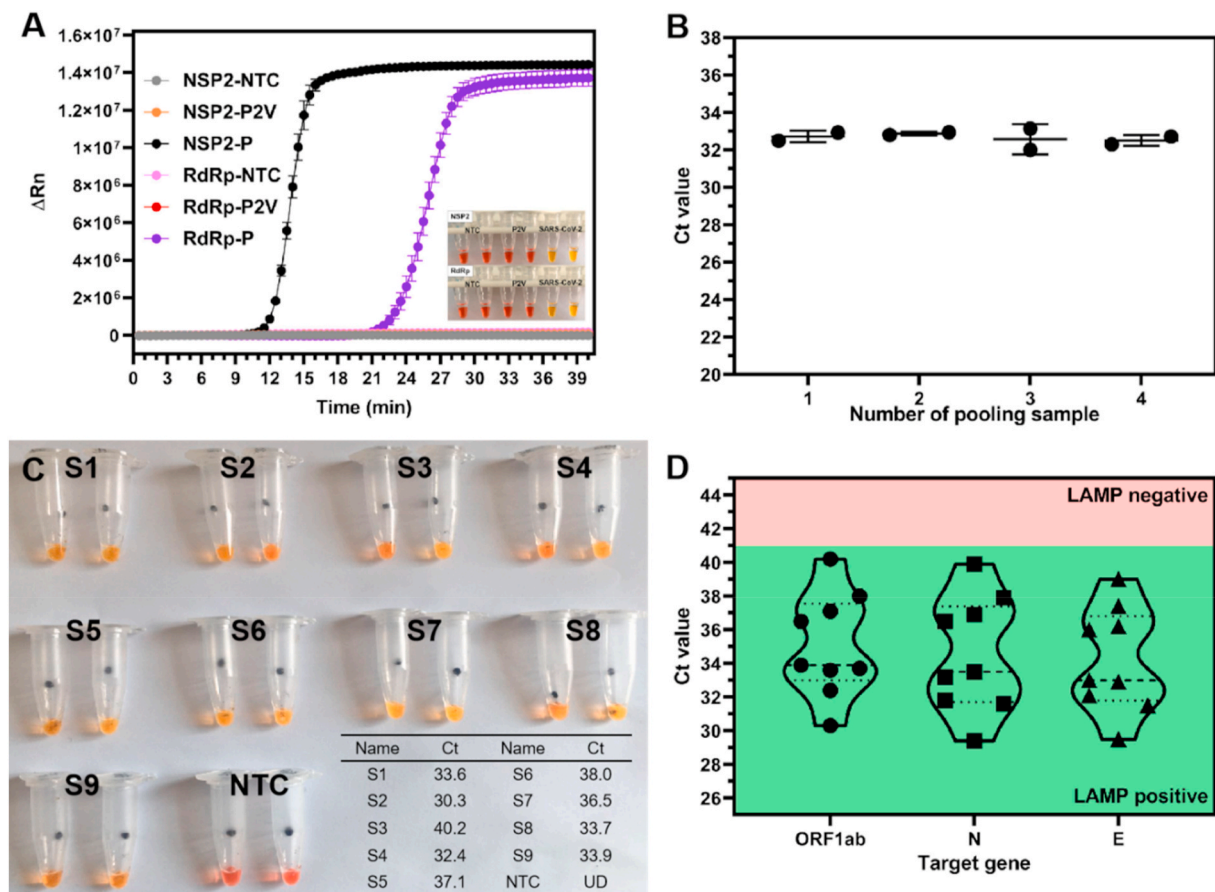


Fig. 4. Specificity and clinical validation of the one-tube colorimetric RT-LAMP assay using clinical SARS-CoV-2 samples. (A) Specificity test for SARS-CoV-2 detection using RdRp and NSP2 primer sets. GX/P2V RNA and one clinical SARS-CoV-2 RNA were used to test the cross-reactivity of LAMP assay. (B) Sample pooling strategies with sample pool sizes of 2–4. (C) Detection of clinical SARS-CoV-2 RNA using one-tube colorimetric RT-LAMP assay. The inset table shows corresponding Ct values (ORF1ab target gene) of the clinical specimens. (D) The Ct values of the clinical samples targeting three different regions of SARS-CoV-2. Ct values were obtained using an authorized 2019-nCoV RT-PCR Detection Kit (20203400299) with a LOD of 300 copies/mL. A Ct value less than 41 indicates a positive result with the sample denoted as LAMP positive. Ct value greater than 41 indicates a negative result with the samples denoted as LAMP negative.

variants, even for samples with lower concentrations of viral RNA (Figs. S8 and S9). Thus, we concluded that these two primer sets, with a very high specificity, were robust for our assay.

In response to the strong demand for higher throughput testing approaches, as well as the global shortage of nucleic acid extraction reagents causing significant delays in testing, 200 μ L 10^4 copies/mL GX/P2V RNA was mixed with an equal volume of the other 1–3 negative samples for implementing pooling test. As shown in Fig. 4B, the Ct values for samples with pool sizes from 1 to 4 were 32.72, 32.87, 32.57 and 32.51, respectively. This results revealed that the yield of the MBs that captured RNA did not decrease, even if the test pool size contained up to 4 specimens, indicating that our assay is suitable for pooling strategies. Finally, we demonstrated the detection of the SARS-CoV-2 RNA from clinical specimens using the one-tube colorimetric RT-LAMP assay. An input volume of 600 μ L of the samples with two replicates was used to characterize our developed assay. Nine samples, confirmed SARS-CoV-2 positive, were all diagnosed with positive, and 20 confirmed SARS-CoV-2 negative samples were all diagnosed as negative (Fig. 4C and S10). These results are in high accordance with the RT-qPCR results, which indicated that the one-tube colorimetric RT-LAMP assay is a highly sensitive and specific method for SARS-CoV-2 RNA diagnosis (Fig. 4D).

4. Conclusions

Herein, we have developed an all-in-one colorimetric RT-LAMP

assay for SARS-CoV-2 detection by integrating viral RNA purification, amplification and detection in a single tube. Following purification, the RNA/Si-OH MBs composite can be directly used as a template for downstream colorimetric RT-LAMP detection. Si-OH MBs are not only compatible with RT-LAMP reaction, but also enhance the reaction. The developed one-tube assay demonstrates an LOD of 200 copies/mL of SARS-CoV-2, requires less than 1 h for performing the test, and is suitable for pooling testing. In addition, the assay is robust and reliable for clinical specimen diagnosis, at a level comparable to the current RT-qPCR gold standard assay. The execution of this promising assay does not require conventional laboratory infrastructure and specialized instruments, which supports its potential use in routine screening and POC testing for COVID-19.

CRedit authorship contribution statement

Yugan He: Methodology, Data curation, Validation, Writing - original draft. Tie Xie: Resources, Data curation. Yigang Tong: Conceptualization, Validation, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgements

This work was financially supported by grants from Ministry of Science and Technology of China (No. 2020YFC0840805), The National Key Research and Development Program of China (2018YFA0903000), the National Natural Science Foundation of China (81672001), Fundamental Research Funds for the Central Universities (No. BUCTRC201917, No. BUCTZY2022) and China MoST Emergency Project on COVID-19 (2020YFC084800).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bios.2021.113330>.

References

- Alessandro Sette, A., Crotty, S., 2021. *Cell* 184, 1–20.
- Baron, Y.M., Flamholz, A., Phillips, R., Milo, R., 2020. *eLife* 9, e57309.
- Bdj Team, 8. www.curaprox.co.uk, 51.
- Behrmann, O., Bachmann, I., Spiegel, M., Schramm, M., Wahed, A., Dobler, G., Dame, G., Huferta, F.T., 2020. *Clin. Chem.* 66 (8), 10478–11054.
- Broughton, J.P., Deng, X., Yu, G., Fasching, C.L., Servellita, V., Singh, J., Miao, X., Sterithorst, J.A., Granados, A., Gonzalez, A.S., Zorn, K., Chiu, C.Y., 2020. *Nat. Biotechnol.* 38, 870–874.
- Color Gnomics. Color SARS-CoV-2 RT-LAMP diagnostic assay. <https://www.color.com>.
- Crozier, A., Rajan, S., Buchan, I., McKee, M., 2021. *Br. Med. J.* 372, n208.
- Feng, R., Huang, M.X., Liang, L.J., Huang, H.T., Hong, Z.S., Yu, J.X., Song, Y.C., Xia, J.Y., Guo, Q.F., Song, T., He, J.F., Yen, H.L., Peiris, M., Wu, J., 2020. *N. Engl. J. Med.* 382, 12.
- Ganguli, A., Mostafa, A., Berger, J., Aydin, M., Sun, F., Ramirez, S., Valera, E., Cunningham, B.T., King, W., Bashir, R., 2020. *Proc. Natl. Acad. Sci. Unit. States Am.* 117 (37), 22724–22735.
- Guglielmi, G., 2020. *Nature* 585 (7826), 496–498.
- Guglielmi, G., 2021. *Nature* 590, 202–205.
- Hogan, C.A., Sahoo, M.K., Pinsky, B.A., 2020. *J. Am. Med. Assoc.* 323 (14), 1967–1969.
- Hu, B., Hua Guo, H., Peng Zhou, P., Shi, z.l., 2020. *Nat. Rev. Microbiol.* 19, 141–155.
- Huang, Z., Tian, D., Liu, Y., Lin, Z., Lai, W.H., Fusco, D., Drouin, A., Yin, X.M., Hu, T., Ning, B., 2020. *Biosens. Bioelectron.* 164, 112316.
- Kambli, P., Mane, V.K., 2016. *Colloids Surf. B Biointerfaces* 141, 546–552.
- Kevadiya, B.D., Machhi, J., Herskovitz, J., Oleynikov, M.D., Blomberg, W.R., Bajwa, N., Soni, D., Das, S., Hasan, M., Patel, M., Senan, A.M., Gorantla, S., McMillan, J., Edagwa, B., Eisenberg, R., Gurumurthy, C.B., Reid, S.M., Punyadeera, C., Chang, L., Gendelman, H.E., 2021. *Nat. Mater.* (in press).
- Klein, S., Khalid, D., Sonntagbuck, V., Heuser, A.M., Meurer, M., Morales, I., Schillak, A., Freishtaedter, A., Ambiel, I., Winter, S.L., Morales, I., 2020. *Viruses* 12 (8), 863–899.
- Koff, W.C., Schenkelberg, T., Williams, T., Baric, R.S., McDermott, A., Cameron, C.M., Cameron, M.J., Friemann, M.B., Neumann, G., Kawaoka, T., Kelvin, A.A., Ross, M.T., Cherry, S.S., Mastro, T.D., Priddy, F.F., Moore, K.A., Ostrowsky, J.T., Osterholm, M. T., Goudsmit, J., 2020. *Sci. Transl. Med.* 13 (579), eabd1525.
- Lam, T., Jia, N., Zhang, Y., Shum, M., Jiang, J., Zhu, H.C., Tong, Y.G., Shi, Y.X., Ni, X.B., Liao, Y.S., Li, W.J., Jiang, B.G., Hu, Y.L., Guan, Y., Cao, W.C., 2020. *Nature* 583, 282–285.
- Li, H.K., Huang, J.H., Lv, J.H., An, H.J., Zhang, X.D., Zhang, Z.Z., Fan, C.H., Hu, J., 2005. *Angew. Chem. Int. Ed.* 44, 5100–5103.
- Li, A.J., Zhou, B.Q., Alves, C.S., Xu, B., Guo, R., Shi, X.Y., Cao, X.Y., 2016. *ACS Appl. Mater. Interfaces* 8, 25808–25817.
- Li, K., Huang, B., Wu, M., Zhong, A., Li, L., Cai, Y., Wang, Z.H., Wu, L.X., Zhu, M.Y., Li, J., Wang, Z.Y., Wu, W., Li, W.L., Bosco, B., Gan, Z.H., Qiao, Q.H., Wu, Q.H., Wang, S.K., Xia, X.Y., 2020. *Nat. Commun.* 11 (1), 1–11.
- Mina, J.M., Andersen, K.G., 2020. *Science* 371, abe9187.
- Pan, Y., Zhang, D.T., Yang, P., Poon, L., Wang, Q.Y., 2020. *Lancet* 20, 411–412.
- Panpradist, N., Wang, Q., Ruth, P.S., Kotnik, J.H., Oreskovic, A.K., Stewart, S.W., Vrana, J., Han, P.D., Beck, I.A., Starita, L.M., Frenkel, L.M., Lutz, B.R., 2021. *EBioMedicine* 64 (7), 103236.
- Poland, G.A., Ovsyannikova, I.G., Kennedy, R.B., 2020. *Lancet* 396 (10262), 1595–1606.
- Rabea, B.A., Cepko, C., 2020. *Proc. Natl. Acad. Sci. Unit. States Am.* 117 (39), 24450–24458.
- Rivett, L., Sridhar, S., Sparkes, D., Routledge, M., Jones, J., Forrest, S., Young, J., Dias, J. P., Hamilton, W.L., Freeis, M., Torok, M.E., Meredith, L., Curran, M.D., Fuller, S., Chaudhry, A., Shaw, A., Samworth, R.J., Bradley, J.R., Dougan, G., Smith, K.G., Lehner, P.J., Matheson, N., Wright, G., Goodfellow, I.G., Baker, S., 2020. *eLife* 9, e58728.
- Tsugunori, N., Hiroto, O., Harumi, M., 2000. *Nucleic Acids Res.* 28, e63.
- 2,1,1,1 Vogels, C.B., Brito, A.F., Wyllie, A.L., Fauver, J.R., Ott, I.M., Kalinich, C.C., Petrone, M.E., Massana, A.C., Muenker, C., Moore, A.J., Foxman, E.F., 2020. *Nat. Microbiol.* 5, 1299–1305.
- Wise, J., 2020. *Br. Med. J.* 371, m4848.
- Wu, F., Zhao, S., Yu, B., Chen, Y.M., Zhang, Y., Wang, W., Song, Z.G., Hu, Y., Tao, Z., Tian, J.H., Pei, Y.Y., Yuan, M.L., Zhang, Y.L., Dai, F.H., Liu, Y., Wang, Q.M., Zheng, J.J., Xu, L., Holmes, E.C., Zhang, Y.Z., 2020. *Nature* 579 (7798), 265–270.