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A novel One-pot rapid diagnostic technology for COVID-19

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HIGHLIGHTS
- The novel One-Pot RT-LAMP approach does not require RNA extraction.
- This one-tube reaction assay is a one-step Diagnosis Method.
- This One-Pot RT-LAMP detection assay is rapid (~45 min).
- Not requiring nucleic acid extraction in One-Pot assay reduces possibility of contamination.

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ABSTRACT
Novel coronavirus disease (COVID-19) caused by SARS-CoV-2 is an ongoing global pandemic associated with high rates of morbidity and mortality. RT-qPCR has become the diagnostic standard for the testing of SARS-CoV-2 in most countries. COVID-19 diagnosis generally relies upon RT-qPCR-mediated identification of SARS-CoV-2 viral RNA, which is costly, labor-extensive, and requires specialized training and equipment. Herein, we established a novel one-tube rapid diagnostic approach based upon formamide and colorimetric RT-LAMP (One-Pot RT-LAMP) that can be used to diagnose COVID-19 without the extraction of specific viral RNA. The technique could visually detect SARS-CoV-2 within 45 min with a limit of detection of 5 copies per reaction in extracted RNA, and about 7.66 virus copies per mL in viral transport medium. The One-Pot RT-LAMP test showed a high specificity without cross-reactivity with 12 viruses including SARS-CoV, MERS-CoV, and human infectious influenza virus (H1N1/H3N2 of influenza A and B virus, etc. We validated this One-Pot RT-LAMP approach by its successful use for the analysis of 45 clinical nasopharyngeal swab samples, yielding results identical to those of traditional RT-qPCR analyses, while achieving good selectivity and sensitivity relative to a commercial RT-qPCR approach. As such, this One-Pot RT-LAMP technology may be a valid means of conducting high-sensitivity, low-cost and rapid SARS-CoV-2 identification without the extraction of viral RNA.

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1. Introduction

The novel coronavirus SARS-CoV-2 [1], which causes a novel coronavirus disease (COVID-19), has rapidly become a global pandemic [2]. As of December 17, 2020, over 70 million confirmed cases and over 1.6 million COVID-19-related deaths have been confirmed worldwide [3]. A rapid and accurate diagnosis of COVID-19 is essential to control this deadly pandemic disease.

To date, approaches for diagnosing COVID-19 based on RNA amplification include RT-qPCR [4–6], reverse transcription recombinase polymerase amplification [7–9], CRISPR-based method [10,11], and RT-LAMP [12–14], etc. RT-PCR is the most commonly used technology for pathogen nucleic acid detection and has been considered as a gold standard for infectious disease diagnostics including SARS-CoV-2 [5,15]. However, this approach is costly, labor-extensive, and requires specialized training and equipment. RT-qPCR analyses also require roughly 1.5 h to yield results and are associated with false-negative rates as high as 30–40% [16–18]. These limitations mean that RT-qPCR approaches
are a suboptimal means of accurately identifying patients with suspected COVID-19, testing their close contacts, or assessing individuals with potentially asymptomatic disease. As such, there is a clear need for the development of a reliable, safe, rapid and POC means of diagnosing COVID-19. Isothermal amplification techniques, such as loop-mediated isothermal LAMP [12–14] and RPA [8,9], have become the methods of choice to detect SARS-CoV-2 without the need for complicated thermocyclers [19,20]. A high-sensitivity RPA method based on lateral flow strips may be cumbersome to achieve the high-throughput analysis of SARS-CoV-2 [21].

The LAMP amplification relies on a set of four to six primers, specially designed to recognize six to eight distinct regions of a target gene, resulting in high efficiency and specificity [19,22,23]. LAMP reaction generates a large amount of DNA and by-product pyrophosphate ion that not only can be judged by fluorescence using an intercalating DNA dye, turbidity (magnesium pyrophosphate formation) [24], but can also be visually observed with the color change of a metal sensitive indicator, for example, HNB [25], malachite green [26], calcein [23], or color change using pH-sensitive indicator dye [27]. When used in combination with reverse transcriptase, RT-LAMP approaches multiple readouts can be used for successful and high-throughput detection of RNA viruses including West Nile virus [28], MERS-CoV [29], SARS-CoV [30], influenza virus [31], and recently, SARS-CoV-2 [12–14], which represents a LOD of 3–5 copies per reaction by using extracted RNA [12,32], viral lysate [33] or unextracted RNA [34–36].

Colorimetric LAMP assays are susceptible to cross-contamination such as aerosol, and may lead to false-positive results of SARS-CoV-2 detection [37,38]. On the other hand, RNA extraction is often the rate-limiting step when conducting RT-LAMP assays as a POC test. As such, we herein designed a novel One-Pot RT-LAMP method to detect SARS-CoV-2 via targeting the nucleocapsid (N) gene. This one-tube reaction utilized a 6% of formamide solution for lysis viral particles and thereby facilitated in situ reverse transcription. Overall, this One-Pot RT-LAMP assay with a LOD of ~7.66 copies per μL in VTM required just 45 min and achieved high sensitivity and overall accuracy when used to evaluate clinical samples. Importantly, this approach can lower SARS-CoV-2 detection time and associated costs (~1.9 dollars per reaction) without incurring a significant risk of contamination. As such, this technique may be of significant value for field testing and for hospitals lacking sufficient instrumentation to support RT-qPCR analyses, thereby aiding efforts to control the COVID-19 global pandemic.

2. Experimental

2.1. Pseudoviral nucleic acid samples

In total, 12 pseudoviral nucleic acid samples (SARS-CoV, MERS-CoV, H1N1/H3N2 of influenza A and B virus, HPIV-1/2/3, AdV-B/E, MP, RSV, Cpn, EV-U/71, CA16, and HCMV) obtained from Guangdong Provincial People’s Hospital, were utilized for evaluating the specificity and effectiveness of the present RT-LAMP assay.

2.2. Control strain details

A SARS-CoV-2 N fragment (28274–29533 nt) in the SARS-CoV-2 complete genome, (accession nos. MN908947.3) was generated in Sangon Biotech Co., Ltd. (Shanghai, China). RT-LAMP assays were conducted using the synthesized nucleotide plasmid (PUC57–SARS-CoV-2 N) as a positive control. Furthermore, a 2019-nCov-N pseudovirus (Da’an Gene Co., Ltd. of Sun Yat-Sen University, China) was used to assess the relative sensitivity of RT-LAMP and RT-qPCR analyses. A QIAamp Viral RNA Minikit (QIAGEN, CA, USA) was used to extract viral RNA from 20 μL of viral samples, after which a Nanodrop instrument was used to evaluate RNA concentration and quality. Nucleotide copy number was assessed with the underlined formula: RNA copies μL−1 = (concentration of nucleotide (g μL−1)/length of nucleotide × 340) × 6.022 × 1023.

2.3. RT-LAMP primer design

Primers specific for the SARS-CoV-2 N gene were designed for this assay using the Primer Explorer v3.0.0 software (http://primerexplorer.jp/elamp3.0.0/index.html) based on sequences in GenBank (MN908947.3) in accordance with reported recommendations [39]. The six designed primers included two loops (forward loop primer LF, backward loop primer LB), two inner (forward inner primer FIP, backward inner primer BIP), and two outer (forward primer F3, backward primer B3) primers. The FIP primer consisted of the F1 complementary sequence (F1C) and the F2 sense sequence, while the BIP primer consisted of B1C and the B2 sense sequence. The respective sites of primers and sequences synthesized in Sangon Biotech Co., Ltd. (Shanghai, China) were shown in Fig. 1 and Table 1, respectively.

2.4. RT-LAMP assay

All RT-LAMP assays were conducted in a designated room with RNase-free pipettes and tubes. Analysis and imaging procedures were conducted in a separate room to avoid potential contamination. Individual 25 μL RT-LAMP reactions were conducted in tubes containing 5 μL of RNA template, 0.8 μM F3/B3 primers, 0.4 μM LF/LB primers, 1.6 μM FIP/BIP primers, 8 mM MgSO4 (Sigma, USA), 1.6 mM dNTPs (Sangon Biotech Co., Ltd., China), 2.5 μL 10 × Buffer (200 mM Tris-HCl 100 mM (NH4)2SO4, 100 mM KC1, 20 mM MgSO4, 1% Triton-100, pH8.8), 0.8 M Betaine (Sigma, USA), 8 U of Bst DNA polymerase mix (Optimized internally [40]), 5 U Reverse
Transcriptase AMV (Takara Biotechnology (Dalian) Co., Ltd., China), 0.12 mM HNB (Macklin Biochemical Co., Ltd., China) for optimizing visualization. The final volume was adjusted to 25 μL with DEPC H2O. For the One-Pot RT-LAMP reaction containing 6% formamide (Sigma-Aldrich, USA), a 5 μL NP swab sample was used as a template, the concentrations of all other reactions’ components mentioned above were maintained for the 25 μL reaction (Fig. 5).

Table 1
Primer sequences used for COVID-19 N gene RT-LAMP detection.

| Primer (outer primer, inner primer) | Sequence (5’ to 3’) |
|-------------------------------------|---------------------|
| F3 (outer forward primer)          | TGCACTGCGTTCTCCTGGTAA |
| FIP (forward inner primer, F1 + F2) | ATGCACCCCGCATTACG |
| BIP (backward inner primer, B1 + B2) | GCGATCAAAACAACGTCGGCCT |
| LF (loop forward primer)           | CAAGTTGAATCTGAGGGTCCAC |
| LB (loop backward primer)          | TGGCTTCTGGTCCACCGC |

Reaction tubes were assembled on ice, after which they were incubated for 45 min at 65 °C. Reaction tubes were then transferred to 80 °C for 5–10 min to inactivate the enzyme and cease the reaction. All RT-LAMP reactions were run in triplicate or more.

2.5. RT-LAMP amplification product analysis

RT-LAMP assay results were visually evaluated based on the color change of the solution of the reaction. The color of the negative reaction remained violet, while the positive reaction turned sky blue. In addition, agarose gel electrophoresis was also used to confirm the RT-LAMP amplification products. Agarose gel (2%, W/V) was prepared with agarose G-10 powder (Biowest) using 1× TAE buffer (20 mM Acetic acid, 40 mM Tris, 1 mM EDTA) stained with the SYBR® Green I dye (Invitrogen™, UK). The RT-LAMP products (3 μL) were run on these gels for 40 min at 120V and 400 mA. Gels were then imaged using a Fusion SoloS. An EDGE imaging system (Vilber Lourmat, France).
2.6. RT-LAMP primer specificity analyses

Primer specificity was assessed by aligning primer sequences to those of the N genes from SARS-CoV (GenBank DQ182595.1), MERS-CoV (GenBank NC019843.3), RSV (GenBank NC018803.1), HIPV (GenBank NC003461.1), H1N1 (GenBank NC026436.1), and Influenza B virus (GenBank NC002208.1). The BLAST Global Alignment tool was used to calculate mismatch rates between primers and these sequences. Primer specificity was also assessed through cross-reactivity analyses with other pathogens [13]. For these assays, SARS-CoV, MERS-CoV, H1N1/H3N2 influenza A virus, Influenza B virus, HPIV-1/2/3, RSV, Adv-B/E, MP, Cpn, EV-U/71, CA16, and HCMV were amplified via RT-LAMP, with DEPC water serving as a negative control.

2.7. RT-LAMP primer sensitivity analyses

The low limit of detection for this RT-LAMP reaction was analyzed using 10-fold gradient dilutions of SARS-CoV-2 RNA extracted from 2019-nCoV-N pseudovirus (5 × 10^-3 - 5 × 10^5 copies per reaction). Diluted samples were analyzed in parallel via both RT-LAMP and RT-qPCR. Comparing the lowest concentration that resulted in a positive reaction to RT-qPCR results, the sensitivity of RT-LAMP analysis was defined.

2.8. RT-qPCR

An RT-qPCR kit (Da’an Gene Co., Ltd.) that was confirmed to detect the ORF1ab gene and the N gene of SARS-CoV-2 was used for RT-qPCR analyses based on provided instructions. Briefly, individual RNA-free tubes used for these analyses contained reaction buffer A (17 μL), reaction buffer B (3 μL), and target RNA template (5 μL). RT-qPCR was then run by the authorized commercial RT-qPCR kit in ABI 7500 Real-time PCR system (Applied Biosoym, USA).

2.9. Formamide concentration determination

A range of formamide (Sigma-Aldrich, USA) concentrations (2, 4, 6, or 8% v/v) were tested in order to facilitate direct nasopharyngeal swab usage while maximizing RT-LAMP amplification specificity. Optimal formamide concentrations were determined using the PUC57-SARS-CoV-2 N plasmid and a 2019-nCoV-N Pseudovirus (Chinese National Institute of Metrology, China) as a template, respectively.

2.10. Clinical samples

Guangdong Provincial People’s Hospital is one of the seven hospitals approved for COVID-19 nucleic acid detection and treatment in Guangzhou City. For this study, our group and the Hou group simultaneously analyzed 30 negative patient samples and 15 positive patient samples via RT-LAMP and RT-qPCR in a double-blind manner. NP swab samples collected using rayon swabs that were soaked in 500 μL of physiological saline and immediately vortexed with 3 mm beads for 15 s (Novastar, China). For RT-LAMP assays, NP swab samples (5 μL) were used directly after collection. For RT-qPCR, a QIaAmp Viral RNA mini kit was used for the extraction of total sample RNA based on provided directions.

2.11. Ethics statement

All participants in the present study provided written informed consent, and the appropriate committees of the participating institutions approved this study. Samples used for these analyses were isolated from standard COVID-19 tests, and thus did not impose any additional burden on these patients.

3. Results and discussion

3.1. LAMP target selection and specific primer design

To design RT-LAMP primers, we began with the conserved SARS-CoV-2 nucleocapsid (N) gene sequence that is used for RT-PCR-based viral detection (MN908947.3) [41,42]. Both the US CDC and the Chinese authorities recommend the use of primers targeting the viral N gene and the ORF1ab region [7,43]. We, therefore, compared the homology of the SARS-CoV-2 N gene sequence to that of several other respiratory virus N genes, revealing 88% nucleotide similarity to SARS-CoV, 51% homology with MERS-CoV and lower 10% homology with RSV, HIPV, H1N1, and Influenza B virus (Fig. 1A). Six primers specific for the SARS-CoV-2 N gene (nucleotides 28274-29533) were subsequently designed for this RT-LAMP assay (Fig. 1B and Table 1). RT-LAMP primer specificity was confirmed via analyzing N gene sequences from different viruses (Fig. 1C), and by using the BLAST Global Alignment tool to compare these sequences to those of other coronaviruses. This analysis revealed these primers to be mismatched by 17.09–53.80% with 14 other analyzed coronaviruses (Table S1), indicating that these coronaviruses are unlikely to yield positive RT-LAMP results [44]. These findings thus confirmed that the designed RT-LAMP primers were specific for the SARS-CoV-2 N gene.

3.2. Test of RT-LAMP assay specificity

The underlined RT-LAMP primers specificity was next tested via examining their ability to amplify nucleic acids from 12 common pseudoviruses including SARS-CoV, MERS-CoV, H1N1/H3N2, HPIV-1/2/3, Adv-B/E, MP, Influenza B virus, Cpn, EV-U/71; CA16, RSV, and HCMV. For each test, 5 × 10^5 copies of viral RNA (Table S2) served as an RT-LAMP template, with color changes of the metal indicator HNB being used to gauge reaction outcomes. In the process of DNA amplification by LAMP, a large amount of by-product pyrophosphate ion was produced, which could bond with metals firmly and form insoluble salts [45]. As a metal indicator, the color of HNB changing from violet to sky blue indicated a positive reaction [25]. Reaction mixtures containing template SARS-CoV-2 N gene DNA yielded positive (sky blue) results, whereas all other reaction mixtures, including a DEPC water negative control, yielded negative (violet) results. Agarose gel electrophoresis was also used to assess these reaction products (Fig. 2), revealing that only samples containing the target gene yielded the amplified fragments, with no amplification being evident for other tested virus RNA. The result of no cross-reaction with these 12 common pseudoviruses indicates that the primers used for detection have high specificity and can be used for the detection of clinical samples.

3.3. Assessment of the relative sensitivity of RT-LAMP and RT-qPCR assays

In order to establish the sensitivity of RT-LAMP assay for SARS-CoV-2, a tenfold serial dilution of extracted SARS-CoV-2 RNA (5 × 10^-3 -5 × 10^5 copies per reaction) was used to conduct parallel RT-LAMP and RT-qPCR assays. The results indicated that the RT-LAMP assay successfully detected approximately 5 copies per reaction (Fig. 3A), whereas RT-qPCR assays exhibited a LOD of ~50 copies per 25 μL reaction (Fig. 3B). These findings thus indicated that this RT-LAMP approach was more sensitive than RT-qPCR as a means of detecting SARS-CoV-2.
In addition, to determine the optimal formamide concentration for use in this assay context, we conducted this RT-LAMP procedure using a combination of Triton X-100 and a range of formamide concentrations (2, 4, 6, or 8% v/v). Only concentrations of 2–6% facilitated positive SARS-CoV-2 N detection (Fig. 4A), whereas 8% of formamide yielded an erroneous negative result. This suggests that high formamide concentrations can adversely impact the RT-LAMP enzymatic reactions. We, therefore, tested this One-Pot RT-LAMP assay as a means of detecting the 2019-nCoV-N pseudovirus by adding 6% formamide with RT-LAMP reaction system (Fig. 5), and we found that both high (8.38 × 10⁷ copies µL⁻¹) and low (9.8 × 10⁴ copies µL⁻¹) viral concentrations were readily detectable without the need for a devoted RNA step (Fig. 4B). Together these results revealed that 6% formamide combined with 0.1% Triton X-100 degraded the virus strongly and effectively, and thereby promoted in situ reverse transcription.

In order to establish the sensitivity of the One-Pot RT-LAMP assay for SARS-CoV-2, Low-concentration 2019-nCoV-N pseudoviruses (9.8 × 10⁷–9.57 × 10⁴ copies µL⁻¹) in 2-fold serial dilutions with VTM were used as templates for test. A 5 µL diluted pseudovirus was added to the reaction system containing 6% formamide. In these reactions, the concentrations of the buffer, primer, polymerase, and other reaction components were kept constant within the 25 µL reaction as mentioned above. As shown in Fig. 4C, the One-Pot RT-LAMP method was able to detect approximately 7.66 copies per µL of pseudovirus-simulated samples. This LOD of the test is 7.66 virus copies per µL in VTM, which corresponds to clinical needs according to reported viral load of most diagnosed COVID-19 patients. For example, a study conducted on 80 hospitalized SARS-CoV-2 positive patients (67 throat swab samples, 42 sputum samples) estimated that the median viral load was 752 copies per µL in the sputum samples and 79.9 copies per µL in throat samples [49]. In another study, the viral load was assessed in the range of 26 copies per µL to 1.4 × 10³ copies per µL, which performed on 1070 specimens collected from 205 COVID-19 patients [50].

3.4. Selection of an optimal formamide concentration for One-Pot RT-LAMP reactions

To achieve a One-Pot RT-LAMP reaction, mild surfactants were identified as the optimal reagents for simultaneous viral lysis and disruption of protein-protein, protein-lipid, and lipid-lipid interactions. Most surfactants, however, facilitate viral lysis most effectively at high temperatures (~100 °C). Commercial RT-LAMP buffers contain 0.1% Triton X-100 as a surfactant, but it is not an effective denaturant in the present assay context. We, therefore, evaluated the ability of a range of mild surfactants to lyse viral particles at 65 °C (data not shown). We ultimately found that a combination of Triton X-100 with the strong nucleic acid denaturant formamide [46–48] yielded optimal viral lysis under these conditions.
3.5. One-Pot RT-LAMP-mediated SARS-CoV-2 detection in clinical samples

To test the clinical utility of our assay, 45 clinical NP swab samples, of which 30 and 15 had been found to be COVID-19 negative and positive by RT-qPCR, respectively, were analyzed in a double-blind manner using this One-Pot RT-LAMP approach. For RT-LAMP assays, NP samples (5 µL) were used as a template for direct detection of SARS-CoV-2 (Fig. 5). As shown in Fig. 6, all 15 positive samples yielded positive results and all 30 negative samples yielded negative results in our RT-LAMP assay, consistent with the obtained results of RT-qPCR analyses of these same samples.

Our study is the first report a one-step Diagnosis Method, adding 6% formamide to RT-LAMP reaction system can lyse the virus and facilitate the reverse transcription in situ, thus achieving “virus-to-result” detection of SARS-CoV-2 successfully with a LOD of 5 copies per reaction in extracted RNA, and about 7.66 virus copies per µL in VTM by carrying out all reaction’s steps in a single closed tube without RNA extraction. Herein, “One-Pot” means that NP samples, formamide and all the reagents related to RT-LAMP amplification, including primers, reverse transcriptase, Bst DNA polymerase, buffer, dNTPs, MgSO4, Betaine metal and sensitive indicator HNB, are all in a single closed tube and then diagnose COVID-19 visually without RNA extraction after isothermal amplification at 65 °C, 45min. Compared with some detection methods based on RNA amplification, our method has competitive advantages (see Table 2): 1) Simplicity and sensitivity. The One-Pot RT-LAMP assay does not require RNA extraction. Some RT-LAMP researches could rapidly detect SARS-CoV-2 with a low LOD (2 or 3 copies per reaction) [12,51], but require an RNA extraction step.

Fig. 4. Identification of optimal RT-LAMP assay formamide concentrations. (A) PUC57-SARS-CoV-2 N gene plasmids were detected via RT-LAMP in the presence of a range of formamide concentrations (2, 4, 6, and 8% v/v). Results were analyzed visually and via gel electrophoresis. Lane 1, PUC57-SARS-CoV-2 N gene plasmid (without formamide); Lane 2, DEPC water as negative control; Lane 3–6, 2%, 4%, 6%, and 8% formamide, respectively; M, 2000 bp DNA marker. (B) Detection 2019-nCoV-N Pseudovirus by One-Pot RT-LAMP with 6% formamide. Lane 1, SARS-CoV-2 N gene plasmid (without formamide); Lane 2, DEPC water as a negative control. Lane 3–4, high and low concentration of 2019-nCoV-N pseudovirus, respectively (without formamide); Lane 5–6, high (8.38 × 10^5 copies µL^-1) and low (9.8 × 10^2 copies µL^-1) concentration of 2019-nCoV-N Pseudovirus, respectively (6% formamide). (C) The LOD of the One-RT-LAMP approach as a means of detecting SARS-CoV-2 was assessed visually and via gel electrophoresis. Positive reactions yielded a sky blue color, while negative reactions were violet. M, 2000 bp DNA marker; Lane 1, plasmid of SARS-CoV-2 N gene; Lane 2, DEPC water as a negative control; Lane 3–13, 9.8 × 10^5–9.57 × 10^3 copies µL^-1 (2-fold serial dilutions), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
Although some RT-LAMP methods have used unextracted swab samples to detect SARS-CoV-2 effectively, a pretreatment of samples lysed with a 95 °C lysis step in TCEP or VTM regent is required, and LOD of the assay is 50 copies per μL [35,36], which is higher than our work (5 copies per reaction in extracted RNA, and about 7.66 virus copies per μL in VTM); Other isothermal amplification
technologies, such as improved RPA approach, report extraction-free SARS-CoV-2 with a low limit of detection (5 viral copies per reaction), however, it is necessary to manually insert the lateral flow strips into the open tube of each sample to visually interpret the results, which is tedious to achieve high throughput [21].

One-tube reaction. Carry-over contamination usually leads to false-positive results in LAMP reactions [37,38]. The reaction system of this method is adding 5 μL NP swab sample to the tube of RT-LAMP reaction solution containing 6% formamide (Fig. 5). This method detects SARS-CoV-2 from NP swabs to visualized results in a single closed tube without RNA extraction, thereby avoiding the possibility of sample contamination.

3) Low cost. Other relevant technologies, such as extraction-free RNA RT-qPCR assay [55], can diagnose COVID-19 rapidly and effectively through NP swabs but require complex and expensive equipment. The equipment of the method in this work only needs thermostatic equipment to visually detect SARS-CoV-2 under isothermal conditions. Compared with our previous work diagnosed COVID-19 by extracting viral RNA with RT-LAMP [32], the cost of One-Pot RT-LAMP reduces from 3.15 dollars to about 1.9 dollars per test.

The clinical NP swabs verification results showed that the specificity (45/45) and sensitivity (45/45) of the One-Pot RT-LAMP is equal to that of RT-qPCR. Based on these advantages, the One-Pot RT-LAMP approach does not rely on an RNA extraction step, it may thus represent a rapid, inexpensive, and more effective means of visually diagnosing COVID-19.

### 4. Conclusions

Herein, we designed a One-Tube RT-LAMP approach to amplify the SARS-CoV-2 N gene using formamide and Triton X-100 as surfactants. By adding formamide to the lysis buffer, we were able to carry out all reaction’s steps in a single closed tube without RNA extraction, enabling rapid, sensitive, and specific viral RNA detection. This reaction was relatively simple, economical, required just 45 min, and did not necessitate the use of complex or costly instrumentation. As such, this protocol may be of value for future efforts to diagnose and control the spread of COVID-19.

### CRediT authorship contribution statement

**Junmin Li**: Methodology, Validation, Investigation, Visualization, Writing - original draft.

**Xuejiao Hu**: Resources, Investigation, Validation, Visualization.

**Xiaoming Wang**: Resources,


**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.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2021.338310.

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