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Research article

# Simultaneous detection of SARS-CoV-2 and pandemic (H1N1) 2009 virus with real-time isothermal platform

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ABSTRACT

The recent ongoing outbreak of novel coronavirus SARS-CoV-2 (known as COVID-19) is a severe threat to human health worldwide. By press time, more than 3.3 million people have died from COVID-19, with many countries experiencing peaks in infections and hospitalizations. The main symptoms of infection with SARS-CoV-2 include fever, chills, coughing, shortness of breath or difficulty breathing, fatigue, muscle or body aches and pains. While the symptoms of the pandemic (H1N1) 2009 virus have many similarities to the signs and transmission routes of the novel coronavirus, e.g., fever, cough, sore throat, body aches, headache, chills and fatigue. And a few cases of serious illness, rapid progress, can appear viral pneumonia, combined with respiratory failure, multiple organ function damage, serious people can die. Therefore, there is an urgent need to develop a rapid and accurate field diagnostic method to effectively identify the two viruses and treat these early infections on time, thus helping to control the spread of the disease. Among molecular detection methods, RT-LAMP (real-time reverse transcription-loop-mediated isothermal amplification) has some advantages in pathogen detection due to its rapid, accurate and effective detection characteristics. Here, we combined the primers of the two viruses with the fluorescent probes on the RT-LAMP detection platform to detect the two viruses simultaneously. Firstly, RT-LAMP method was used respectively to detect the two viruses at different concentrations to determine the effectiveness and sensitivity of probe primers to the RNA samples. And then, the two virus samples were detected simultaneously in the same reaction tube to validate if testing for the two viruses together had an impact on the results compared to detecting alone.

We verified the detection efficiency of three highly active BST variants during RT-LAMP assay. We expect that this assay can effectively and accurately distinguish COVID-19 from the pandemic (H1N1) 2009, so that these two diseases with similar symptoms can be appropriately differentiated and treated.

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## 1. Introduction

The COVID-19 pandemic is spreading globally and the disappearance likelihood for COVID-19 in the short term is low. Due to the similar symptoms between the pandemic (H1N1) 2009 and COVID-19, the molecular diagnostics assay to separate these two is in high demand before flu season comes in different countries [1, 2, 3, 4, 5, 6]. Here we expanded our iLACO (*i*sothermal *LA*MP based method for *CO*VID-19) platform for testing of SARS-CoV-2 and the pandemic (H1N1) 2009 viruses [7], and establish a simultaneous diagnosis method for COVID-19 and the pandemic (H1N1) 2009 in real-time [8, 9, 10, 11]. Novel coronavirus SARS-CoV-2 detection methods are varied, some for one site of the virus, some for more than two sites of the virus, the reaction time and detection instrument are different [12, 13, 14]. While laboratory techniques for detecting the pandemic (H1N1) 2009 virus include molecular diagnostics, viral detection, and other methods [15], molecular diagnostics are currently commonly used to detect infection with the pandemic (H1N1) 2009 virus as well.

In the molecular diagnosis of RNA virus infection, the real-time reverse transcription polymerase chain reaction (RT-PCR) technique to detect viral nucleic acid is a reliable method for diagnosing viral infection [16, 17]. This method has been widely used by Center for Disease Control and Prevention and other relevant departments worldwide. Recently RT-qPCR based methods were developed and applied worldwide by multiple research and disease control centers [17]. However, RT-qPCR has very high requirements for the purity of samples and detection equipment, and the detection time is relatively long, about 2 h. This feature is slightly more challenging to implement in the face of large samples and remote areas. In addition, RT-qPCR needs trained personnel and sophisticated facilities for sample processing. These disadvantages limit its practical application in many cases, and thus can delay the required rapid prescription and administration of antiviral agents to patients.

Loop-mediated isothermal amplification (LAMP) is a rapid and reliable sequence-specific isothermal nucleic acid amplification technique in these detection methods for SARS-CoV-2 and the pandemic (H1N1) 2009 virus detection [18, 19, 20, 21]. The target nucleic acid was amplified under isothermal conditions, usually between 60 and 65 °C [22, 23]. The reaction time varied from 15-40 min, depending on the loading of virus in the collected samples [7] in the iLACO detection platform. Our previous experiments demonstrated the feasibility and application potential of the iLACO platform in the detection of SARS-CoV-2 [7]. However, colorimetric isothermal detection was limited to single target amplification, and here we expanded iLACO method with a fluorescent probe for simultaneous real-time detection of SARS-CoV-2 and the pandemic (H1N1) 2009 virus. Standard LAMP primers for SARS-CoV-2 and the pandemic (H1N1) 2009 virus were used in the dual detection strategy, the primers contained quenchers and the double annealing region of the fluorophore, the fluorescence signal gain of the two viruses could be obtained through chain separation to detect and distinguish the two viruses [24, 25, 26].

This paper reports on the simultaneous detection and diagnostic evaluation of real-time, one-step RT-LAMP detection of SARS-CoV-2 ORF1ab gene and the pandemic (H1N1) 2009 virus hemagglutinin (HA) gene (Figure 1 A and D).

## 2. Materials and methods

### 2.1. Commercial reagents

The reagents of Bst 2.0 WarmStart DNA polymerase, Bst 3.0 DNA polymerase, and WarmStart RTx Reverse Transcriptase were obtained from New England Biolabs. We obtained dNTP Mix from Thermo Fisher Scientific. We synthesized all of the primers and probes from Tsingke.

### 2.2. Clinical samples

In this study, excess RNA samples from patients suspected of SARS-CoV-2 infection were used based on routine clinical diagnosis, chest

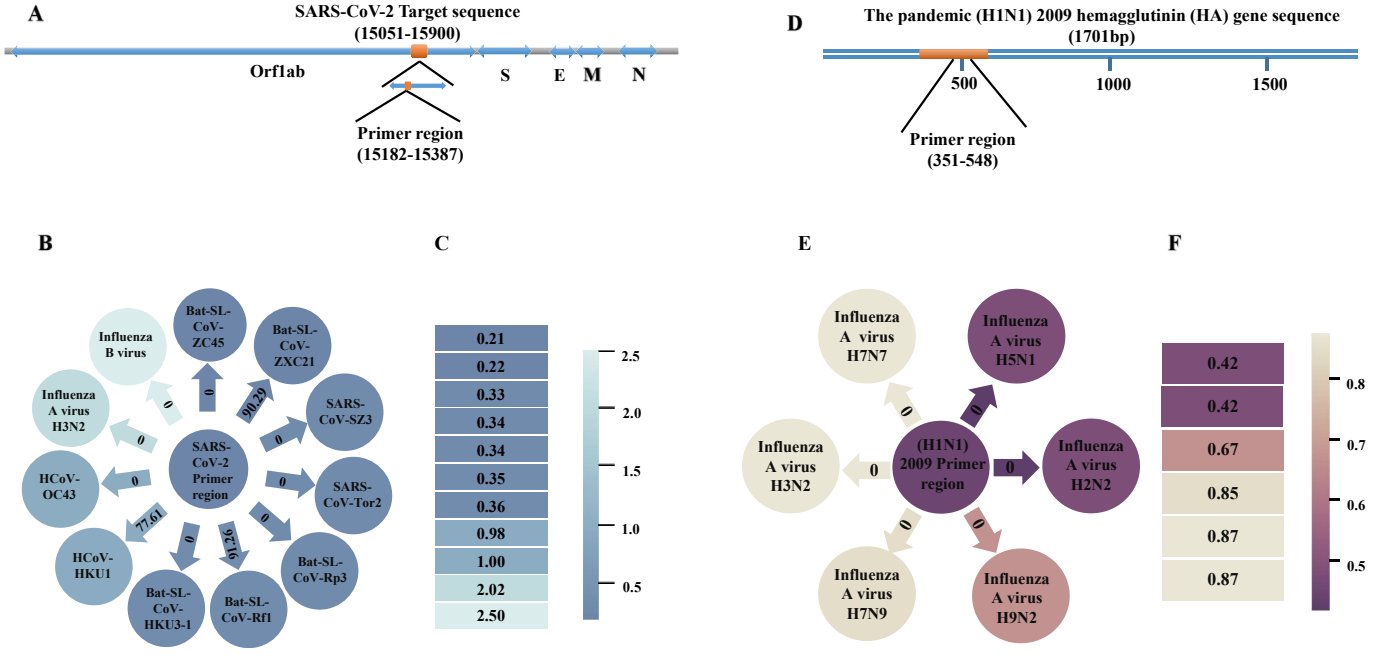


**Figure 1.** The primers sequences analysis of SARS-CoV-2 and the pandemic (H1N1) 2009, and homology analysis of these two viruses with other viruses respectively. A: The position of the target sequence and primer region on the complete genome sequence of SARS-CoV-2. B: Homology analysis of the primer sequence of SARS-CoV-2 and other viruses' genomes by BLAST software. The percentage of virus sequence similarity are shown in the arrows. C: The genetic distance results between SARS-CoV-2 and other viruses (seven similar coronaviruses, two influenza viruses, and two other coronaviruses) by MEGA software. D: The position of the target sequence and primer region on HA gene of the pandemic (H1N1) 2009. E: Homology analysis of the primer sequence of the pandemic (H1N1) 2009 and other subtypes viruses' genomes by BLAST software. The percentage of virus sequence similarity are shown in the arrows. F: The genetic distance results between the pandemic (H1N1) 2009 and other subtypes of influenza A virus (H2N2, H3N2, H5N1, H7N7, H7N9, H9N2) by MEGA software.

imaging, and epidemiological evidence. No information identifiable to the patient was collected. The only data collected from the sample was specimen type (248 nasopharyngeal swabs). We obtained the 248 nasopharyngeal swabs samples (18 positive samples, 230 negative samples) for SARS-CoV-2 detection and 4 of the pandemic (H1N1) 2009 positive patient' samples from the Shenyang Center for Disease Control and Prevention. Samples of SARS-CoV-2 were collected between January 15th to February 24th. We conducted the study on February 26th. Before the detection, the RNA samples of two viruses were extracted using the QIAamp Viral RNA Mini Kit (Qiagen) and were tested with Taqman RT-qPCR, which Ct values under 37 were called as positive, while Ct values were not determined or above Ct 37 were called as negative. Before RT-LAMP experiment, each sample was inactivated at 95 °C for 15 min and stored at -80 °C. We obtained SARS-CoV-2 and the pandemic (H1N1) 2009 virus samples that could be obtained from the CDC of Shenyang for this experiment. Since it was the outbreak period in COVID-19, so there were more SARS-CoV-2 samples than the pandemic (H1N1) 2009 virus, which was one of the limitations in the experiment.

### 2.3. RNA extraction

RNA of two virus samples was extracted from clinic samples with virus infection during the epidemic in Shenyang with the Thermo nuclear acid extraction workstation in the P2 laboratory of CDC Shenyang. Before the experiment, the concentration of the virus RNA sample was measured with Qubit 3.0 (Thermo Fisher).

Samples were collected and analyzed in P2 laboratory by the local CDC in Shenyang. The internal use of samples were agreed under the medial and ethical rules for each participating individual, and the written informed consent was waived.

Design of RT-LAMP assay primers and probes for SARS-CoV-2 and the pandemic (H1N1) 2009 influenza virus.

For SARS-CoV-2, the primers and probes' design were based on the primers used in the iLACO detection platform before [7], because after comparison of several groups of primers, this group of primers showed high detection efficiency and specificity. The FIP and BIP regions of LAMP internal primers were selected to design the probes [25]. For SARS-CoV-2, we added a dark quencher BHQ2 (Q-FIP) probe at the 5′ end of FIP and the 3′ end of F1c carried HEX fluorophore (green fluorophore, Fd) (Figure 2A) [25, 28, 29]. The primers and probes sequence of SARS-CoV-2 virus including: FIP (5′-AGGTGAGGGTTTTCTACATCACTATATTGGAACAAGCAAATTCTATGG-3′), Q-FIP:Fd (Q-FIP: "5′-BHQ2-AGGTGAGGGTTTTCTACATCACTATATTGGAACAAGCAAATTCTATGG-3′, Fd: 5′-ATAGTGATGTAGAAAACCCTCACCT-HEX-3′), BIP (5′-ATGGGTTGGGATTATCCTAAATGTGTGCGAGCAAGAACAAGTG-3′), F3/B3(5′-CCACTAGAGGAGCTACTGTA-3′/5′-TGACAAGCTACAACACGT-3′), LF/LB(5′-CAGTTTTTAACATGTTGTGCCAACC-3′/5′-GAGCCATGCCTAACATGCTTAG-3′). For the pandemic (H1N1) 2009 virus, the 5′ end of FIP was designed with a dark quencher BHQ1 (Q-FIP), and the 3′ end of F1c carried FAM fluorophore (blue fluorophore, Fd). The primers and probes of the pandemic (H1N1) 2009 virus were: FIP(5′-CGAGTCATGATTGGGCCATGACAGTGTCATCATTTGAAAGGTTT-3′), Q-FIP:Fd(5′-BHQ1-CGAGTCATGATTGGGCCATGACAGTGTCATCATTTGAAAGGTTT-3′, Fd:5′-TCATGGCCCAATCATGACTCG-FAM-3′), BIP(5′-AAGGTGTAACGGCAGCATGTCCGAATTTCCTTTTTTAACTAGCCAT-3′), F3/B3(5′-GCTAAGAGAGCAATTGAGC-3′/5′-ATGTAGGATTTGCTGAGCT-3′), LF/LB(5′-ACTTGTCTTGGGGAATATCTC-3′/5′-ATGCTGGAGCAAAAAGCT-3′). Before conducting the test, Q-FIP and Fd oligos in these two primers and probes sets were annealed by heating up to 95 °C at 50 μM and slowly cooling the mixture at 2° per minute to room temperature to form the fluorescence reporter duplexes [24, 25]. All oligonucleotides were purchased from Tsingke with HPLC (high-pressure liquid chromatography) purification and dissolved in DEPC-treated water upon arrival.

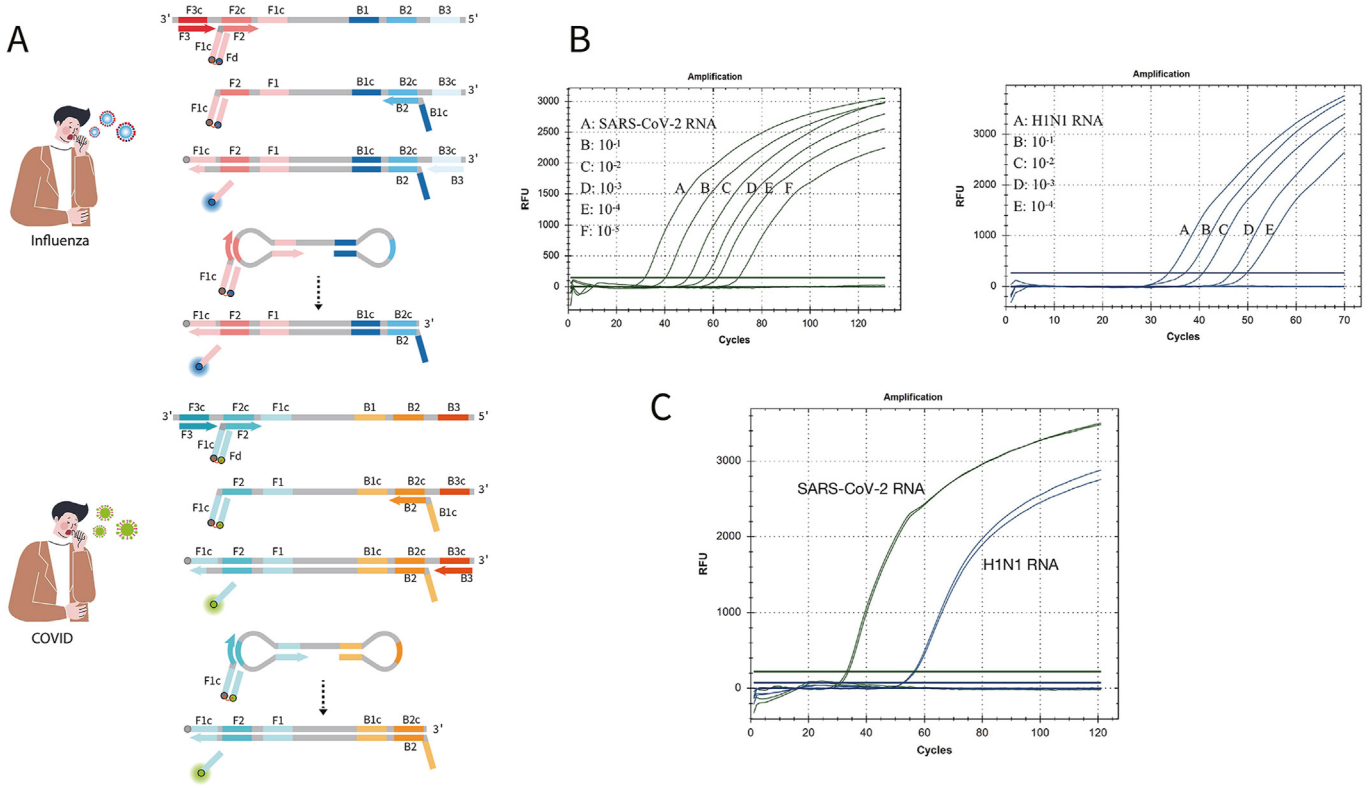


**Figure 2.** Detection of SARS-CoV-2 and the pandemic (H1N1) 2009 virus amplification by release of quenching with real-time isothermal platform. (A) Schematic diagram of probe design in the pandemic (H1N1) 2009 and SARS-CoV-2. (B) Multiplex detection in different concentration of SARS-CoV-2 and the pandemic (H1N1) 2009 virus. (C) Simultaneous detection of SARS-CoV-2 and the pandemic (H1N1) 2009 RNA samples in real-time isothermal reaction.

### 2.4. RT-LAMP reaction conditions

We first tested the performance of iLACO-dual with in vitro transcribed virus RNA fragments for SARS-CoV-2 and 2009 H1N1 influenza virus [7, 30]. When the RT-LAMP assays were performed with Bst 2.0 WarmStart DNA polymerase, each reaction condition (20 μL) consisted of 2 μL Isothermal Amplification Buffer (New England Biolabs), 2.8 μL dNTP Mix (10 mM), 0.5 μL WarmStart RTx Reverse Transcriptase (New England Biolabs), 1 μL Bst 2.0 WarmStart DNA polymerase, 2 μL RT-LAMP primer mix (0.8 μM FIP, 0.8 μM Q-FIP: Fd, 1.6 μM BIP, 0.2 μM F3/B3, 0.4 μM LF/LB), 1 μL RNA mix of SARS-CoV-2 and H1N1, 10.7 μL DEPC water. Bst 3.0 DNA polymerase (New England Biolabs, USA) was the same as Bst 2.0 WarmStart DNA polymerase, except the KCl is 150 mM in Isothermal Amplification Buffer instead of 50 mM for Bst 2.0.

For homemade Bst variant Bst v7.16, the Isothermal Amplification Buffer contains 10 mM $(NH4)_2SO4$, 50 mM KCl, 8 mM MgSO4, 0.1% Tween 20, and all components were mixed in DEPC water, then adjusted pH to 7.5 with 1M KOH and measured by pH paper (Supelco). The other reagents of the reaction condition (20 μL) were the same as Bst 2.0 and Bst 3.0 DNA polymerase.

## 3. Results and discussion

### 3.1. Primer region mapping

We selected the regions of the ORF1ab (SARS-CoV-2) and hemagglutinin (HA, the pandemic (H1N1) 2009) as target regions (Figure 1A and D). We used the online software Primer Explorer V5 (http://primerexplorer.jp/lampv5e/index.html) to design the RT-LAMP primers [7]. After the specificity analysis, we retained one primer set with several pairs of loop primers for SARS-CoV-2 and 2009 H1N1 influenza virus. To assure the primer specificity, we compared the sequence of SARS-CoV-2 with other viruses genome (include seven similar coronaviruses, two influenza viruses, and two other coronaviruses) by the online tool BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=MegaBlast&PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&DATABASE=n/a&QUERY=&SUBJECTS=) [26] to calculate the homology (Figure 1B). The results represent the percentage of sequence similarity comparison between SARS-Cov-2 and other viruses, and are shown in the arrows. Results indicated that the primer sequence of SARS-CoV-2 showed no significant similarity to the following viruses, which are Bat-SL-CoV-ZC45, SARS-CoV-SZ3, SARS-CoV-Tor2, Bat-SL-CoV-Rp3, Bat-SL-CoV-HKU3-1, HCoV-OC43, Influenza A virus H3N2 and Influenza B virus. The similarities between SARS-CoV-2 and Bat-SL-CoV-ZXC21, Bat-SL-CoV-Rf1, HCoV-HKU1 virus sequence are 90.29%, 91.26% and 77.61% respectively. For primer specificity analysis of the 2009 H1N1 influenza virus, we compared the sequence with other subtypes of influenza A virus genome, include two mainly infects humans subtypes H2N2 and H3N2, and highly pathogenic avian influenza viruses H5N1, H7N7, H7N9, H9N2 (Figure 1E). The numbers in the arrows represent the percentage of similarity between the sequence of the pandemic (H1N1) 2009 virus and the sequences of other subtypes. Sequence alignment showed no significant similarities between the pandemic (H1N1) 2009 virus and the other subtypes (H2N2, H3N2, H5N1, H7N7, H7N9, H9N2). The genetic distance generated by MEGA software was used to compare the degree of genetic difference between SARS-CoV-2 and other viruses (seven similar coronaviruses, two influenza viruses, and two other coronaviruses) [34]. A low value indicates that the genetic distance is close, which means that the common origin between viruses is close. The results were shown in a heat map with reference to the genetic distance matrix (Figure 1C). We showed the genetic distance data between SARS-CoV-2 and all viruses shown in Figure 1B in the figure. It can be seen from the results that the genetic distance between SARS-CoV-2 and Bat-SL-CoV-ZC45 is the closest (0.21), indicating that the common origin between these two viruses is close. The genetic distance between SARS-CoV-2 and Influenza B virus is the largest (2.50), indicating that the common origin between SARS-CoV-2 and this virus is relatively far. We used the same method to compare the genetic difference analysis between the pandemic (H1N1) 2009 virus and other subtypes of influenza A virus, and the results are shown in Figure 1F. The results show that the pandemic (H1N1) 2009 has relatively close homology with influenza A virus H5N1 and influenza A virus H2N2 (0.42), and relatively far from influenza A virus H7N7 homology (0.87).

### 3.2. Compariation and optimization of RT-LAMP enzymes

We choosed Bst 2.0 WarmStart, Bst 3.0 DNA Polymerase, and Home-Made Bst Variant Bst V7.16 to improve detection efficiency and optimize LAMP reagents. The iLACO-dual reaction conditions for the three different enzymes were the same: 120 cycles were carried out in RT-qPCR, each cycle of 30s, and the reaction temperature was set at 65 °C. Samples were tested with Taqman RT-qPCR, in which Ct values under 80 were called as positive, while Ct values were not determined or above Ct 80 were called as negative. The results showed that when Bst 2.0 WarmStart and Bst 3.0 DNA polymerase were used for dual fluorescent probe detection, the Bst 2.0 WarmStart DNA polymerase showed better detection performance, and the fluorescence signal detection of both FAM and HEX channels were faster (Table 1). We further tested the homemade Bst variant Bst v7.16, and the experimental results showed that the detection efficiency of Bst V7.16 was comparable to that of Bst 2.0 WarmStart DNA polymerase [27] (Table 1). Compared with the other two DNA polymerases, the detection efficiency of Bst V7.16 was more prominent, which provided an optimization direction for LAMP reagent optimization. However, its detection specificity and stability still need more experimental verification support, so we chose Bst 2.0 WarmStart DNA polymerase as the future research object to conduct the iLACO-Dual test.

### 3.3. Assay sensitivity

This experiment aims to compare and analyze the feasibility of detecting two kinds of viruses in the same reaction tube through separate and joint detection of the two viruses and whether the joint detection impacts the detection results. The sensitivity of the RT-LAMP detection method was verified while the two viruses were detected separately. Similar experiments were also conducted in the iLACO detection platform and compared with RT-qPCR [7]. In the detection of the two viruses respectively, RT-LAMP showed good detection sensitivity.

Before the test, we measured the RNA concentration of two viruses, SARS-CoV-2 8.16 ng/μL and 13.49 ng/μL for the pandemic (H1N1) 2009 virus RNA. We used Bst 2.0 WarmStart DNA polymerase to detect the two viruses. However, to avoid false positives, the reaction conditions and components should be optimized to maximize the sensitivity and reduce false positives. We optimized the concentration of the primer and verified the most suitable concentration of KCl for Bst 2.0 WarmStart DNA polymerase, which is 50mM. To check the detection limit of RT-LAMP, we prepared multiple reactions containing serial dilutions of synthesized ORF1ab gene and HA gene (10 times dilution). As shown in Figure 2B, RT-LAMP detection platform is very sensitive, two kinds of viral RNA can be detected at low concentrations (1:100000 dilution for SARS-CoV-2 RNA, 1:10000 for the pandemic (H1N1) 2009 virus RNA). The sensitivity of RT-LAMP to detection of SARS-CoV-2 was higher than that of the pandemic (H1N1) 2009 virus. This difference was also reflected in the experimental results of the joint detection of the two viruses.

We then matched the specific primers and viral RNA of the two viruses with a 1:1 ratio in the simultaneous detection of two clinical virus-positive samples. Then 2 μL of the primer mixture and 1 μL of the RNA sample mixture were taken for simultaneous detection. There were two blank control groups and two repeated experimental groups for each virus, respectively. In the process of simultaneous detection, the positive experimental groups of the two viruses showed good repeatability (Figure 2C). As shown from the test results in Figure 2C, the Ct value for SARS-CoV-2 was around 30, while the Ct value of the pandemic (H1N1)

**Table 1.** Comparison of commercial DNA polymerase with homemade Bst variant in RT-LAMP detection.

| Bst variants | Multiplex detection of SARS-CoV-2 and the pandemic (H1N1) 2009 virus samples | | Simultaneous detection of SARS-CoV-2 and the pandemic (H1N1) 2009 virus samples | |
|---|---|---|---|---|
| | SARS-CoV-2 (Ct) | (H1N1)2009 (Ct) | SARS-CoV-2 (Ct) | (H1N1)2009 (Ct) |
| Bst 2.0 Warmstart | 25.0 | 29.0 | 27.0 | 44.0 |
| Bst 3.0 | >80.0 | >80.0 | >80.0 | >80.0 |
| Bst V7.16 | 18.2 | 22.0 | 19.0 | 25.0 |

2009 virus was about 45. Compared with the individual detection results of each virus, the influence of the simultaneous detection on the experimental results was not significant, and the detection sensitivity of RT-LAMP to the two viruses was consistent with that of the separate detection. The detection sensitivity of RT-LAMP to SARS-CoV-2 was higher than that of the pandemic (H1N1) 2009 virus.

RT-LAMP sequence-specific isothermal nucleic acid amplification has become a growing field in molecular diagnosis, with its main advantages in providing rapid and sensitive detection. The detection cost of RT-LAMP depends significantly on the purification method of primers (PAGE, HPLC or desalt) and the different ordering companies of detection reagents. Here we compared the dosage and reference price of three different enzymes in one reaction system, the reference price for Bst 2.0 WarmStart DNA Polymerase and Bst 3.0 DNA Polymerase is similar, about $0.55/reaction (6.4 units). For homemade Bst variant Bst V7.16 the reference price is about $0.11/reaction (6.4 units). In addition, real-time isothermal amplification techniques often provide similar or better detection limits than PCR, with relatively short response times [7, 31, 32]. Secondly, due to their advantages in efficiency, cost and detection time, these methods show relatively obvious advantages when large quantities of samples are tested [33].

In this study, we designed specific fluorescent probe primers for two kinds of viruses and used real-time isothermal fluorescence amplification technology to simultaneously detect SARS-CoV-2 and the pandemic (H1N1) 2009 viruses in the same reaction tube. We first tested the two viruses separately to verify the effectiveness of the designed probe primers and test samples. To verify the sensitivity of RT-LAMP method to the detection of the two viruses, we carried out concentration gradient dilution of the RNA of the two viruses. The results showed that the sensitivity of RT-LAMP detection method to SARS-CoV-2 was better than that of the pandemic (H1N1) 2009 virus under the same reaction system. In the experiment of simultaneous detection of two positive virus samples, two sets of replicates were performed for each virus to verify the accuracy of the results. The results showed that the reproducibility of the test was good, and the fluorescence values detected at the same time were not significantly different from those detected alone. The results showed that the 1:1 mixture of probe primers and viral RNA of the two viruses did not significantly impact each other. The sensitivity of RT-LAMP to SARS-CoV-2 was slightly higher than that of the pandemic (H1N1) 2009 virus, which was consistent with the results of detection respectively.

In comparing and optimizing primers and reaction reagents, we also got some valuable suggestions, primer combination, fluorescence channel setting, and concentration of each reaction component will affect the detection results. Therefore, it is essential to analyze the primers and fluorescent channels and the concentration of reaction reagents for the highest sensitivity in RT-LAMP detection. In addition, in the RT-LAMP reaction process, the operating specifications must be strictly followed to avoid cross-contamination between samples to avoid inaccurate results and false positives. As the number of novel coronavirus infections continues to increase, we hope that this work will effectively detect SARS-CoV-2 and the pandemic (H1N1) 2009 virus during the flu season. Secondly, in the experiments of comparing different commercially available reagents with homemade reagents, the homemade Bst variant showed a shorter detection time. We hope that after more stability and accurate research experiments, the homemade reagents can be applied to more detection fields.

## 4. Conclusion

In summary, we developed a method for simultaneous detection of SARS-CoV-2 and the pandemic (H1N1) 2009 viruses using dual-fluorescence real-time isothermal platform. During simultaneous testing of two virus-positive samples, the test showed consistent results with separate trials, and the two viruses did not significantly interfere with each other's detection effect. The dual fluorescence pathway multiple detection methods mentioned in this experiment provide reference data for simultaneous detection of multiple viruses, verifying the feasibility of simultaneous detection of two viruses in the same reaction tube. After continuous experiments and optimization, we hope the simultaneous detection of two or even multiple viruses can be put into clinical and other applications, which may contribute to reducing labor cost, detection cost and detection time.

## 5. Ethical statement

Sample collection and analysis were approved in the P2 laboratory by the local CDC of Shenyang city. We obtained 248 SARS-CoV-2 RNA samples and 4 (H1N1) 2009 virus RNA samples from Shenyang Center for Disease Control And Prevention (Shenyang CDC) and conducted the study on February 26th. We had access to information that could identify individual participants during or after data collection through Shenyang CDC. The internal use of samples was agreed under the medial and ethical rules of each participating individuals.

## Declarations

### *Author contribution statement*

Lin Yu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Jingyao Wang: Performed the experiments; Analyzed and interpreted the data.

Xuelong Li, Weihua Chen, Vicent Pelechano: Analyzed and interpreted the data.

Lingling Mao, Yi Sui, Xing Guo: Contributed reagents, materials, analysis tools or data.

Xiushan Yin: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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### Data availability statement

Data will be made available on request.

### Declaration of interests statement

The authors declare the following conflict of interests: Simultaneous detection of SARS-CoV-2 and pandemic (H1N1) 2009 virus with real-time isothermal platform, which we have filed a patent application describing this method.

### Additional information

No additional information is available for this paper.

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