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# Letter to the Editor

## Rapid Detection of COVID-19 Coronavirus Using a Reverse Transcriptional Loop-Mediated Isothermal Amplification (RT-LAMP) Diagnostic Platform

***To the Editor:***

The recent outbreak of a novel coronavirus SARS-CoV-2 (also known as 2019-nCoV) threatens global health, raising serious cause for concern. It is urgent to develop rapid, accurate, and onsite diagnostic methods to effectively identify individuals with early infections, administer timely treatment, and control disease spread *(1)*. For viral RNA infections, particularly acute respiratory infection, probe coupled RT-qPCR of respiratory secretions is routinely used to detect causative viruses *(2)*. However, RT-qPCR has many limitations such as the need for high purity samples, trained personnel, and sophisticated facilities for sample processing and the access to expensive laboratory instruments; in addition, long reaction times (around 2 h) are required. Loop-mediated isothermal amplification (LAMP) combined with reverse transcription (RT-LAMP) allows the direct detection of RNA *(3, 4)*. This system can be coupled with a pH indicator present in the reaction mix to allow readout of the amplification reaction by change in color *(5)*.

Here we describe a LAMP-based method named iLACO (*i*sothermal LAMP based method for COVID-19) for rapid detection of the SARS-CoV-2. We selected a fragment of the ORF1ab as the target region and used the online software, Primer Explorer V5, to design the RT-LAMP primers. We ensured the primer specificity by comparing the target sequence with other viral genomes, including 9 corona and 2 influenza viruses using the NCBI BLAST tool.

We validated our method on an RNA sample extracted from a SARS-CoV-2 (RT-qPCR verified) positive patient. We observed a color change from pink to light yellow in the reaction tubes after 20 min of incubation at 65 °C; we further confirmed the size of the DNA amplification product using electrophoresis. The concentrations of the primers used in our method were as follows: 0.2 µmol/L of each outer primer (F3 5′-CCACTAGAGGAGCTACTGTA-3′ and B3 5′-TGACAAGCTACAACACGT-3′), 1.6 µmol/L of each inner primer (FIP 5′-AGGTGAGGGTTTTCTACATCACTATATTGGAACAAGCAAATTCTATGG-3′ and BIP 5′-ATGGGTTGGGATTATCCTAAATGTGTGCGAGCAAGAACAAGTG-3′), 0.4 µmol/L of each loop primer (LF 5′-CAGTTTTTAACATGTTGTGCCAACC-3′ and LB 5′-TAGAGCCATGCCTAACATGCT-3′). iLACO showed similar performance when we compared the samples from SARS-CoV-2 RNA or cDNA, indicating the one-step isothermal amplification is sufficient.

We further optimized the reaction protocols for potential field and bedside use. We validated the efficiency of iLACO in 1.5 mL tubes incubated in a water bath at 65 °C (WarmStart Colorimetric LAMP 2X Master Mix, M1800, NEB with manual) and found that 20 min reaction time was sufficient for a color change with a virus RNA concentration of 1000 copies per µL (i.e., using 1 µL sample in total 20 µL reaction). We recommend adding 20 µL of mineral oil after adding all the required solutions to avoid evaporation. To check the detection limit of iLACO, we made serial dilutions of synthesized ORF1ab gene (from 1 000 000 to 0.1 copies per µL). iLACO could detect as low as 10 copies of ORF1ab gene. Samples with copy number below 10 copies per µL failed to change color, even with extended incubation time up to 2 h. To further expand the iLACO detection capability, we added SYBR green dye (1:10 000 stock solution, S7563 from Thermo Fisher) into the reaction mix and checked the color change with a Gel imaging system. We also selected a new type of nucleic acid dye, GeneFinder™ (D039 from Bridgen), which has enhanced fluorescent signal and sensitivity. By exposure to blue light, green fluorescence was observed clearly with naked eye in the positive reaction with 100 copies per µL, whereas it remained pink in the negative control (Fig. 1).

We then evaluated the performance of iLACO in a total of 248 samples from COVID-19 patients diagnosed in Shenyang province, China. We were able to detect 89.9% (223/248) of samples with positive signals. The 25 false-negative samples were further tested with Taqman RT-qPCR and showed the Ct values above 35, indicating very low viral loads. We then ran iLACO on 11 samples (each in triplicate) with Ct values varying between 35 and 37, and obtained random color changes within the repeats, indicating that a concentration below 60 copies per µL was the detection threshold. Currently, most of the RT-qPCR reactions in China for SARS-CoV-2 test use 5 µL sample as input. We therefore checked whether increasing the sample volume would facilitate the detection. However, increasing the volume to 5 µL RNA sample led to variable results. This was most likely due to the presence of Tris or EDTA in the RNA dilution buffer when an automatic RNA extraction workstation is used. This could be optimized by adjusting the concentrations of the

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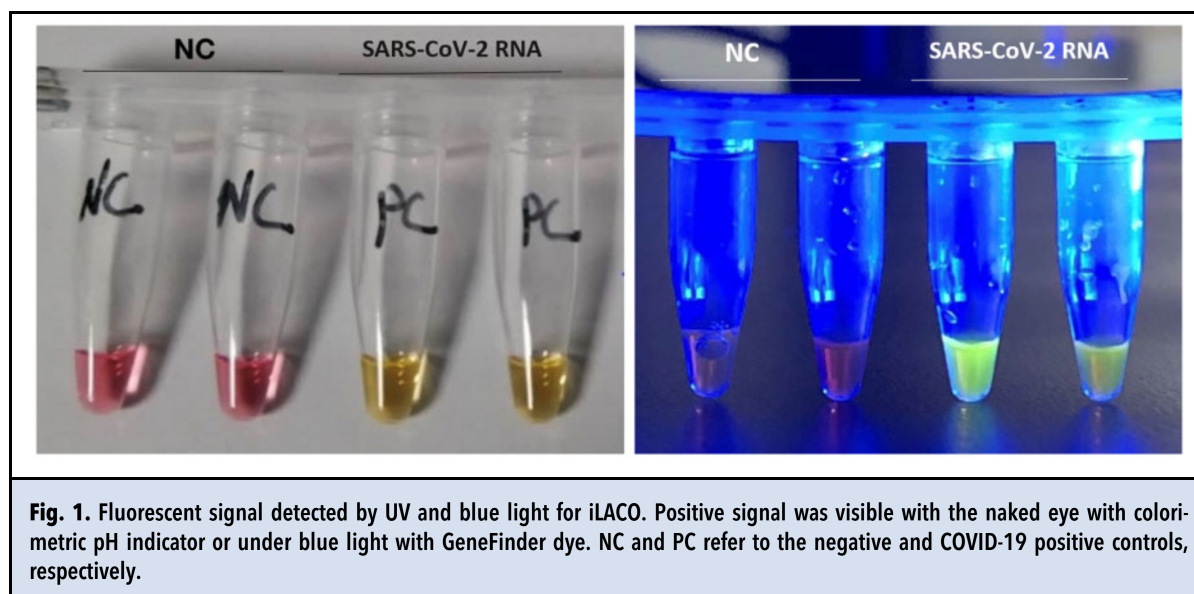


**Fig. 1.** Fluorescent signal detected by UV and blue light for iLACO. Positive signal was visible with the naked eye with colorimetric pH indicator or under blue light with GeneFinder dye. NC and PC refer to the negative and COVID-19 positive controls, respectively.

buffers used. We recommend always using a positive and a negative control sample resuspended in the same buffers used for patient RNA isolation.

In summary, we have developed a RT-LAMP-based method, optimized for the detection of SARS-CoV-2. Our method is robust, accurate, and simple to use. We hope that this method will contribute to continuing efforts to contain the still spreading SARS-CoV-2.

**Author Declaration:** A version of this article was previously posted as a preprint on medRxiv as https://www.medrxiv.org/content/10.1101/2020.02.20.20025874v1.

**Ethical Statement:** Sample collection and analysis of samples were approved in the P3 laboratory by the local CDC of Shenyang city. The internal use of samples was agreed under the medial and ethical rules for each participating individual.

**Author Contributions:** *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

X. Yin, W. Chen, V. Pelechano, X. Hao, S. Wu and L. Yu conceived the study and wrote the manuscript. X. Yin, L. Mao, X. Hao, S. Wu, L. Yu, X. Dong collected the data. X. Yin, W. Chen, L. Mao, X. Hao, S. Wu.and L. Yu processed and analyzed the data. X. Yin, W. Chen and V. Pelechano edited the final manuscript, all authors have read and approved the manuscript. X. Yin supervised the whole work.

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