Rapid detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform

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The recent outbreak of a novel coronavirus SARS-CoV-2 (also known as 2019-nCoV) threatens global health, given serious cause for concern. It is urgent to develop rapid, accurate and onsite diagnosis methods in order to effectively identify these early infects, treat them on time and control the disease spreading *(1)*. For RNA virus infections, especially acute respiratory infection, probe coupled RT-qPCR from 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, as well as requiring long reaction times (around 2 hs). 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 allowing 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 target region and used the online software Primer Explorer V5 (http://primerexplorer.jp/lampv5e/index.html) to design the RT-LAMP primers. We ensured the primer specificity by comparing the target sequence with other viral genomes, including nine corona and two influenza viruses using the NCBI BLAST tool.

We validated our method on the 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 minutes 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 followed: 0.2 μM of each outer primer (F3 5'-CCACTAGAGGAGCTACTGTA-3' and B3 5'-TGACAAGCTACAACACGT-3'), 1.6 μM of each inner primer (FIP 5'-AGGTGAGGGTTTTCTACATCACTATATTG

GAACAAGCAAATTCTATGG-3' and BIP 5'-ATGGGTTGGGATTATCCTAAA TGTGTGCGAGCAAGAACAAGTG-3'), 0.4 μM of each loop primer (LF 5'-CAGTTTTTAACATGTTGTGCCAACC-3' and LB 5'-TAGAGCCATGCCTAA CATGCT-3'). iLACO showed similar performance when we compared the samples from SARS-CoV-2 RNA or cDNA, indicating the one-step isother mal amplification is sufficient.

We further optimized the reaction protocols for potential field and bed-side usages. We validated the efficiency of iLACO in 1.5 ml tubes incubated in a water bath at 65° (WarmStart Colorimetric LAMP 2X Master Mix, M1800, NEB with manual) and found that twenty-minutes reaction time was sufficient for a color change with a virus RNA concentration of 1000 copies per μL (i.e. using 1μL sample in total 20 μL reaction). We recommend to add 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 can detect as low as 10 copies of ORF1ab gene. Samples with copy number below 10 /μL failed to change color even with extended incubation time up to 2 hours. To further expand the iLACO detection capability, we added SYBR green dye (1:10000 stock solution, S7563 from Thermo Fisher) into the reaction mix and checked the color change with a Gel imaging system. We also chose a new type of nucleic acid dye GeneFinder^{TM} (D039 from Bridgen), which has enhanced fluorescent signal and sensitivity. By exposing under 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 with in total 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 run iLACO on eleven 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 the RT-qPCR reactions in China for SARS-CoV-2 test use 5 μL sample as input. We thereby checked whether increasing the sample volume would facilitate the detection. However, increasing the volume to 5 μL RNA sample leads to variable results. This is most likely due to the presence of Tris or EDTA in the RNA dilution buffer when automatic RNA extraction workstation is used. This could be optimized by adjusting the concentration of used buffers. We recommend to use always a positive and a negative control sample resuspended in the same buffers used for patient RNA isolation.

In summary, we developed a RT-LAMP-based method, optimized for the detection of SARS-CoV-2. Our method is robust, accurate, and simple to use. We thus hope that it would contribute to our continuing effort to containing the still spreading SARS-CoV-2.

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 of each participating individuals.

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Conflict of interest

Xiushan Yin and Wei-Hua Chen are co-founders for Biotech & Biomedicine Science (Shenyang) Co. Ltd and Pluri Biotech Co.Ltd. Xiushan Yin is the co-founder for Nanog Biotech Co.Ltd.

Author Contributions

All authors confirmed they have met the following 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.

Contributions of the authors were as follows: 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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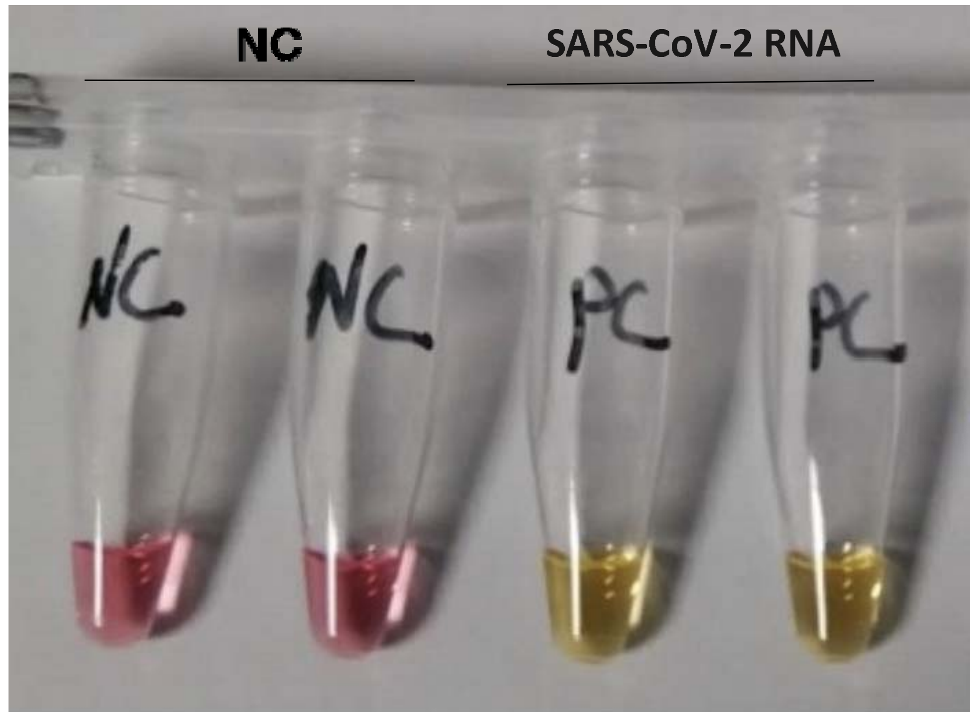
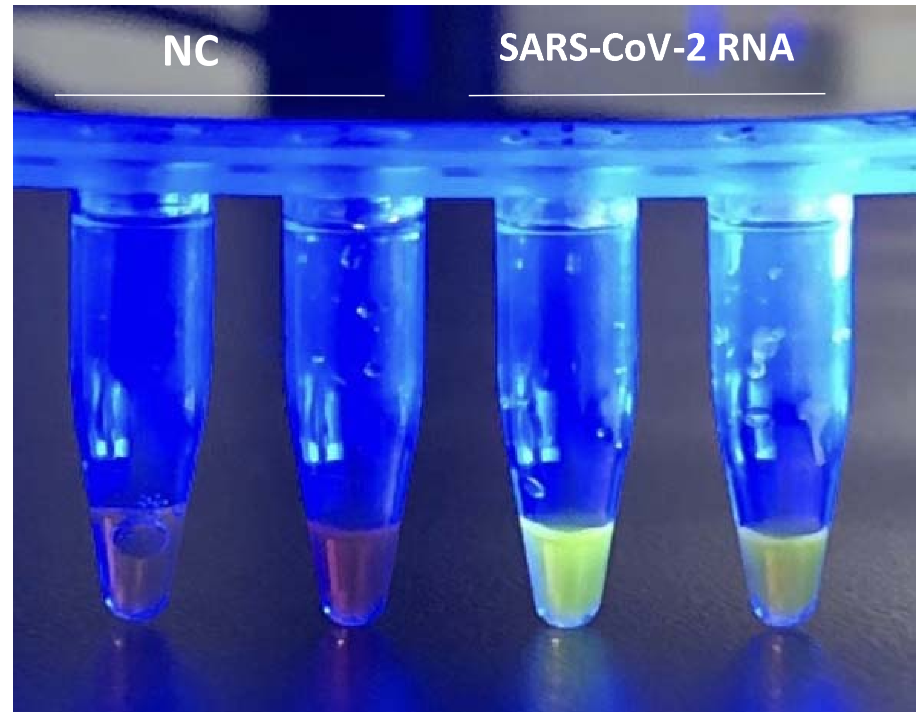
Figure legends

Figure.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 refers to the negative and COVID-19 positive control respectively.


NC
SARS-CoV-2 RNA
NC
NC
PC
PC


NC
SARS-CoV-2 RNA