Supporting Information

Rapid One-pot Detection of SARS-CoV-2 based on Lateral Flow Assay in Clinical Samples

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Table of Contents:
Experimental section .............................................................................................................................................. S2
Table S1. Detailed sequences for the current study ............................................................................................ S4
Table S2. Detailed sequences of synthetic pathogenic microorganism gene fragments. .................................... S6
Table S3. Details of 12 synthetic samples. ........................................................................................................... S14
Table S4. Results of RT-qPCR and RT-LAMP-LFA for clinical samples. ......................................................... S15
Figure S1. RT-LAMP reaction with and without dUTP. .................................................................................... S16
Figure S2. Illustrations of SARS-CoV-2 Orf1ab and N gene targeted by the primers ....................................... S17
Figure S3. pH of RT-LAMP system treated with 50mM NaOH. ....................................................................... S18
Figure S4. Confusion matrix and ROC analysis of RT-LAMP-LFA method with clinical samples. .. S19
Figure S5. Diagnostic results of 6 clinical Mycoplasma infection samples .................................................... S20
**Experimental section**

**Preparation of DNA Oligonucleotides.** The oligonucleotide primers used in RT-LAMP assay are listed in Table S1 and purchased from Sangon (Shanghai, China). The storing solutions of DNA oligonucleotides (10 μM) were obtained by dispersing them with nuclease-free water. The synthetic positive plasmids of all pathogenic microorganism used in this study are listed in Table S2, which were synthesized by Tsingke Biotech (Beijing, China). All plasmids were resuspended at 10^{12} copy/mL in nuclease-free water and stored at -20°C.

**Pseudovirus and RNA purification.** Pseudovirus of SARS-CoV-2 were provided by Zeesan Biotech (Xiamen, China), product batch number: 20020101. The pseudovirus was composed of an adenovirus capsid with RNA fragments containing Orf1ab and N gene sequences of SARS-CoV-2 virus. All pseudovirus were resuspended at 10^{10} copy/mL in nuclease-free water and stored at -20°C. Viral RNA in pseudovirus was extracted using the QIAamp Viral RNA Mini Kit from Qiagen of Canada according to the manufacturer’s instructions.

**Reaction System of the RT-LAMP Preamplification.** The RT-LAMP amplification was performed by incubating 0.2 μM of each of forward outer primer (F3) and backward outer primer (B3), and 0.8 μM of forward inner primer (FIP) and backward inner primer (BIP) with a certain concentration of template in 25 μL of 1×isothermal master mix (ISO-RT004, OptiGene, UK), and adding 4 μM Fluorescein-12-dUTP (ThermoFisher Scientific, United States) for lateral flow readout. SybrGreen I dye (Solarbio, China) was used in fluorescence detection for qPCR. The reaction was performed at 63°C for 30 min by the LightCycler 480 PCR instrument (Roche). The resultant mixture was then stored at 4°C until used for visual detection or gel electrophoresis imaging.

**Gel electrophoresis analysis.** The gel electrophoresis analysis was conducted by mixing 5 μL of RT-LAMP samples and 1 μL of 6× loading buffer (New England Biolabs, United States) together at room temperature. The mixtures were then loaded into the notches of a freshly prepared 10% native-PAGE for electrophoresis at a constant voltage of 100 V. Finally, the gel was immersed in 3× Gelred dye solution (Sangon, China) for 15 minutes before being scanned by the Gel Doc EZ imager with an Image Lab analysis software (BioRad, United States).
**Lateral flow assay readout.** After completion of the LAMP preamplification step, 20 µL of amplicon was combined with 80 µL of the chromatography buffer. A lateral flow strip (Ustar, China) was then added to the reaction tube and a result was visualized after approximately 2 min. Only a single band close to the top of the strip (Control line) indicated a negative result, while when another band close to the sample application pad (Test line) appearances indicated a positive result.

**Specificity of the LAMP Assay.** The specificity of the RT-LAMP assay was evaluated using synthetic plasmids of 13 common pathogenic microorganisms, including nucleoprotein gene of five different human coronaviruses (SARS-CoV, MERS-CoV, HCoV-NL63, HCoV-229E and HCoV-HKU1), Influenza A virus (H7N9, H5N1, H1N1 and H3N2) and Influenza B virus; 16S ribosomal RNA of *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Streptococcus pneumoniae*.

**Preparation of the clinical RNA samples.** The clinical RNA samples used in this research were isolated from anonymized surplus swab samples from the clinical laboratory of Central Hospital of Loudi. All positive samples had been analyzed using a standard RT-qPCR to confirm the presence of SARS-CoV-2. The study was approved by the Ethics Committee at the Central Hospital of Loudi. All methods were performed in accordance with the approved guidelines.
Table S1. Detailed sequences for the current study

| Pathogens | Name     | Sequence (5’→3’)                                                                 |
|-----------|----------|----------------------------------------------------------------------------------|
| SARS-CoV-2 (N gene) | N1-FIP* | Biotin-TTCCCCCTACTGCTGCCTGGAGTTCTCATACGTAUTGCGG |
|           | N1-BIP*  | Biotin-TTCTCTGCTAGAATGGCTGGCTGTCAAGCAGCAGCAGAAAG |
|           | N1-F3*   | GGCAGTCAAGCTCTTCTCTC                                                             |
|           | N1-B3*   | TTGCTCTCAAGCTGGTTCGA                                                             |
| SARS-CoV-2 (Orf1ab gene) | O1-FIP* | Biotin-TTGTACTCAAACATACGGCATAAACCTCAATTAAAACACAGTCTGTACC |
|           | O1-BIP*  | Biotin-GCTGATGCGCAATCGGTATTAGTGACTAGTACTAGTGTGCTT |
|           | O1-F3*   | TTGTGCTAATGACCTGTC                                                             |
|           | O1-B3*   | TCAAAAGGCTCTGATACGA                                                             |
| SARS-CoV-2 (N gene) | N2-FIP  | Biotin-TTCTCTACTGCTGCCTGGAGGCTGAGGTAUTGCTGCTCTTTCAATACAGTCTCTTTC |
|           | N2-BIP  | Biotin-TCGTACTGCTGAATGGCTGGCTGTCAAGCAGCAGCAGCAAAAG |
|           | N2-F3   | GCCAAAAGGCTCTTACGCA                                                             |
|           | N2-B3   | TTGCTCTCAAGCTGGTTCGA                                                             |
| SARS-CoV-2 (Orf1ab gene) | O2-FIP  | Biotin-GGTCATTAGGCACAAGTTTAGTAGTATCAAATCCCTAAAGGATTTGTC |
|           | O2-BIP  | Biotin-AACACAGTCTGTACCGGTACTGAAGCATGGTTCCGAGGT |
|           | O2-F3   | CCCTGTCACACATAGTCA                                                             |
|           | O2-B3   | AACGATTGTGCACTAGCT                                                             |
| SARS-CoV-2 (N gene) | N3-FIP  | Biotin-TCTGCCTAGAAGCTCTTGGCAAATGCTCAGTCATACGTAUTGCTAC |
|           | N3-BIP  | Biotin-GGCCTAGGAGCCTCTCAGTGTCGAGTTGAAAGGT |
|           | N3-F3   | CACCGGAAATCTCCTGTAAC                                                             |
|           | N3-B3   | CCAGGCCATCTCAGGAGGA                                                             |
| SARS-CoV-2 (Orf1ab gene) | O3-FIP  | BiotinATAACCTTTTGACATACCGCAGACCCCTGTTGGGTTTACACT |
|           | O3-BIP  | Biotin-GTATGTTGTGATCACTCCCGAGATGCACTTACACCAGAACC |

S4
|     | O3-F3       | AAATACCTACAACCTTGTGCTAA |
|-----|-------------|-------------------------|
|     | O3-B3       | ATCACTACTAGTCCTGTC      |
|     | O5-F3       | AAATACCTACAACTTGTGCTAA |
|     | O5-B3       | TCTGCAAGCAGCAGCAAAG    |
|     | O4-F3       | ACTTAAACACAGCTGTACCA    |
|     | O4-B3       | TCAAAGCCCTGTATACGA      |
| SARS-CoV-2 | N4-FIP     | Biotin-TGGCAGCTACGTTAGAAGGACTGGAACACGTTCCTCAGCTAGC |
| N gene | N4-BIP     | Biotin-TTCAACTCCAGCGCAGTGAGCAGCAGCATCACGGC |
|     | N4-F3       | GCTGCAATCGTGCTACAAGT    |
|     | N4-B3       | TCTGCAAGCAGCAGCAAAG    |
| SARS-CoV-2 | O4-FIP     | Biotin-TGACTGAAGCATGGAGGGTTCGCTTACGTTGATGTGGAAAG |
| Orf1ab gene | O4-BIP     | Biotin-GCTGATGCACAATCGTCTTCTTTAAGCAGCATCTACTAGTGCTGT |
|     | O4-F3       | ACTTAAAAACACAGCTGTACCA |
|     | O4-B3       | TCAAAGCCCTGTATACGA      |
| SARS-CoV-2 | N5-FIP     | Biotin-CCTACTGCTGCTGGAGTTGAAGCCCTTTTCGTTCTCTGATAC |
| N gene | N5-BIP     | Biotin-GCGGTGATGCTGCTTTGCTTTTGCCCTTTACCAGACA     |
|     | N5-F3       | GGCTTCTACGCAGAAGGGA     |
|     | N5-B3       | GTGACAGTTTTGCGCTTGTG    |
| SARS-CoV-2 | O5-FIP     | Biotin-TGGTTTTAAGTGTAAACACACAGGAGGTAAGTATGTACAATAACCTAC |
| Orf1ab gene | O5-BIP     | Biotin-AGTCTGTACCGTCTGCTGCTTTCGACCGTACCAGCATGG |
|     | O5-F3       | ATCCTAAAGGATTTTGTGACTT |
|     | O5-B3       | CCGTTTTAAAAACGATTGTGCA |
| H5N1 | FIP*        | Biotin-CGCACGAGACTTCTGTGTCCCTCAACTGCTGCTACTCATC |
|      | BIP*        | Biotin-GCAAGGATCACAATCCCTGCGAGGTTCGACTCCCTTTACTC |
|      | F3*         | GCCTCAAGCGAAACATGGA    |
|      | B3*         | CCCCTTCGCTTTATCATCCGAA |
| H7N9 | FIP*        | Biotin-GCTCCAGATCTCTCCTCGGGAGAGAATGGATCCCGGATGTG |
|      | BIP*        | Biotin-TGCAGGTGCAAGCAGTGAAGGCGGCTGTTGATCCCTCGT |
|      | F3*         | AGAGCTCTCGTGCTACTG     |
|      | B3*         | CCATTTTCGCTTCCAGAA    |

* Selected primer.
Table S2. Detailed sequences of synthetic pathogenic microorganism gene fragments.

| Name  | Sequence (5’-3’)                                                                 |
|-------|-------------------------------------------------------------------------------|
| SARS-CoV | ATGTCTGATAATGGACCCCAATCAAACCAACGTAGTGCCCCCACCAGATTACATTTGGTGACCCACAGATTCAACGAGGTGGCTCACTCAATCCACATGAGCTGGAGAATAGCAGACGAGGACCCCTGCTGCACCTCGTGCTGTTTCCTTTGCCGATAACAATGAATAAACAAATACAAACCTATCTCGAGGTAGAGGACGTAATCCAAAACCAACGGAGCTGCACCAAATAACACTGTCTCTTGGTACACTGGGCTTACCCAACACGGGAAAGTCCCTCTTACCTTTCCACCTGGGCAGGGTGTACCTCTTAAAGGCCAAACACAGGCGACAGGACAAATTAATACCGGGAATGGAATTAAGCAACTGGCTCCCAGGTGGTACTTCTACTACACTGGAACTGGACCCGAAGCAGCACTCCCATTCCGGGCTGTTAAGGATGGCATCGTTTGGGTCCATGAAGATGGCGCCACTGATGCTCCTTCAACTTTTGGGACGCGGAACCCTAACAATGATTCAGCTATTGTTACACAATTCGCGCCCGGTACTAAGCTTCCTAAAACTTCCACATTGAGGGGACTGGAGGCAATAGTCAATCATCTTCAAGAGCCTCTAGCTTAAGCAGAAACTCTTCCAGATCTAGTTCACAAGGTTCAAGATCAGGAAACTCTACCCG |
| HCoV-NL63 | ATGGCTAGTGTAAAAATTGGGCCGATGACAGAGCTGCTAGGAGAAGAAATTCTCCTCTCTTCTATTTGTGGTATGTCGTAAGGGCACCACCATGATAGGTGCTATCTTGTTGATAGTGTAATTCCAGGTGAACTTATTGTTCATCGTTAGTTGTTAGTTGTTGAGGATCGCTCTAATAACTCATCTCGTGCAGCAGTCGTTCTTCAACTCGTAACAACTCACGAGACTCTTCTCGTAGCCTTCAAGACAACAGTCTCGCACTCGTTCTGATTCTAACCAGTCTTCTTCAGATCTTGTTGCTGCTGTTACTTTGGCTTTAAAGAACTTAGGTTTTGATAACCAGTCGAAGTCACCTAGTTCTTCTGGTACTTCCACTCCTAAGAAACCATAAAGCTCCTTCTTCACACCCAGGGCTGATAAGCCTTCTCAGTTGAAGAAACCTCGTTGGAAGCGTTGTTCCTACCAGAGAGGAAAATGTTATTCAGTGCTTTGGTCCTCGTGATTTTAATCACAATATGGGGGAATTGCAGATCTTGTACAGAATGGTGTTGATGCCAAAGGTTTTCCACAGCTTGCTGAATTGATTCCTAATCAGGCTGCGTTATTCTTTGATAGTGAGGTTAGCACTGATGAAGTGGTGATAATGTTCAGATTACCTACACCTACAAAATGCTCTGTAAGGATAATAAAGACCTCTTTCTACAAGTTGGAATGTTGAAAAACGTTTCAATTTGAGATGATGCAGTTTCTCTTCTCCAGAATCTAAACCATTGGCTGATGATGATTCCAGCCATTATAGAAATTGTCAACGAGGTTTTGCATTAA |
| HCoV-229E | ATGGCTACAGTCAAAATGGGGCGATGACAGAGCTGCTAGGAGAAGAAATTCTCCTCTCTTCTATTTGTGGTATGTCGTAAGGGCACCACCATGATAGGTGCTATCTTGTTGATAGTGTAATTCCAGGTGAACTTATTGTTCATCGTTAGTTGTTAGTTGTTGAGGATCGCTCTAATAACTCATCTCGTGCAGCAGTCGTTCTTCAACTCGTAACAACTCACGAGACTCTTCTCGTAGCCTTCAAGACAACAGTCTCGCACTCGTTCTGATTCTAACCAGTCTTCTTCAGATCTTGTTGCTGCTGTTACTTTGGCTTTAAAGAACTTAGGTTTTGATAACCAGTCGAAGTCACCTAGTTCTTCTGGTACTTCCACTCCTAAGAAACCATAAAGCTCCTTCTTCACACCCAGGGCTGATAAGCCTTCTCAGTTGAAGAAACCTCGTTGGAAGCGTTGTTCCTACCAGAGAGGAAAATGTTATTCAGTGCTTTGGTCCTCGTGATTTTAATCACAATATGGGGGAATTGCAGATCTTGTACAGAATGGTGTTGATGCCAAAGGTTTTCCACAGCTTGCTGAATTGATTCCTAATCAGGCTGCGTTATTCTTTGATAGTGAGGTTAGCACTGATGAAGTGGTGATAATGTTCAGATTACCTACACCTACAAAATGCTCTGTAAGGATAATAAAGACCTCTTTCTACAAGTTGGAATGTTGAAAAACGTTTCAATTTGAGATGATGCAGTTTCTCTTCTCCAGAATCTAAACCATTGGCTGATGATGATTCCAGCCATTATAGAAATTGTCAACGAGGTTTTGCATTAA |
GTGTTAGGCGCAAGAAATTCAGAACCAGAGATACCACACTTCAATCAAAA
GCCTCCCCAAATTGTTGTTACTGTTGAAGAACACTCTCCCGTGCTCCCT
TCCCGGTCTCAGTCGAGGTCGCAGAGTCGCGGTCGTGGTGAAATCCAAA
CTCTAATCTCCGAACCTCAGGGAAGATTCACATGTCGAGGATCG
ACATCATGAAGGCCAGTTGGTGCTGCCCTCAAATTTTTGTTGACAGG
CCTAGGAAAAAGATAGACAGCTGCAAAACCGGGTACTCTCTAGCTTTCA
GATAAACATGCAGACTTCTCTTCCTTCCACCTCCCAATTTGCGCTCT
AACAGGCAATCCAAACTTGGGTAAGTTTCTTGAGGAGTTAAATGCATTCA
CTAGAAAAGTCAAAACACTCCTCTTCTTTAACCTTAGTGACTAGAATTC
AACCCATCTCAAACATCCCTGCAATCGTGAAACCAGTCGATGAAAG
TTCTATTGAAACTGACATAATTTAGGAAGTTAAACTCTAA

HCoV-HKU1

ATGCTTCTTACTCCGCGGTATTATGTTGAAAGCTTCTTTCTGGAA
TCGTTCCGAACTTCTCCACAAAGAAAAATCTTCTTGCTGAAACCATCTGGCA
AATTACCAAAACCTTTATAGAGGCAGAAAACCCAACCTTAATTCACTGCT
GTTCTACTCAACAAAGAGGAATACTACATACTCCCAAAATTATCTCCTCCT
GAGATCTAATTTTCAAAAAAGGTAGAGACTTTAATTTTCAGATGGTC
AAAGAGATCCCGCCATCTCTCTGGCTGAGTTGTGGCTCTAAAATGCTGCTCT
CAGAGGCTAATGTCTTCCAAAGGATGCTGAAACATTTTCTGTTTGAACTA
CTAGAAAAGTCAAAACACTCCTCTTCTTTAACCTTAGTGACTAGAATTC
AACCCATCTCAAACATCCCTGCAATCGTGAAACCAGTCGATGAAAG
TTCTATTGAAACTGACATAATTTAGGAAGTTAAACTCTAA

ATCCTATATGTGAAGACTCTGTTGCTTAA
| Chlamydia pneumoniae | GGAATAATGACTTCGTTTGGTTATTTAGTGCGGAAGGGTTAGTAGTACATAGATACTTCAGAATCCCTACATTGGGAGGAACGCTGGTGGGTAACGGCTCACCAAGGCGACGATACATAGCCGACCTGAGAGGGGTGATCGGCCACACTGGGACTGAGACACGCCGAGACTCCTACGGGAGGCTGCAGTCGAGAATCTTTCGCAATGGACGAAAGTCTGACGAAGCGACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTAAGAGAAGAACGAGTGTGAGAGTGGAAAGTTCACACTGTGACGGTATCTTAACAGAAAGGGACGGCTAACTACGTGCCAGCAGCAGGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTAGATAAGTCTGAAGTTAAAGGCTGTGGCTTAACCATAGTAGGCTTTGG |
| Streptococcus pneumoniae | ATTTGATCCTCGCTAGCAAGCAGACGCTGGGCGGTCTCAATACAGTCAAGTGAGAAGGAGAGTGGATGTTGCATGACATTTGCTTAAGGTGCACTTGCATCACTACCAGATGGACCTGCGTTGTATTAGCTAGTGTTGGGGTAACGGCTCACCAAGGCGACGATACATAGCCGACCTGAGAGGGGTGATCGGCCACACTGGGACTGAGACACGCCGAGACTCCTACGGGAGGCTGCAGTCGAGAATCTTTCGCAATGGACGAAAGTCTGACGAAGCGACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTAAGAGAAGAACGAGTGTGAGAGTGGAAAGTTCACACTGTGACGGTATCTTAACAGAAAGGGACGGCTAACTACGTGCCAGCAGCAGGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTAGATAAGTCTGAAGTTAAAGGCTGTGGCTTAACCATAGTAGGCTTTGG |
| Mycoplasma pneumoniae |
|-----------------------|
| AACTGGTTAAGTGCAAGAGGGGAGAGTTGGAAATTCATTGATGTGAG |
| CCGTGGATTGAGTGGAGGACGTCGCTAGCTGGGAAAGCTTGGGAGCAGCAACAG |
| GATTAGATACCCCTGGATCCACGGTCTGTAACATGACTAGTGGTCTTAA |
| GACCCCTTCCGCGTTAGTGCCTAAGCCCTAATAGCAACTCCCGCTCTCTCTCT |
| CCGGACAGAGGTGACAGGTGCTCTGTCCTGCGGACAGAGGGCAGGACAGGCTC |
| TCTGGCTTGAAGTCCCGCTAGGATTGCGAGCATGTGGTTTAATCCGAGCATCAGG |
| CAAGGCTCTGCTAGTTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTT |
| ACCATTTGCTCAGTTGCGCTAGTTGCGAGAGTTTTCCTCTTCGAGGACAGAGGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGCCTAATAACCGGAG |
| GAAGGTGGGAGTACGTGCAAATCATCATGCCCCTTATGACCTGGGCTACACGTGCTACAATGGCTGGTACAACGAGTCGCAAGCCGGTGACGGCAAGCTAATCTCTTAAAGCCAGTCTCAGTTCGGATTGTAGGCT |
| GCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGT |
| GAATACGTTCCCGGGCCTTGTACACACCCCGGTCACACCAGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCGTAAGGAGCCAGCCGCCTAAAGGTGGGATAGATGATTGGGGTGAAGTCTGTAACAAGGTCAGCCGTTTGGGAGA |
| Mycoplasma pneumoniae | TTAACGCTGGCGGCATGCTCATTAATATCATGCAAGTTCGATCGAAAGGTAGTAATATCCTTAAAGGGAGGCGGATGCTGATTAACACGTATCCACCTACCTCATATAATG |
| GGGATAACATGTTGAAAGAAGCTAGCTAATACCGCATAAAGAAACTTTGGTT |
| GCCAGAATACAAAGTTGAAAGGACCTCGAAGGTTGGTAAATTCGACGGTAGGA |
| GAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| CTTGGACTGTACATAGGTAAGTAAAGCCTGTTAGGAAAGACAGGTCG |
| AGCCCATATCTACCGGGAGCACAGTAGGGAATTTCTCATAATGAGGCT |
| GAAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| GCGGTAATACATAGGTCGCAAGCGTCTTATCCGGATTTATGGGCGTAAAAGC |
| AACGAGCCAGGGGATGAAAGCTCTGGTGTAAAGGCGACTGCTTAAAC |
| GTTTGAGCTTGCAATGAGGCTGAAATGCATTGAGGTAATGACAGG |
| ATACGCTGGCGGCATGCTCATTAATATCATGCAAGTTCGATCGAAAGGTAGTAATATCCTTAAAGGGAGGCGGATGCTGATTAACACGTATCCACCTACCTCATATAATG |
| GGGATAACATGTTGAAAGAAGCTAGCTAATACCGCATAAAGAAACTTTGGTT |
| GCCAGAATACAAAGTTGAAAGGACCTCGAAGGTTGGTAAATTCGACGGTAGGA |
| GAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| CTTGGACTGTACATAGGTAAGTAAAGCCTGTTAGGAAAGACAGGTCG |
| AGCCCATATCTACCGGGAGCACAGTAGGGAATTTCTCATAATGAGGCT |
| GAAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| GCGGTAATACATAGGTCGCAAGCGTCTTATCCGGATTTATGGGCGTAAAAGC |
| AACGAGCCAGGGGATGAAAGCTCTGGTGTAAAGGCGACTGCTTAAAC |
| GTTTGAGCTTGCAATGAGGCTGAAATGCATTGAGGTAATGACAGG |
| ATACGCTGGCGGCATGCTCATTAATATCATGCAAGTTCGATCGAAAGGTAGTAATATCCTTAAAGGGAGGCGGATGCTGATTAACACGTATCCACCTACCTCATATAATG |
| GGGATAACATGTTGAAAGAAGCTAGCTAATACCGCATAAAGAAACTTTGGTT |
| GCCAGAATACAAAGTTGAAAGGACCTCGAAGGTTGGTAAATTCGACGGTAGGA |
| GAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| CTTGGACTGTACATAGGTAAGTAAAGCCTGTTAGGAAAGACAGGTCG |
| AGCCCATATCTACCGGGAGCACAGTAGGGAATTTCTCATAATGAGGCT |
| GAAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| GCGGTAATACATAGGTCGCAAGCGTCTTATCCGGATTTATGGGCGTAAAAGC |
| AACGAGCCAGGGGATGAAAGCTCTGGTGTAAAGGCGACTGCTTAAAC |
| GTTTGAGCTTGCAATGAGGCTGAAATGCATTGAGGTAATGACAGG |
| ATACGCTGGCGGCATGCTCATTAATATCATGCAAGTTCGATCGAAAGGTAGTAATATCCTTAAAGGGAGGCGGATGCTGATTAACACGTATCCACCTACCTCATATAATG |
| GGGATAACATGTTGAAAGAAGCTAGCTAATACCGCATAAAGAAACTTTGGTT |
| GCCAGAATACAAAGTTGAAAGGACCTCGAAGGTTGGTAAATTCGACGGTAGGA |
| GAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| CTTGGACTGTACATAGGTAAGTAAAGCCTGTTAGGAAAGACAGGTCG |
| AGCCCATATCTACCGGGAGCACAGTAGGGAATTTCTCATAATGAGGCT |
| GAAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| GCGGTAATACATAGGTCGCAAGCGTCTTATCCGGATTTATGGGCGTAAAAGC |
| AACGAGCCAGGGGATGAAAGCTCTGGTGTAAAGGCGACTGCTTAAAC |
| GTTTGAGCTTGCAATGAGGCTGAAATGCATTGAGGTAATGACAGG |
| ATACGCTGGCGGCATGCTCATTAATATCATGCAAGTTCGATCGAAAGGTAGTAATATCCTTAAAGGGAGGCGGATGCTGATTAACACGTATCCACCTACCTCATATAATG |
| GGGATAACATGTTGAAAGAAGCTAGCTAATACCGCATAAAGAAACTTTGGTT |
| GCCAGAATACAAAGTTGAAAGGACCTCGAAGGTTGGTAAATTCGACGGTAGGA |
| GAAGCTTTATGAGGCAATGCGGCTCTTTCAGCCAGGCTAACAGGAGTGAAGTT |
| Sequence | Source |
|----------|--------|
| AGTTGGTCTCACGTTCAAGGTTGGGGCTGCAATTCTCGTCCTCATGAAGTCGGAATCACTAGTAATCGCGAATCAGC | Influenza B virus |
| TATGTCGCGGTGAATACGTTCTCGGGTCTTGTACACACCGCCCGTCAAACTATGAAAGCTGGTAATATTTAAAAACGTGTTGCTAACCATTAGGAAGCGCATGTCAAGGATAGCACCGGTGATTGGAGTTAAGTCGTAACAAGGTACCCCTACGAGAACGTGGGGGTGGATCCTCCTTT | |
| AGAATTCTATTGGCTGCCACTGATGACAAGAAAACCGAGTTCCAGAAGAAAAAGAATGCCAGAGATGTCAAAGAAGGAAAGAAGAAATAGATCACAACAAAACAGGAGGCACCTTTTACAAGATGTTAAGAGATGATAAACAACATTAGATCACAAAGGATAGAATAAGTGGTTGCAATCAAAG | Influenza A virus H1N1 |
| ATGGCGTCTCAAGGCACCAAACGATCATATGAACAAATGGAGACTGGTGGGGAGCGCCA | |
| GGTGCTTTCTGGAATCGGGAGATTCTACATCCAAATGTGCACTGAACTCAAACTCAGTGATTATGATGGACGACTAATCCAGAATAGCATAACAATAGAGGATTGTCGCTTTCTGCTTTTGATGAGAGAAGAAATAAATACCTAAAGAGCAGACAGTTCCAGAAGATCAGGTGCTACTGGTGTTGCAATCAAAGGAGGTGGAACCTTAGTGGCTGAAGCCATTCGGTTTATAGGAAGAGCAATTGGCAGACAGGGCTATTGAGAGACATCAAAGCCAAGACTGCC | |
| AGAGTATCGAAGGCTGCTTCAGTTCAAGGTTGGGGCTGCAATTCTCGTCCTCATGAAGTCGGAATCACTAGTAATCGCGAATCAGC | Influenza B virus |
| TATGTCGCGGTGAATACGTTCTCGGGTCTTGTACACACCGCCCGTCAAACTATGAAAGCTGGTAATATTTAAAAACGTGTTGCTAACCATTAGGAAGCGCATGTCAAGGATAGCACCGGTGATTGGAGTTAAGTCGTAACAAGGTACCCCTACGAGAACGTGGGGGTGGATCCTCCTTT | |
| AGAATTCTATTGGCTGCCACTGATGACAAGAAAACCGAGTTCCAGAAGAAAAAGAATGCCAGAGATGTCAAAGAAGGAAAGAAGAAATAGATCACAACAAAACAGGAGGCACCTTTTACAAGATGTTAAGAGATGATAAACAACATTAGATCACAAAGGATAGAATAAGTGGTTGCAATCAAAG | Influenza A virus H1N1 |
| ATGGCGTCTCAAGGCACCAAACGATCATATGAACAAATGGAGACTGGTGGGGAGCGCCA | |
| GGTGCTTTCTGGAATCGGGAGATTCTACATCCAAATGTGCACTGAACTCAAACTCAGTGATTATGATGGACGACTAATCCAGAATAGCATAACAATAGAGGATTGTCGCTTTCTGCTTTTGATGAGAGAAGAAATAAATACCTAAAGAGCAGACAGTTCCAGAAGATCAGGTGCTACTGGTGTTGCAATCAAAGGAGGTGGAACCTTAGTGGCTGAAGCCATTCGGTTTATAGGAAGAGCAATTGGCAGACAGGGCTATTGAGAGACATCAAAGCCAAGACTGCC | |
| Influenza A virus H3N2 | GATTTGTCCCTTCCAGGGCGGGAGTCTTCGAGCTCTCGGACGAAAGG <br> GCAACGACCCACGATCGTGCCCTTCTTTGACATGAGTAATGGAAGGGTGCTT <br> ATTTCTCCGGAGACAAATGCAAGAGGATATGACGTTGGAAG | TTTGAAAAAGGGGATTTCTTTGGAAGGACCCTTTCAAAACTACT <br> CCAACCGTATACAGGCTAAGGAAACACTAATCAACAGAGGCCCCTCTCAGGCCC <br> AAATCAGTGTGCAAACCTGCATTTTCATGCAAGGAAACCTCCTCAATTGA | CAAATTCAACCATCATCGGCCAGATTGACTCGGGAATACGGAAGGAAGACC <br> TCAGACATGAGGGCAAAATACATGAGATGGAAGGTGCAAAACCAAGAAATGTCCTTCCGTGGGCG | 
| Influenza A virus H5N1 | AGCAAAAGCGAGGGTGAATATCATACTACCTGACTAGTGCACTCACAACATCGGG <br> CTCTCAGGGGACCCAAAAACGATCTTATGAAACAGATGAAACACTGTTGGGAAGG <br> AAGCAGGAAATGGTGACTCGAGATTGAGTTGAACTGGTGGAGAACGCCAGAATGCTACTGAGATCAGAGCATCTGTTGGAAGAATGGTTGGTGGAATTGGGAGGTTTTATATACAGATGTGCACTGAGCAAAAGCAATGGA | AGCAAAAGCGAGGGTGAATATCATACTACCTGACTAGTGCACTCACAACATCGGG | 

**S12**
Influenza A virus H7N9

TCTTCCAGGGCCGGAGGTCTTCGAGCTCTCGGACGAAAAGGCAACG
AACCCGATCGTGCCTTCCTTTGACATGAGTAATGAAGGATCTTATTTCTT
CGGAGACAATTCAGAGGAATATGACAATTGAAGAAAAATACCTCTTGT
CTACT
Table S3. Details of 12 synthetic samples.

| Sample | Pseudovirus concentrations | Sample type       |
|--------|----------------------------|------------------|
| 1      | $1.5 \times 10^4$ copies/mL | Nasopharynx swab |
| 2      | $1.5 \times 10^4$ copies/mL | Nasopharynx swab |
| 3      | $1.5 \times 10^4$ copies/mL | Nasopharynx swab |
| 4      | $1.5 \times 10^5$ copies/mL | Nasopharynx swab |
| 5      | $1.5 \times 10^5$ copies/mL | Nasopharynx swab |
| 6      | $1.5 \times 10^6$ copies/mL | Nasopharynx swab |
| 7      | $1.5 \times 10^6$ copies/mL | Nasopharynx swab |
| 8      | $1.5 \times 10^6$ copies/mL | Nasopharynx swab |
| 9      | $1.5 \times 10^7$ copies/mL | Nasopharynx swab |
| 10     | $1.5 \times 10^7$ copies/mL | Nasopharynx swab |
| 11     | $1.5 \times 10^7$ copies/mL | Nasopharynx swab |
| 12     | $1.5 \times 10^7$ copies/mL | Nasopharynx swab |
Table S4. Results of RT-qPCR and RT-LAMP-LFA for clinical samples.

| Sample | RT-qPCR (Ct value) | RT-LAMP-LFA |
|--------|--------------------|-------------|
| 1      | 31                 | Positive    |
| 2      | 33                 | Positive    |
| 3      | 30                 | Positive    |
| 4      | 29                 | Positive    |
| 5      | 30                 | Positive    |
| 6      | 32                 | Positive    |
| 7      | 28                 | Positive    |
| 8      | 32                 | Positive    |
| 9      | Not detected       | Negative    |
| 10     | Not detected       | Negative    |
| 11     | Not detected       | Negative    |
| 12     | Not detected       | Negative    |

For RT-qPCR: Ct < 35: positive; Ct > 35: retest; Ct = not detected: negative.
For RT-LAMP-LFA: Positive: Both C and T lines are red; Negative: C line is red, T line is not red; Retest: C line is not red.
**Figure S1.** Comparison of RT-LAMP amplification efficiency with and without dUTP.

The fluorescence kinetics data are presented as the mean ± s.d., n = 3 independent experiments.
Figure S2. Illustrations of SARS-CoV-2 Orf1ab and N gene targeted by the primers.
Figure S3. pH of RT-LAMP system treated with 50mM NaOH.
Figure S4. Confusion matrix and ROC analysis of RT-LAMP-LFA method with clinical samples. Clinical samples: 8 SARS-CoV-2 infection samples and 4 healthy donors.
Figure S5. Diagnostic results of 6 clinical Mycoplasma infection samples. The negative control contains NaOH-treated water as amplification template, and the positive control contains 1000 copies SARS-CoV-2 pseudovirus as amplification template.