Supporting Information

Single and Two-Stage, Closed-Tube, Point of Care, Molecular Detection of SARS-CoV-2

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Supplementary Methods

qRT-PCR

We carried out RT-PCR with the CDC Emergency Use Authorization (EUA) primers/probes (IDT, Catalog number: 10006713). Five microliters of extracted SARS-CoV-2 RNA and the premixed primers/probes at the CDC’s recommended concentrations were added to the reaction buffer (New England Biolabs, Luna Universal Probe One-Step RT-qPCR kit, cat #E3006L). RT-PCR was carried out with a Thermal Cycler (BioRad, Model CFD3240) with the temperature profile of 55°C for 10 min, and then 95°C for 1 min, followed by 45 cycles of amplification (95°C for 15 s and 60°C for 1 min).

Calibration Curves

To obtain a calibration curves, we determined the threshold time as a function of template concentration from a dilution series of purified SARS-CoV-2 RNA genome (USA-WA1/2020 strain). This calibration curve was verified with a Twist Synthetic SARS-CoV-2 RNA Control (MN908947.3, Twist Bioscience).

Verification of the Specificity of Our LAMP Assay

RNAs of coronaviruses from different genera [Alphacoronavirus (PEDV and TGEV); Gammacoronavirus (IBV); and Deltacoronavirus (PDCoV)], MERS-CoV (Betacoronavirus) cDNA, and SARS-CoV (Betacoronavirus) synthetic DNA (Table S2) were used as negative controls to verify the specificity of our LAMP assay.

Infectivity of Coronavirus and RNA Integrity after Heat Treatment

To reduce the burden on our BSL3 facilities, we used avian infectious bronchitis gammacoronavirus (IBV) isolate (10^{3.2} EID_{50} /ml) as a surrogate for SARS-Cov-2 to examine the effect of heat treatment on coronavirus infectivity and on RNA degradation.

Coronavirus Infectivity after Heat Treatment. We incubated 400 µl aliquots of IBV in 1.5 ml cryotubes submerged in a water bath at 56°C, 70°C, and 95°C for 5 min, 10 min, and 30 min. Immediately after incubation, the tubes were placed on ice. The 50% embryo infective dose (EID_{50}) titer was then determined with the Reed–Muench method. Later, we verified in BSL3 experiments that the optimal deactivation conditions identified for IBV are also applicable to SARS-CoV-2.
Coronavirus RNA Quality with RNase inhibitor protection.

We evaluated the stability of coronavirus RNA of the heat-treated samples in the presence and in the absence of RNase inhibitor by monitoring the threshold cycle as a function of sample preparation conditions. We used the RNase inhibitor iNtRON (optimal working temperature 42°C, Cat. 25011, iNtRON Biotechnology, Seongnam, Korea), RNase inhibitor RNasin® (recommended temperature from 50 to 70°C, Cat. N2615, Promega), and a home-made RNase inhibitor TCEP/EDTA\(^3\). RNA was extracted with the RNA extraction kit (Qiagen, Cat. No. 52904/52906) and quantified with real-time RT-qPCR\(^4\).

Block Heater

An inexpensive, portable block heater (Fig. 5) was developed in house. Our block heater consists of a PID controller (STM32F103C8T6), heating silk nickel chrome wire (HAZY XH-RS2090), two Pt1000 temperature sensors, an LCD screen, control buttons, an aluminum heating block, a buzzer (12V, Risym) to alert the user of scheduled operations, and an OEM power supply that works with an adjustable step-down regulated power supply module (Risym LM2596S-ADJ) to provide 5V DC to the PID controller and 12V to other system components. The various components were packaged in a 3D-printed box.

Supplementary Results and Discussion

Comparison of Direct RT-LAMP with OptiGene Reaction Mix and NEB Colorimetric Master Mix

We repeated our experiments using NEB colorimetric master mix (NEB #M1800) (Figure S8). The NEB master mix includes phenol red – a pH indicator that reacts to proton produced during polymerase, changing from purple to yellow (Figure S8A). We obtained similar threshold times for virions in saline, water, and saliva in the presence of TCEP/EDTA and after incubation at 95°C for 5 min (Figure S8B). In contrast to the OptiGene master mix, the NEB buffer mix performed poorly in the absence of pre-incubation (95°C, 5 min) (Figure S8C). We suspect that this is caused by differences in the reaction mix compositions. The OptiGene reaction mix includes detergent that lyses the virus and appears to be absent in the NEB reaction mix,
The pH-based colorimetric detection is, however, inappropriate for testing saliva because saliva's acidity varies from one person to another and depends on diet. Hence, in the absence of pH control, the NEB colorimetric test occasionally produces false positives. Indeed, we have observed, in a few cases (Figure S8D), an immediate color change when sample was added to the reaction mix, prior to incubation. The LCV dye has the advantage over phenol red, being less sensitive to pH variations.
Figure S1: Target region and sequences of LAMP primers for SARS-CoV-2- ORF1ab gene: A) SARS-CoV-2 amplicon sequence with the LAMP primers’ positions indicated; arrows show the extension direction. B) Comparison of the targeted sequence of the ORF1ab gene with other human coronaviruses (SARS-CoV, MERS-CoV, HCoV-HKU1, HCoV-229E, HCoV-OC43 and HCoV-NL63).
Figure S2: Target region and sequences of LAMP primers for SARS-CoV-2 N gene: A) SARS-CoV-2 amplicon sequence with the LAMP primers' positions indicated; arrows show the extension direction. B) Comparison of the targeted N gene sequence with other human coronaviruses (SARS-CoV, MERS-CoV, HCoV-HKU1, HCoV-229E, HCoV-OC43 and HCoV-NL63).
Figure S3: Quantification of inactivated SARS-CoV-2 with contrived samples. (A) RT-PCR amplification curves of $5 \times 10^6$, $5 \times 10^5$, $5 \times 10^4$, $5 \times 10^3$, 500, 50, 5, and 0 (no template control) copies of SARS-CoV-2 RNA genome per reaction (all experiments in duplicate). (B) Threshold PCR cycle (Ct) as a function of the log of SARS-CoV-2 RNA genome copies per reaction. (C) Amplification curves of ~40 copies per microliter spiked in VTM, saline, water, and saliva behaved similarly.
Figure S4: RT-RPA detection of SARS-CoV-2 ORF1ab and N gene with LAMP F3/B3 primers as forward and backward primers. (A) RT-RPA amplification curves targeting the ORF1ab gene in the presence of $5 \times 10^4$, $5 \times 10^3$, $5 \times 10^2$, 50, 5, and 0 copies of SARS-CoV-2 RNA per reaction. (B) Threshold time of ORF1ab RT-RPA correlates with the template concentration ($n = 3$). (C) RT-RPA amplification curves targeting the N gene in the presence of $5 \times 10^4$, $5 \times 10^3$, $5 \times 10^2$, 50, 5, and 0 copies of SARS-CoV-2 RNA per reaction. (D) Threshold time of N gene RT-RPA correlates with the template concentration ($n = 3$). In both cases, RT-RPA sensitivity is about 50 copies/reaction. For real time amplicon monitoring, we used EXO-RPA probe (Table S5). The RT-RPA threshold time may have been adversely affected by our use of shorter (18~22 nt) forward and backward primers than commonly used in RPA (28-35 nt).
Figure S5: Comparison among LAMP, RT-PCR, and Closed-Tube Penn-RAMP for SARS-CoV-2 Detection. (A) LAMP, (B) PCR, (C) closed-tube Penn-RAMP detection of 70000, 7000, 700, 70, 7, and 0 (no template control) copies per reaction. The limits of detection of LAMP, PCR, and closed-tube Penn-RAMP are, respectively, 70, 70, and 7 copies per reaction. The threshold time of LAMP (D), threshold cycle of PCR (E), and threshold time of single-tube Penn-RAMP (F) as functions of the log of synthesized DNA (Table S2) with a sequence equivalent to SARS-CoV-2 (n = 3). These experiments were carried out before SARS-CoV-2 samples became available in the USA. (This data was published in our preprint in ChemRxiv<sup>5</sup>).
Figure S6: Effect of heat treatment on the stability of coronavirus-IBV RNA: IBV-RT-PCR threshold cycle as a function of heat treatment conditions: in the absence of RNase inhibitors (gray bar), in the presence of commercial low working temperature (42°C) RNase inhibitor iNtRON (black bar), and in the presence of homemade RNase inactivating mixture (striped bar). \(10^{3.2}\) EID\(_{50}\) /ml IBV isolate.
Figure S7: The effect of medium type: VTM, saline, water, and saliva on PCR amplification. Similar quantities of SARS-CoV-2 virions were spiked in various media, incubated at 95°C for 5 min, RNA-purified, and subjected to RT-PCR. SARS-CoV-2 RNA degrades in VTM during incubation.
Figure S8: Detection of SARS-CoV-2 with NEB colorimetric LAMP buffer. (A) Visual detection of SARS-CoV-2 with RT-LAMP. (B) The threshold time of as a function of medium type in the presence of TCEP/EDTA and thermal incubation at 95°C for 5 min. (C) The threshold time as a function of medium type in the presence of TCEP/EDTA but without pre-heating. 4 μL of ~40 RNA genomes/μL were added to 25 μL NEB colorimetric LAMP reaction mix (NEB #M1800). (D) Occasionally, the NEB colorimetric LAMP buffer changed color due to sample’s acidity (false positive). All experiments were carried out with the N gene LAMP primer set. RT = room temperature.
| Coronavirus | GISAID or Genbank accession ID | Specimen source | Country | Patient age | Collection date |
|-------------|---------------------------------|-----------------|---------|-------------|-----------------|
| SARS-COV-2  | EPI_ISL_402119                  | Alveolar lavage fluid | China   | 49          | 2019-12-30      |
| SARS-COV-2  | EPI_ISL_402120                  | Alveolar lavage fluid | China   | 61          | 2020-01-01      |
| SARS-COV-2  | EPI_ISL_402121                  | Alveolar lavage fluid | China   | 32          | 2019-12-30      |
| SARS-COV-2  | EPI_ISL_402123                  | Bronchoalveolar lavage fluid | China   | 65          | 2019-12-24      |
| SARS-COV-2  | EPI_ISL_402124                  | Bronchoalveolar lavage fluid | China   | 49          | 2019-12-30      |
| SARS-COV-2  | EPI_ISL_402126                  | Throat swab       | Japan   | 30          | 2020-01-14      |
| SARS-COV-2  | EPI_ISL_402127                  | Bronchoalveolar lavage fluid | China   | 32          | 2019-12-30      |
| SARS-COV-2  | EPI_ISL_402128                  | Bronchoalveolar lavage fluid | China   | 52          | 2019-12-30      |
| SARS-COV-2  | EPI_ISL_402129                  | Bronchoalveolar lavage fluid | China   | 40          | 2019-12-30      |
| SARS-COV-2  | EPI_ISL_402130                  | Bronchoalveolar lavage fluid | China   | 56          | 2019-12-30      |
| SARS-COV-2  | EPI_ISL_403932                  | Endotracheal aspirates | China   | 66          | 2020-01-14      |
| SARS-COV-2  | EPI_ISL_403933                  | Endotracheal aspirates | China   | 65          | 2020-01-15      |
| SARS-COV-2  | EPI_ISL_403936                  | Throat swab       | China   | 68          | 2020-01-17      |
| SARS-COV-2  | EPI_ISL_403937                  | Nasal swab        | China   | 49          | 2020-01-18      |
| SARS-COV-2  | EPI_ISL_403962                  | Nasopharyngeal swab and Throat swab | Thailand | 61          | 2020-01-08      |
| SARS-COV-2  | EPI_ISL_403963                  | Nasopharyngeal swab and Throat swab | Thailand | 74          | 2020-01-13      |
| SARS-COV-2  | EPI_ISL_404253                  | Sputum            | USA     | 63          | 2020-01-21      |
| SARS-COV-2  | EPI_ISL_404895                  | Oropharyngeal swab | USA     | 30          | 2020-01-19      |
| TGEV        | Genebank NC_038861.1            | --               | --      | --          | --              |
| PDCoV       | Genebank KX022605.1             | --               | --      | --          | --              |
Table S2: Sequences and concentrations of SARS-CoV-2 LAMP primers.

| Amplicon     | Primer name | Sequence (5’ to 3’)                                      | Concentration (μM) |
|--------------|-------------|---------------------------------------------------------|--------------------|
| ORF1ab       | F3          | TGCTTCAGTCAGCTGATG                                      | 0.2                |
|              | B3          | TTAATTTGTCATCTTCCGTCCTT                                  | 0.2                |
|              | FIP         | TCAGTACTAGTGCTGTGCC-CACAATCGTTTTTTAAACGGGT              | 1.6                |
|              | BIP         | TCGTATACAGGGGTCTCTGACATCTA-TCTTGGAAAGCGACAACAA          | 1.6                |
|              | Loop F      | CTGCACATTACACCGCAA                                      | 0.8                |
|              | Loop B      | GTAGCTGTTTTTGCTAAATTCC                                  | 0.8                |
| N gene       | F3          | CGGCAGTCAAGCCTCTCC                                      | 0.2                |
|              | B3          | TTGCTCTCAAGCTGGTTCAA                                    | 0.2                |
|              | FIP         | TCCCCTACTGCTGCTGAG-CGTTCCCTATCAGTAGCG                   | 1.6                |
|              | BIP         | TTCTCCTGCTAGAATGGCTGCG-TCTGTCAAGCAGCAGCAAG              | 1.6                |
|              | Loop B      | AATGGCGGGTATGCTGCTCT                                     | 0.8                |

Table S3. Synthesized DNA targets

| Source        | Genebank Accession | -- | -- | -- | -- |
|---------------|--------------------|----|----|----|----|
| PEDV          | NC_003436.1        | -- | -- | -- | -- |
| IBV           | NC_001451.1        | -- | -- | -- | -- |
| HCoV-229E     | NC_002645.1        | -- | -- | -- | -- |
| OC43          | KX344031.1         | -- | -- | -- | -- |
| NL63          | JX504050.1         | -- | -- | -- | -- |
| HKU1          | KF686346.1         | -- | -- | -- | -- |
| SARS-CoV      | NC_004718.3        | -- | -- | -- | -- |
| MERS-CoV      | NC_019843.3        | -- | -- | -- | -- |

Synthesized SARS-CoV-2 DNA (ORF1ab Fragment, 619 nt)
### Synthesized SARS-CoV-2 DNA (N gene Fragment, 599 nt)

| DNA Sequence                                                                 |
|------------------------------------------------------------------------------|
| 5'- CTG CTA AAG CTT ACA AAG ATT ATC TAG CTA GTG GGG GAC AAC CAA TCA CTA       |
| ATT GTG TTA AGA TGT TGT GTA CAC ACA CTG GTA CTG GTC AGG CAA TAA CAG TTA       |
| CAC CGG AAG CCA ATA TGG ATC AAG AAT CCT TTG GTG GTG CAT CGT GTT GTC           |
| TGT ACT GCC GTT GCC ACA TAG ATC ATC CAA ATC CTA AAG GAT TTT GTG ACT TAA       |
| AAG GTA AGT TAC CTG GTA CAC CTG CTA CAA CTT GTA GTC AAT CTG TGG TGG GTT TTA   |
| CAC TTA AAA ACA CAG TCT GTG GCC TCT GCG GTA TGT GGA AAG GTT ATG GCT           |
| GTA GTT GTG ATC AAC TCC GCG AAC CCA TGC TTC AGT CAG CTG ATG AAC AAT CTG TTT   |
| AAG ATG AGC TGG TTT TGG TGC TAA ATT CCT AAA AAC TAA TTG TTG TGC CTT CCA AGA |
| AAA GGA CGA AGA TGA CAA TTT AAT TGA TTC TTA CTT TGT AGT TAA GAG ACA CAC     |
| TTT CTC TAA CTA CCA ACA TGA AGA AAC AAT TTA TAA TTT ACT T -3 '               |

### Synthesized SARS DNA (ORF1ab Fragment, 619 nt)

| DNA Sequence                                                                 |
|------------------------------------------------------------------------------|
| 5'- GGA CTT CCC TAT GGT GCT AAC AAA GAC GGC ATC ATA TGG GTT GCA ACT GAG       |
| GGA GCC TTG AAT ACA CCA AAA GAT CAC ATT GGC ACC CGC AAT CCT GCT AAG           |
| AAT GCT GCA ATC GTG CTA CAA CTT CCT CAA GGA ACA ACA TGG CCA AAA GCC          |
| TTC TAC GCA GAA GGC AGA AGA GGA GGC ATG CAA GCC TCT TCT CGT TCC TCA         |
| TCA CTG AGT CGC AAC AGT TCA AGA AAT TCA ACT CCA GCC AGC AGT AGG GGA ACT      |
| TCT CCT GCT AGA ATG GCT GCC AAT GGC GGT GAT GCT CCT TTG CTG CTG CTG ATT GTC |
| CTG CTG GTT CAC AGG ATG TTG AGT AAC CAG TTG GAG AAA ATG TCT GGT GAA GGC     |
| CAA CCA AAA CAA GGC CAA ACT GTC ACT AAG AAA TCT GCT GAG ACT TCT AAG AGG CTG |
| CAA CTA ATG AAA TCG CTA CCA ACA TGA AGA AAC AAT TTA TTA TTT ACT T -3 '        |

### Synthesized SARS DNA (N gene Fragment, 272 nt)

| DNA Sequence                                                                 |
|------------------------------------------------------------------------------|
| 5'- TGC CAA AAG GCC TAT CAT AGG AGG ATT ACC TAG CAA GTG GAG GAC AAC CAA TCA CCA |
| ACT GTG TGA AGA TGT TGT GTA CAC ACA CTG GTA CAG GAC AGG CAA TTA CTG          |
| TAA CAC CAG AAG CTA ACA TGG ACC AAG AGT CCT TTG GTG GTG CTT CAT GTT GTC      |
| TGT ATT GTA GAT GCC ACA TTG ACC ATC CAA ATC CTA AAG GAT TCT GTG ACT TGA     |
| AAG GTA ATG AGC TCC AAA TAC CTA CCA CTG TTT GTG CTA ATG ACC CAG TGG TTG     |
| TTA CAC TTA GAA ACA CAG TCT GTA CCG TCT GCG GAA GTG GAA AAG GTT AAT GCT      |
| GCT GTA GTT GTG ACC AAC TCC GCG AAC CCT TGA TGC AGT CGG ATG CAT AAA CTA CTT |
| TTG TAA ACG GGT TTG CGG TGT AAG TGC AGC CCG TCT TAC ACC GTG CGG CAC AGG CAC |
| TAG TCA TTG TGG CTA GAG GCC TCT TGG TTA TGA TAT TTA AAA AAG AGT TGC TGG CTT |
| CCA GGA GAA GGA TGA AGG AGG CAA TTT ATT AGA ATG TGA CTA TCA AGG CAA TTT     |
| ATT AGA CTC TTA CTT TGT AGT TAA GAG GCA TAC TAT GTC TAA CTA CCA ACA TGA     |
| AGA GAC TAT TTA TAA AAA CTT GGT GCT TGG TGG TGG TGG GTT TGG TGG TGG TGG TCC |
| GCC CCC AGC GCT TCA GCG TGC TGG TGC TGG TGC TGG TGG TGG TGG TGG TGG TGG TGG |

### Synthesized SARS DNA (N gene Fragment, 272 nt)

| DNA Sequence                                                                 |
|------------------------------------------------------------------------------|
| 5'- TGC CAA AAG GCC TAT CAT AGG AGG ATT ACC TAG CAA GTG GAG GAC AAC CAA TCA CCA |
| ACT GTG TGA AGA TGT TGT GTA CAC ACA CTG GTA CAG GAC AGG CAA TTA CTG          |
| TAA CAC CAG AAG CTA ACA TGG ACC AAG AGT CCT TTG GTG GTG CTT CAT GTT GTC      |
| TGT ATT GTA GAT GCC ACA TTG ACC ATC CAA ATC CTA AAG GAT TCT GTG ACT TGA     |
| AAG GTA ATG AGC TCC AAA TAC CTA CCA CTG TTT GTG CTA ATG ACC CAG TGG TTG     |
| TTA CAC TTA GAA ACA CAG TCT GTA CCG TCT GCG GAA GTG GAA AAG GTT AAT GCT      |
| GCT GTA GTT GTG ACC AAC TCC GCG AAC CCT TGA TGC AGT CGG ATG CAT AAA CTA CTT |
| TTG TAA ACG GGT TTG CGG TGT AAG TGC AGC CCG TCT TAC ACC GTG CGG CAC AGG CAC |
| TAG TCA TTG TGG CTA GAG GCC TCT TGG TTA TGA TAT TTA AAA AAG AGT TGC TGG CTT |
| CCA GGA GAA GGA TGA AGG AGG CAA TTT ATT AGA ATG TGA CTA TCA AGG CAA TTT     |
| ATT AGA CTC TTA CTT TGT AGT TAA GAG GCA TAC TAT GTC TAA CTA CCA ACA TGA     |
| AGA GAC TAT TTA TAA AAA CTT GGT GCT TGG TGG TGG TGG GTT TGG TGG TGG TGG TCC |
| GCC CCC AGC GCT TCA GCG TGC TGG TGC TGG TGC TGG TGG TGG TGG TGG TGG TGG TGG |
| **Table S4**: Sequences of EXO-RPA probes* |
|------------------------------------------|
| **ORF1ab EXO-RPA probe**                  |
| 5'-TGTCGTATACAGGGCTTTTGACATCTACAA[FAM-dT]G[THF][BHQ-dT]AAAGTAGCTGGTTTTG[3'-block] |
| **N gene EXO-RPA probe**                  |
| 5'-TAGAATGGCTGGCAATGGCGGTGATGCTGC[FAM-dT][THF][BHQ-dT]TGCTTTGCTGCTGCTT [3'-block] |

*ORF1ab EXO-RPA probe was designed in house, and N gene EXO-RPA probe sequence is from literature\(^6\).
Table S5: IBV infectivity after various heat treatments.

| Virus | Temperature | Time (Minutes) | Pre-treatment virus titer (EID<sub>50/ml</sub>) | Post-treatment virus titer (EID<sub>50/ml</sub>) |
|-------|-------------|----------------|-----------------------------------------------|-----------------------------------------------|
| IBV   | 56 °C       | 30             | 10<sup>3.2</sup>                              | 0                                             |
|       | 70 °C       | 5              | 10<sup>3.2</sup>                              | 0*                                            |
|       | 70 °C       | 10             | 10<sup>3.2</sup>                              | 0                                             |
|       | 70 °C       | 30             | 10<sup>3.2</sup>                              | 0                                             |
|       | 95 °C       | 5              | 10<sup>3.2</sup>                              | 0                                             |
|       | 95 °C       | 10             | 10<sup>3.2</sup>                              | 0                                             |
|       | 95 °C       | 30             | 10<sup>3.2</sup>                              | 0                                             |

* A few virus particles remained infectious.
Table S6. Effect of RNase inhibitor during heat treatment of COVID-19 patient samples.

| Patient No. | Sample type | CDC RT-PCR with purified RNA | CDC RT-PCR with purified RNA | CDC RT-PCR with purified RNA |
|-------------|-------------|------------------------------|------------------------------|------------------------------|
|             |             | Lysis buffer inactivation     | Heating inactivation (56 °C, 1 hour) in the absence of RNase inhibitor | Heating inactivation (56 °C, 1 hour) in the presence of RNase inhibitor |
| 228         | NP/OP/VTM   | ++                           | +                            | ++                           |
| 234         | NP/OP/VTM   | ++                           | -                            | -                            |
| 235         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 240         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 242         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 247         | NP/OP/VTM   | ++                           | -                            | ++                           |
| 248         | NP/OP/VTM   | ++                           | +                            | +                            |
| 251         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 258         | NP/OP/VTM   | +                            | +                            | +                            |
| 256         | NP/OP/VTM   | ++                           | +                            | +                            |
| 257         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 258         | NP/OP/VTM   | +                            | +                            | +                            |
| 256         | NP/OP/VTM   | ++                           | ++                           | ++                           |
| 257         | NP/OP/VTM   | ++                           | ++                           | ++                           |
| 260         | NP/OP/VTM   | +                            | +                            | +                            |
| 262         | NP/OP/VTM   | ++                           | ++                           | ++                           |
| 263         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 266         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 264         | OP/VTM      | +                            | +                            | +                            |
| 264         | OP/H₂O      | +                            | +                            | +                            |
| 266         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 266         | NP/OP/H₂O   | +++                          | +++                          | +++                          |
| 269         | NP/VTM      | +++                          | +++                          | +++                          |
| 269         | NP/OP/H₂O   | +++                          | +++                          | +++                          |
| 272         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 272         | NP/OP/H₂O   | +++                          | +++                          | +++                          |
| 272         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 272         | NP/OP/H₂O   | +++                          | +++                          | +++                          |
| 275         | NP/OP/VTM   | +                            | +                            | +                            |
| 275         | NP/OP/H₂O   | +                            | +                            | +                            |
| 279         | NP/OP/VTM   | +++                          | +++                          | +++                          |
| 279         | NP/OP/H₂O   | +++                          | +++                          | +++                          |
| 283*        | NP/OP/VTM   | -                            | -                            | -                            |
| 283*        | NP/OP/H₂O   | +                            | +                            | +                            |
| 284*        | NP/OP/VTM   | +++                          | +++                          | +++                          |
| Sample | Sample Type | RT-PCR Ct | Treatment |
|--------|-------------|-----------|-----------|
| 284*   | NP/OP/H₂O   | +++       | TCEP/EDTA |
| 288*   | NP/OP/VTM   | -         | Rnasin®   |
| 288*   | NP/OP/H₂O   | +         | TCEP/EDTA |
| 290    | NP/OP/VTM   | +++       | Rnasin®   |
| 290    | NP/OP/H₂O   | +++       | TCEP/EDTA |
| 291    | NP/OP/VTM   | +++       | Rnasin®   |

1. “+++++”, “++++”, “+++”, “+” indicates, respectively, positive when Ct <20, <24, <28, <32, <36. “-” indicates negative.
2. Shaded area compares test results of samples treated with Rnasin® with untreated samples.
3. Different swabs from the same patient (labeled with “*”) provide different RT-PCR Ct, which is likely caused by variations in swab sample collection.
| Patient No. | Sample type | CDC RT-PCR with purified RNA | RT-LAMP with “dirty” sample | RT-RAMP with “dirty” sample |
|------------|-------------|-----------------------------|-----------------------------|-----------------------------|
| 227        | NP/VTM      | -                           | -                           | -                           |
| 228        | NP/OP/VTM   | +                           | Positive                    | -                           |
| 234        | NP/OP/VTM   | -                           | -                           | -                           |
| 235        | NP/OP/VTM   | +++                         | Positive                    | Positive                    |
| 232        | NP/VTM      | -                           | -                           | -                           |
| 232        | OP/VTM      | -                           | -                           | -                           |
| 233        | NP/VTM      | -                           | -                           | -                           |
| 233        | OP/VTM      | -                           | -                           | -                           |
| 240        | NP/OP/VTM   | +++                         | Positive                    | Positive                    |
| 242        | NP/OP/VTM   | +++++                       | Positive                    | Positive                    |
| 244        | NP/OP/VTM   | -                           | -                           | -                           |
| 245        | NP/OP/VTM   | -                           | -                           | -                           |
| 247        | NP/OP/VTM   | ++                          | Positive                    | -                           |
| 246        | NP/OP/VTM   | -                           | -                           | -                           |
| 248        | NP/OP/VTM   | +                           | Positive                    | -                           |
| 251        | NP/OP/VTM   | +++++                       | Positive                    | Positive                    |
| 252        | NP/OP/VTM   | -                           | -                           | -                           |
| 233        | NP/OP/VTM   | -                           | -                           | -                           |
| 237        | NP/OP/VTM   | -                           | -                           | -                           |
| 248        | NP/OP/VTM   | +                           | Positive                    | -                           |
| 251        | NP/OP/VTM   | +++                         | Positive                    | Positive                    |
| 252        | NP/OP/VTM   | -                           | -                           | -                           |
| 255        | NP/OP/VTM   | -                           | -                           | -                           |
| 252        | NP/OP/VTM   | -                           | -                           | -                           |
| 253        | NP/OP/VTM   | +                           | Positive                    | -                           |
| 255        | NP/OP/VTM   | -                           | -                           | -                           |
| 256        | NP/OP/VTM   | +++                         | Positive                    | Positive                    |
| 257        | NP/OP/VTM   | +++                         | Positive                    | Positive                    |
| 258        | NP/OP/VTM   | +                           | Positive                    | -                           |
| 254        | NP/OP/VTM   | -                           | -                           | -                           |
| 256        | NP/OP/VTM   | ++                          | Positive                    | -                           |
| 254        | NP/OP/VTM   | -                           | -                           | -                           |
| 257        | NP/OP/VTM   | ++                          | Positive                    | -                           |
| 260        | NP/OP/VTM   | +                           | Positive                    | -                           |
| 259        | NP/OP/VTM   | -                           | -                           | -                           |
| 262        | NP/OP/VTM   | ++                          | Positive                    | -                           |
| 263        | NP/OP/VTM   | +++                         | Positive                    | Positive                    |
| 263        | NP/OP/VTM   | -                           | -                           | -                           |
| 266        | NP/OP/VTM   | +++                         | Positive                    | Positive                    |

“++++”, “+++++”, “+++”, “++”, “+” indicates, respectively, positive when Ct <20, <24, <28, <32, <36. “−” indicates negative.
Table S8: Comparison of VTM with H₂O as a swab collection medium for direct LAMP detection of "dirty" patient swab sample.

| Patient No. | Sample type | CDC RT-PCR with purified RNA, Ct | LAMP with “dirty” sample, Tt |
|-------------|-------------|---------------------------------|-----------------------------|
|             |             | Lysis buffer inactivated         | Heat inactivated (56 °C, 1 hour) Adding RNase inhibitor |
| 279         | NP/OP/VTM   | +++                             | ++                           |
| 279         | NP/OP/H₂O   | +++                             | +++                          |
| 284         | NP/OP/VTM   | +++                             | +++                          |
| 284         | NP/OP/H₂O   | +++                             | +++                          |
| 288         | NP/OP/VTM   | +                               | -                            |
| 288         | NP/OP/H₂O   | ++                              | +                            |
| 290         | NP/OP/VTM   | +++                             | +++                          |
| 290         | NP/OP/H₂O   | +++                             | +++                          |
| 291         | NP/OP/VTM   | +++                             | +++                          |
| 291         | NP/OP/H₂O   | +++                             | +++                          |

1. "++++", "+++", "++", "+" indicates, respectively, positive when Ct<24, <28, <32, <36 for RT-PCR, and Tt<10, <12, <14, <18 min for RT-LAMP. Ct = threshold cycle; Tt = threshold time.

2. The threshold cycle Ct is defined as the cycle number until the fluorescent signal increases above the baseline level to ~10% of the saturation level.

3. The threshold time Tt is defined as the reaction time until the fluorescent signal increases above the baseline level to ~10% of the saturation level.
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