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## Supplementary information

# Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA

In the format provided by the authors and unedited

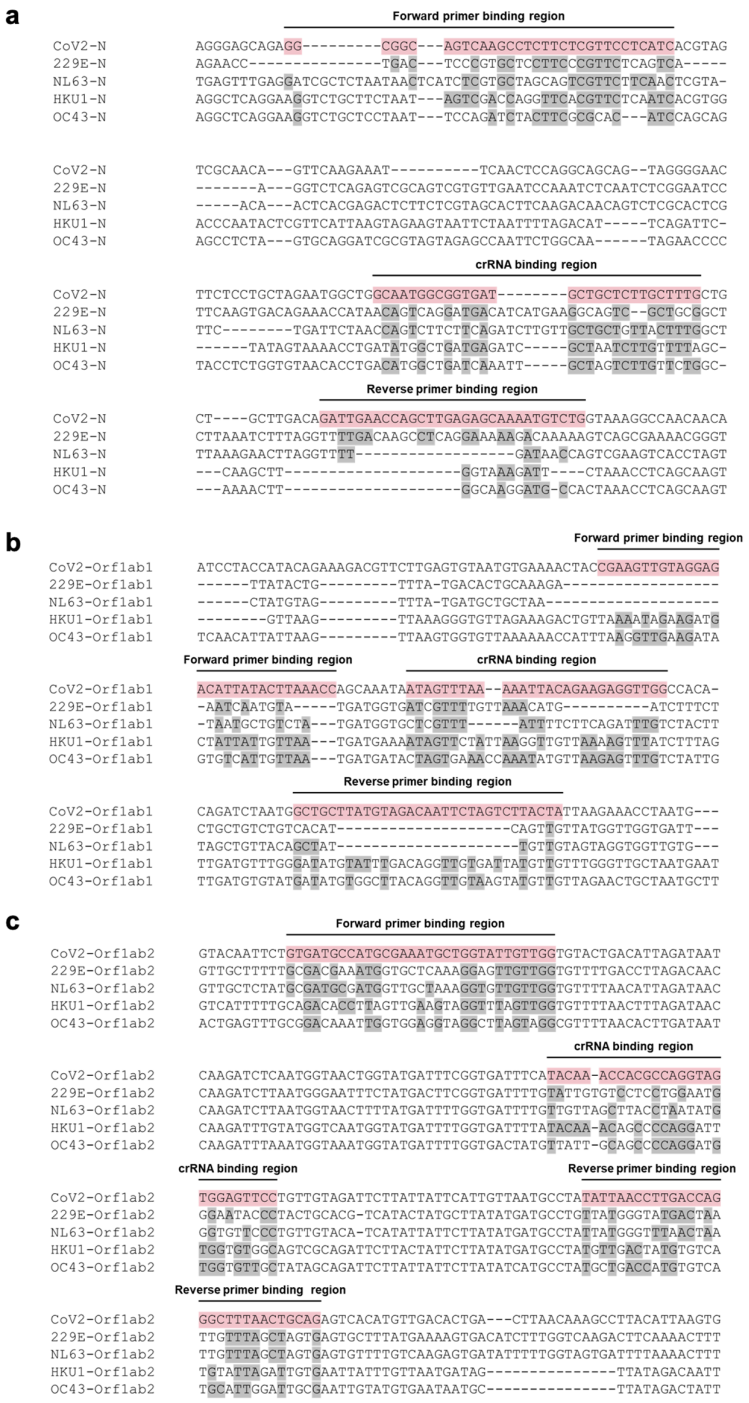


**Figure S1. Predicted specificity of SHERLOCK-SARS-CoV-2 detection via other chosen gene regions.** Alignments of N (**a**), and Orf1ab (**b,c**) gene regions of SARS-CoV-2 with homologous gene regions in hCoV-OC43, hCoV-HKU1, hCoV-NL63 and hCoV-229E are shown. Matches nucleotides of RPA primer and crRNA binding regions of SARS-CoV-2 S gene (coloured in pink) to those of other viruses are highlighted in grey.

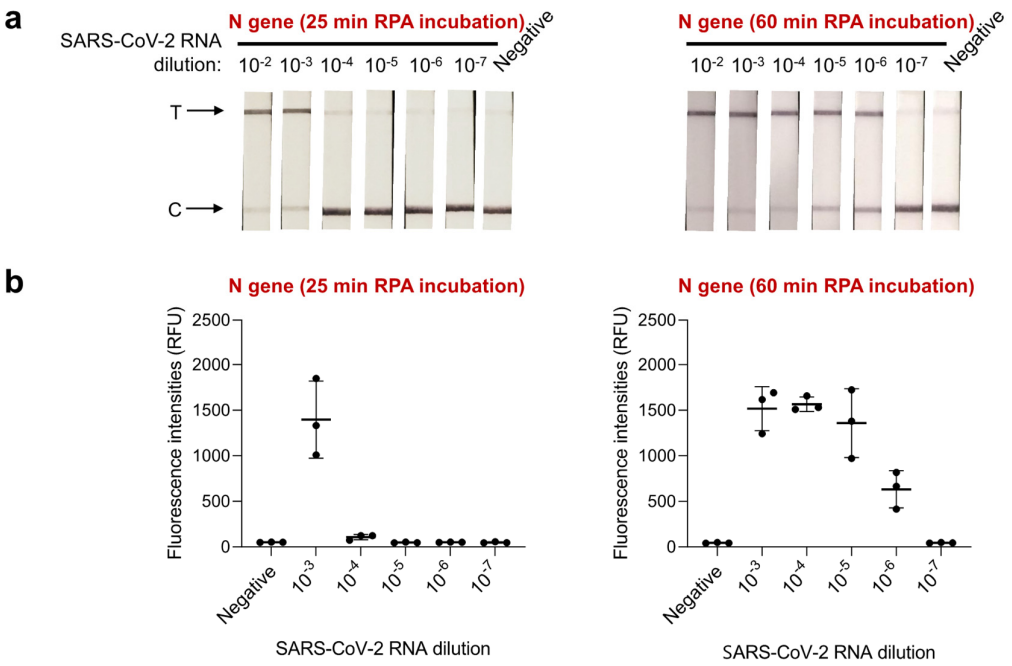


**Figure S2. Extending RPA duration improves the limit of SHERLOCK detection for SARS-CoV-2 N gene.** RPA was performed for 25 min (our conventional condition) vs 60 min. **a,** with lateral flow readout. Representative flow strips for each serial dilution of SARS-CoV-2 RNA are shown. **b**, with fluorescence readout. Fluorescence signals generated after 20 min of CRISPR-Cas13a reaction at each dilution were quantified. Error bars, ± s.d. from three technical replicates.

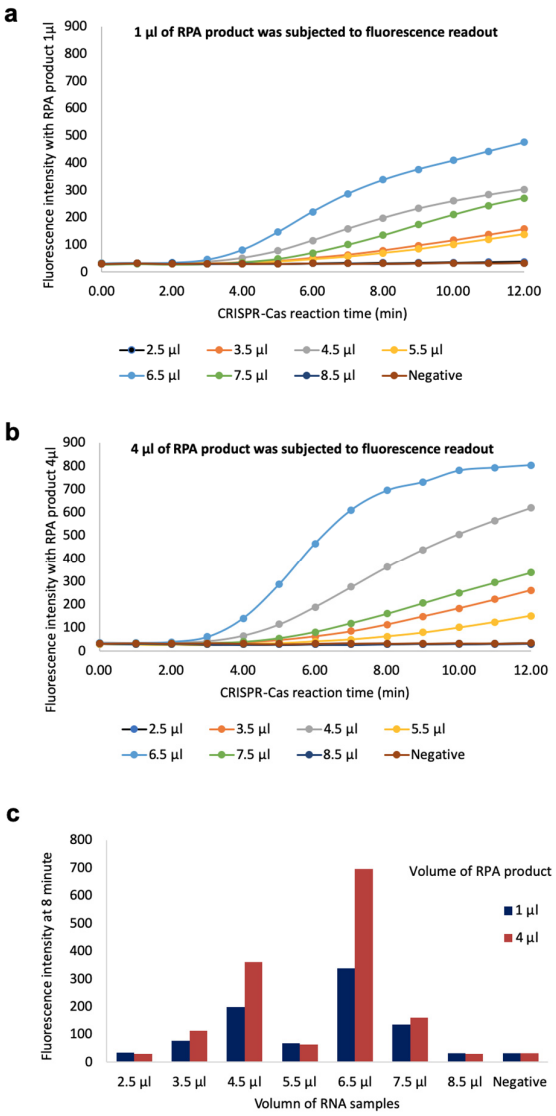


**Figure S3. Optimisations for 1) RNA input amount and 2) RPA reaction amount to be transferred to CRISPR-Cas13a detection step. a, b**, kinetics of fluorescence signal generation is shown for each RNA input amount (shown in µL; stock RNA contains ~30 copies per µL). 1 µL (**a**) and 4 µL (**b**) of RPA reactions was transferred to CRISPR-Cas13a detection step. **c**, Quantification of fluorescence intensity after 8 min of CRISPR-Cas reaction for different conditions.

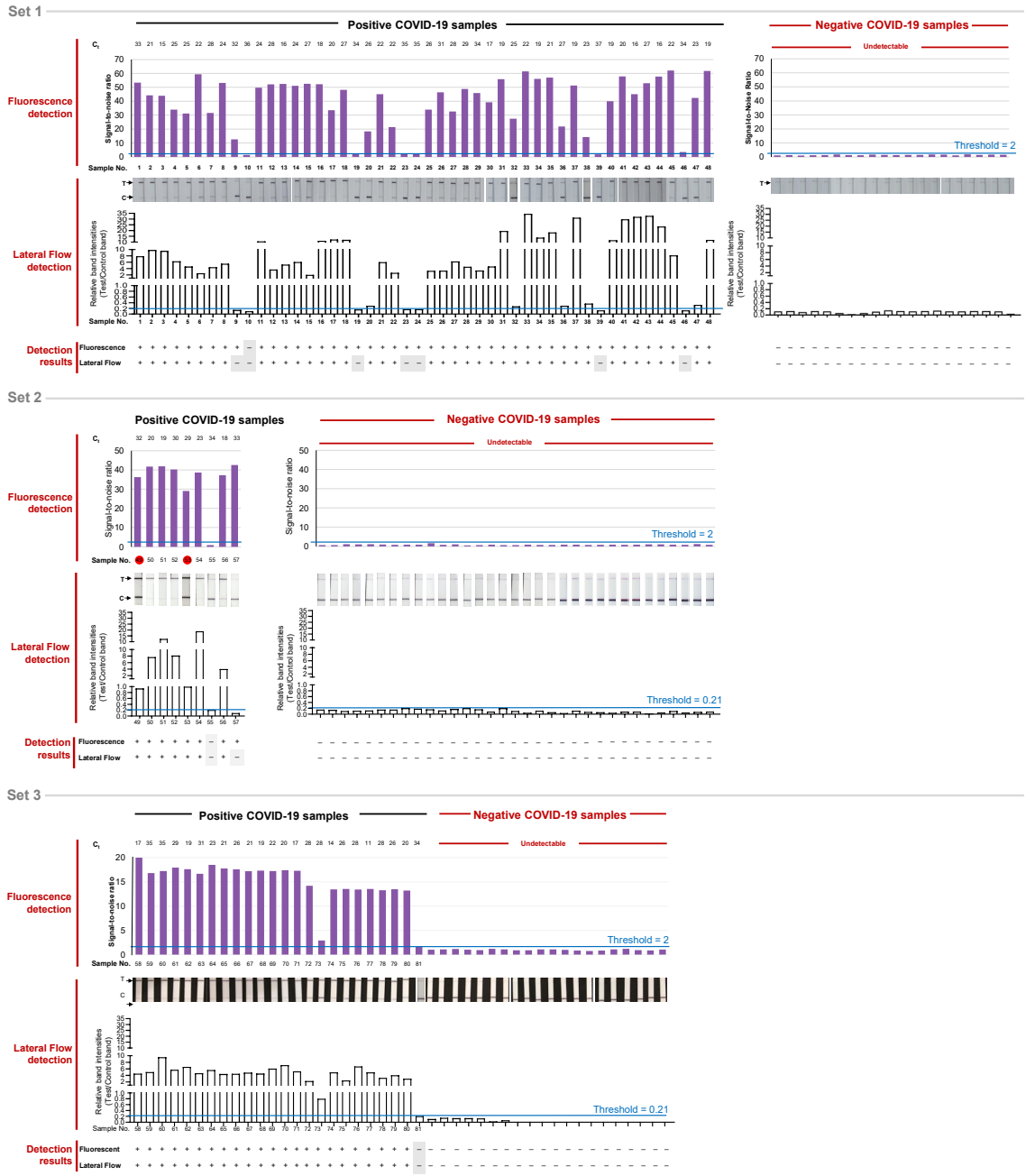


**Figure S4. Fluorescence and lateral flow results from SHERLOCK detection of SARS-CoV-2 in 154 nasopharyngeal and sputum clinical samples.** Lateral flow and fluorescence detection for clinical samples (n=154) of which 81 were positive for COVID-19 and 73 were negative for COVID-19. For the lateral flow readout, an appearance of a coloured test band, often in concurrence with disappearance of a coloured control band, indicated a positive COVID-19 result of clinical samples. For fluorescence readout, we set the threshold (blue line) of signal-to-noise of fluorescence intensities (with noise being fluorescence intensity from water-as-input negative sample performed in parallel) for a positive result to be 2. Red circle indicates the samples that were re-analysed with newly extracted RNA from the same clinical samples.

| For 5 reaction | RPA pellet | Primer mix (10 μM) | Reverse transcriptase (200U/μL) |
|---|---|---|---|
| 1X | 1 tubes | 5 μl | 1 μl |
| 2X | 2 tubes | 10 μl | 2 μl |

| Experiment | RPA pellet | Primer | Reverse transcriptase | Final concentration of MgOAc (mM) |
|---|---|---|---|---|
| 1 | 1X | 1X | 1X | 14 |
| 2 | 1X | 2X | 1X | 14 |
| 3 | 1X | 1X | 2X | 14 |
| 4 | 1X | 1X | 1X | 20 |
| 5 | 2X | 2X | 2X | 14 |

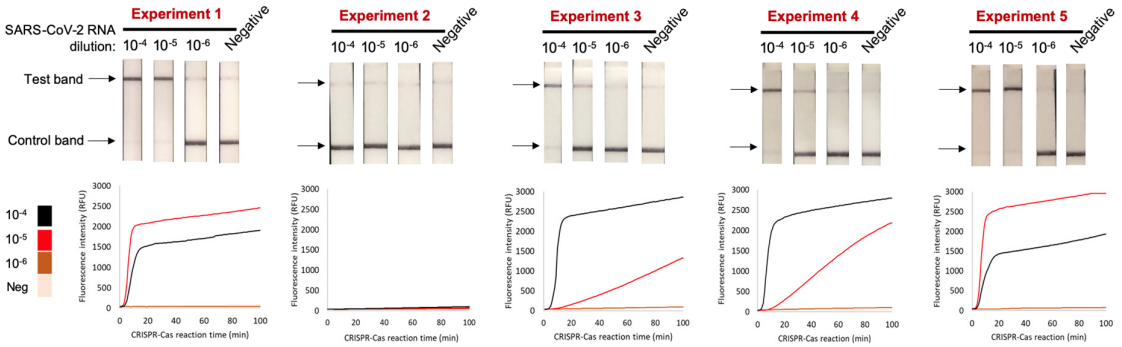


**Figure S5. Systematic optimisations of the RT-RPA reaction for higher RNA input volume.** To combat stochastic nature of the RPA amplification at low viral titre, we increased the RNA input volume to the RPA reaction, but had to compensate for the higher total reaction volume (and decreased RPA efficiency) by supplying some components in extra. We found it is best to supply all reaction components at twice the amount (experiment 5) to ensure high detection signal at lower viral RNA concentrations.

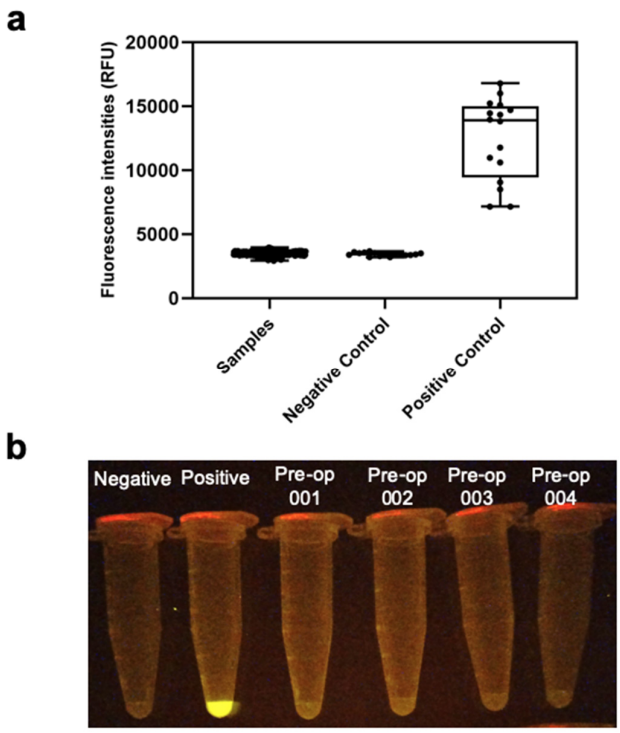


**Figure S6. SHERLOCK detection of SARS-CoV-2 as a part of the pre-operative assessment for surgical patients**. **a**, SHERLOCK with fluorescence detection of SARS-CoV-2 S gene on 380 clinical samples collected from surgical patients at Siriraj Hospital. Quantification of end-point fluorescence by CFX96 RT-PCR system after 30-min of the CRISPR-Cas reaction was shown for each clinical sample. **b**, representative image of fluorescence detection of SARS-CoV-2 by SHERLOCK via a phone camera and a blue-LED transilluminator. 4 SHERLOCK detection of pre-operative (pre-op) samples are shown here. In both **a** and **b**, *in vitro*-transcribed S gene RNA was used as positive controls in the RPA step, while DEPC-treated water was added as input for negative controls.

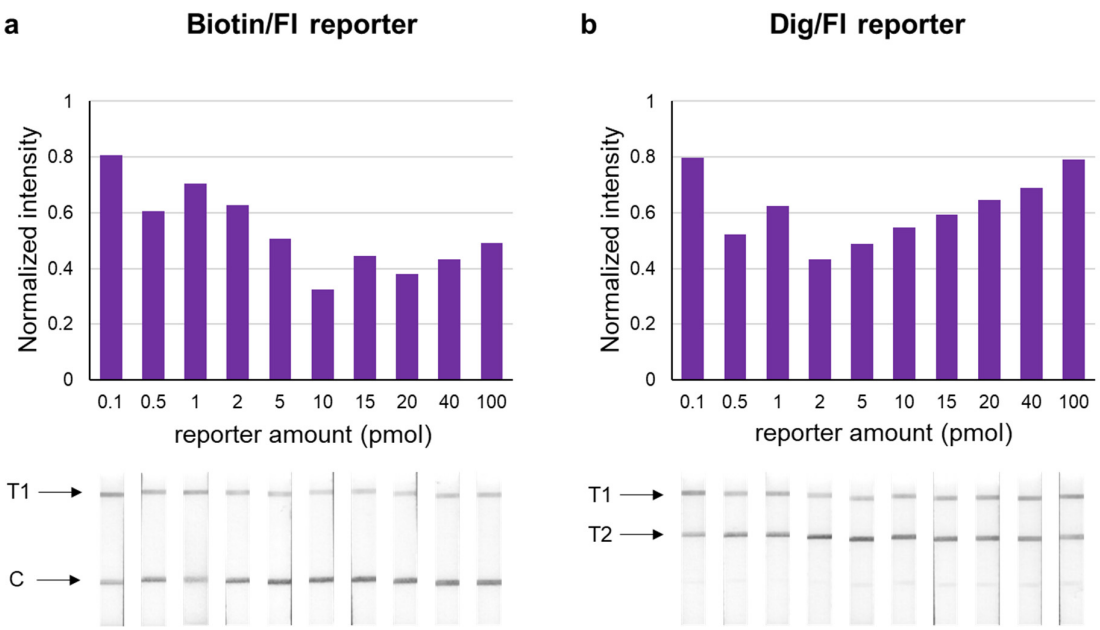


**Figure S7. Optimisations of the amount of Biotin-FI SHERLOCK reporter (a) and DIG-FI RNase reporter (b).** The varying amounts of each reporter in 20 µL DEPC-treated water were tested using 80 µL of assay buffer and HybriDetect 2T lateral flow strips (Milenia Biotech) to provide readout. The intensities of T1 line were normalised by the T1 line of DEPC-treated water sample without any reporters. 20 pmol and 2 pmol were chosen as optimal amount for biotin/FI and DIG/FI reporters respectively (for generating minimal amount of background escapee at T1 band).

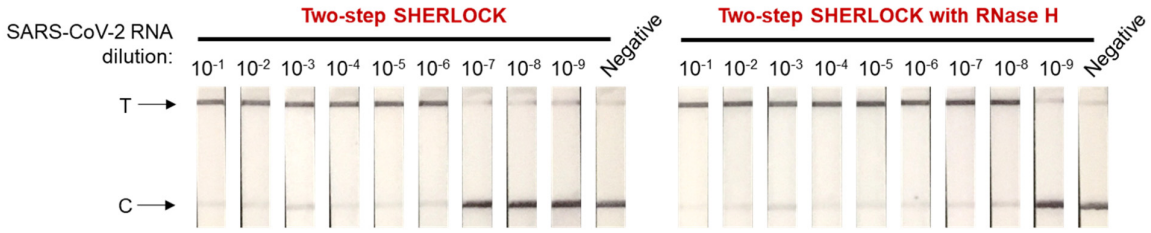


**Figure S8. Addition of RNase H improves SHERLOCK detection limit of SARS-CoV-2 RNA.** Left, the limit of detection of SARS-CoV-2 S gene using the two-step SHERLOCK protocol we had used for our clinical validation study. Right, the improved limit of detection upon addition of RNase H (Ambion, 0.1 U/μL) to the RPA reaction; other reaction components and conditions were identical to the clinical validation study protocol.

| SARS-CoV-2 RNA dilution | $C_t$ from RT-qPCR (N gene) |
|---|---|
| $10^{-2}$ | 19.6 |
| $10^{-3}$ | 23.5 |
| $10^{-4}$ | 26.8 |
| $10^{-5}$ | 30.1 |
| $10^{-6}$ | 33.5 |
| $10^{-7}$ | Undetectable |
| Negative (water) | Undetectable |

**Table S1. Serial dilutions of RNA extracted from cultured SARS-CoV-2 in Vero cells and their corresponding $C_t$ values from RT-qPCR (N gene)**

| Characteristic | Total (N = 152) | SARS-CoV-2 Positive (N = 79) |
|---|---|---|
| **Sex–no. (%)** | | |
| Male | 74 (49) | 46 (58) |
| Female | 78 (51) | 33 (42) |
| **Age—year** | | |
| Average | 39 | 39 |
| Median (interquartile range) | 37 (26-50) | 36 (26-50) |
| Range | 2-87 | 13-82 |
| **Estimated duration between symptom onset and diagnosis—days** | | |
| Average | N/A | 5 |
| Median (interquartile range) | N/A | 4 (1.5-6) |
| Range | N/A | 1-28 |
| **Symptoms—no. (%)** | | |
| Upper respiratory infections (URI) | N/A | 45 (57) |
| Fever | N/A | 14 (17.7) |
| Pneumonia | N/A | 10 (12.7) |
| Bronchitis | N/A | 1 (1.3) |
| Diarrhoea | N/A | 1 (1.3) |
| Asymptomatic | N/A | 8 (10) |

**Table S2. Characteristics of patients for SHERLOCK method validation**.
We performed SHERLOCK detection of SARS-CoV-2 on excess RNA extracts from nasopharyngeal and throat swab samples from 152 patients with suspected SAR-CoV-2 infection, who were confirmed to be positive (79 in total) or negative (73 in total) for COVID-19 by RT-PCR. Sample collection and RNA extraction were performed at Siriraj Hospital and the Department of Microbiology, Siriraj Hospital, respectively, between March 3 and April 10, 2020. RNA extracts were used without any personally identifiable information collected. For two patients, follow-up studies after drug treatment were performed and two additional RNA samples were collected during treatment for each patient and confirmed positive for COVID-19 by RT-PCR, bringing the total number of clinical samples we analysed with SHERLOCK to 154 samples.

| Name | Sequence | Sources | Note | REF |
|---|---|---|---|---|
| **RT-RPA primer** | | | | |
| SL_S-RPA-Forward_v1 | gaaattaatacgactcactatagggAGGTTTCAAACTTTACTTGCTTTACATAGA | IDT | Lower-case letters indicate indicate overhang T7 promotor sequence | 17 |
| SL_S-RPA-Reverse_v1 | TCCTAGGTTGAAGATAACCCACATAATAAG | IDT | - | 17 |
| SL_Orf1ab-RPA-Forward_v1 | gaaattaatacgactcactatagggCGAAGTTGTAGGAGACATTATACTTAAACC | IDT | Lower-case letters indicate overhang T7 promotor sequence | 17 |
| SL_Orf1ab-RPA-Reverse_v1 | TAGTAAGACTAGAATTGTCTACATAAGCAGC | IDT | - | 17 |
| Sl_Orf1b-RPA-Forward_v1 | gaaattaatacgactcactatagggTGATGCCATGCGAAATGCTGGTATTGTTGG | IDT | Lower-case letters indicate overhang T7 promotor sequence | This study |
| Sl_Orf1b-RPA-Reverse_v1 | CTGCAGTTAAAGCCCTGGTCAAGGTTAATA | IDT | - | This study |
| Sl_N-RPA-Forward_v1 | gaaattaatacgactcactatagggCGGCAGTCAAGCCTCTTCTCGTTCCTCATC | IDT | Lower-case letters indicate overhang T7 promotor sequence | This study |
| Sl_N-RPA-Reverse_v1 | CAGACATTTTGCTCTCAAGCTGGTTCAATC | IDT | - | This study |
| **crRNA** | | | | |
| 13a_SL_S-crRNA_v1 | gauuuagacuaccccaaaaacgaaggggacuaaaacGCAGCACCAGCUGUCCAACCUGAAGAAG | Synthego | Lower-case letters indicate scaffold sequence | 17 |
| 13a_SL_Orf1ab-crRNA_v1 | gauuuagacuaccccaaaaacgaaggggacuaaaacCCAACCUCUUUCUGUAAUUUUUAAACUAU | Synthego | Lower-case letters indicate scaffold sequence | 17 |
| 13a_Sl_Orf1b-crRNA_v1 | gauuuagacuaccccaaaaacgaaggggacuaaaacGGAACUCCACUACCUGGCGUGGUUUGUA | Synthego | Lower-case letters indicate scaffold sequence | This study |
| 13a_Sl_N-crRNA_v1 | gauuuagacuaccccaaaaacgaaggggacuaaaacAAAGCAAGAGCAGCAUCACCGCCAUUGC | Synthego | Lower-case letters indicate scaffold sequence | This study |
| **Reporter** | | | | |
| Cas13a_reporter | /FAM/mArArUrGrGrCmAmArArUrGrGrCmA/Bio/ | IDT | Lateral Flow Reporter | 17 |
| RNaseAlert Substrate | - | IDT | Flurorescent Reporter | - |
| PolyU reporter | /FAM/rUrUrUrUrU/Iowa Black Quencher/ | IDT | Flurorescent Reporter | 17 |
| RNase reporter | /FAM/mAUGAGCmA/DIG/ | Genscript | Lateral Flow Reporter | This study |
| **IVT templates for gene fragments production** | | | | |
| T7-3G | GAAATTAATACGACTCACTATAGGG | IDT | - | This study |
| SL_S-gene_T7_anti_pctrl | ACAGTCTACAGCATCTGTAATGGTTCCATTTTCATTATATTTTAATAGAAAAGTCCTAGGTTGAAGATAACCCACATAATAAGCTGCAGCACCAGCTGTCCAACCTGAAGAAGAATCACCAGGAGTCAAATAACTTCTATGTAAAGCAAGTAAAGTTTGAAACCTAGTGATGTTAccctatagtgagtcgtattaatttc | IDT | - | This study |

## Table S3. RPA primers, crRNAs, and RNA reporters