# SUPPLEMENTARY MATERIALS

# Clinical Validation of Engineered CRISPR/Cas12a For Rapid SARS-CoV-2 Detection

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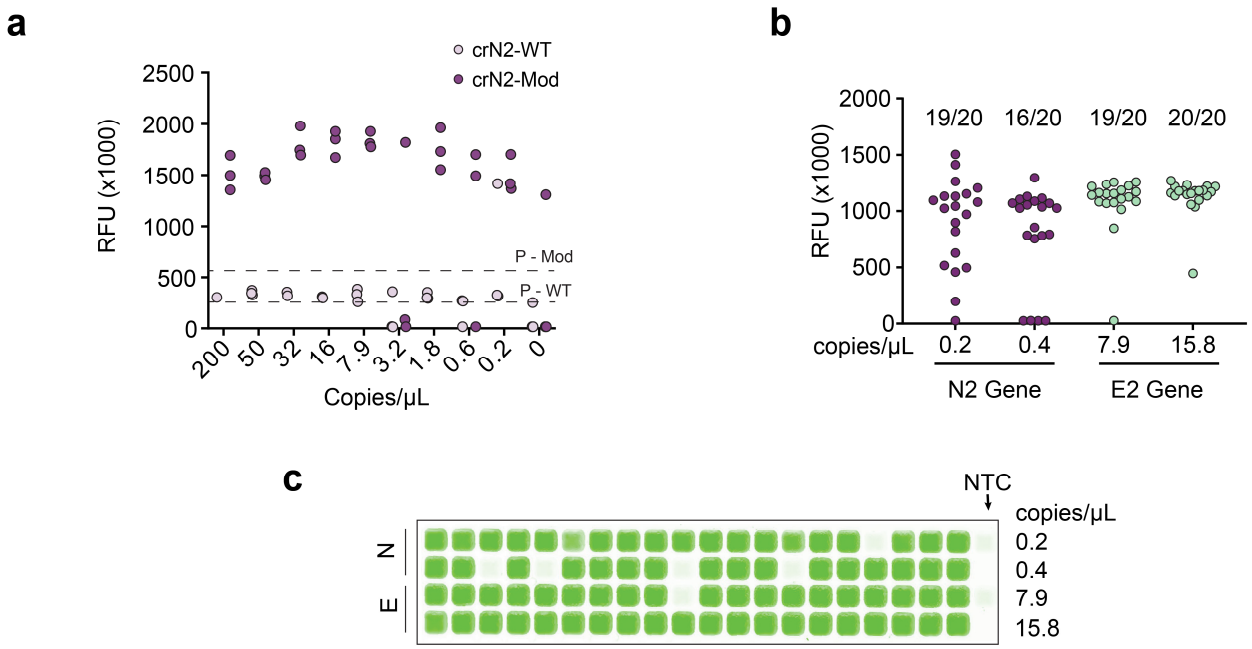
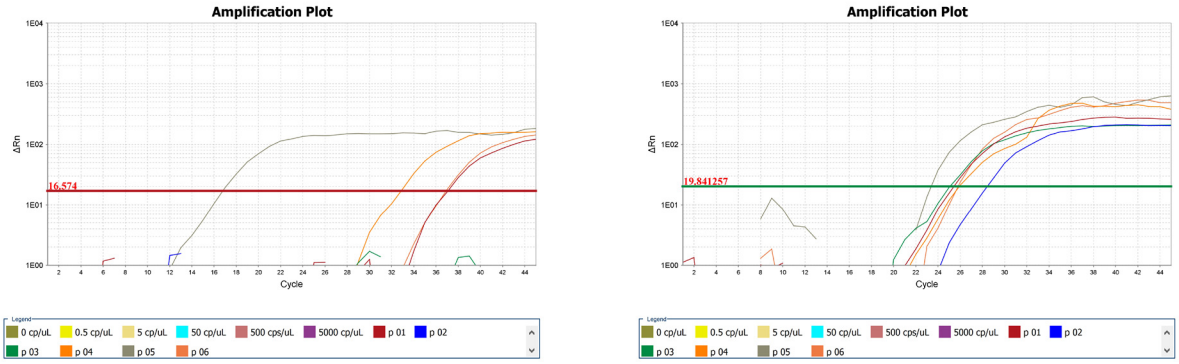


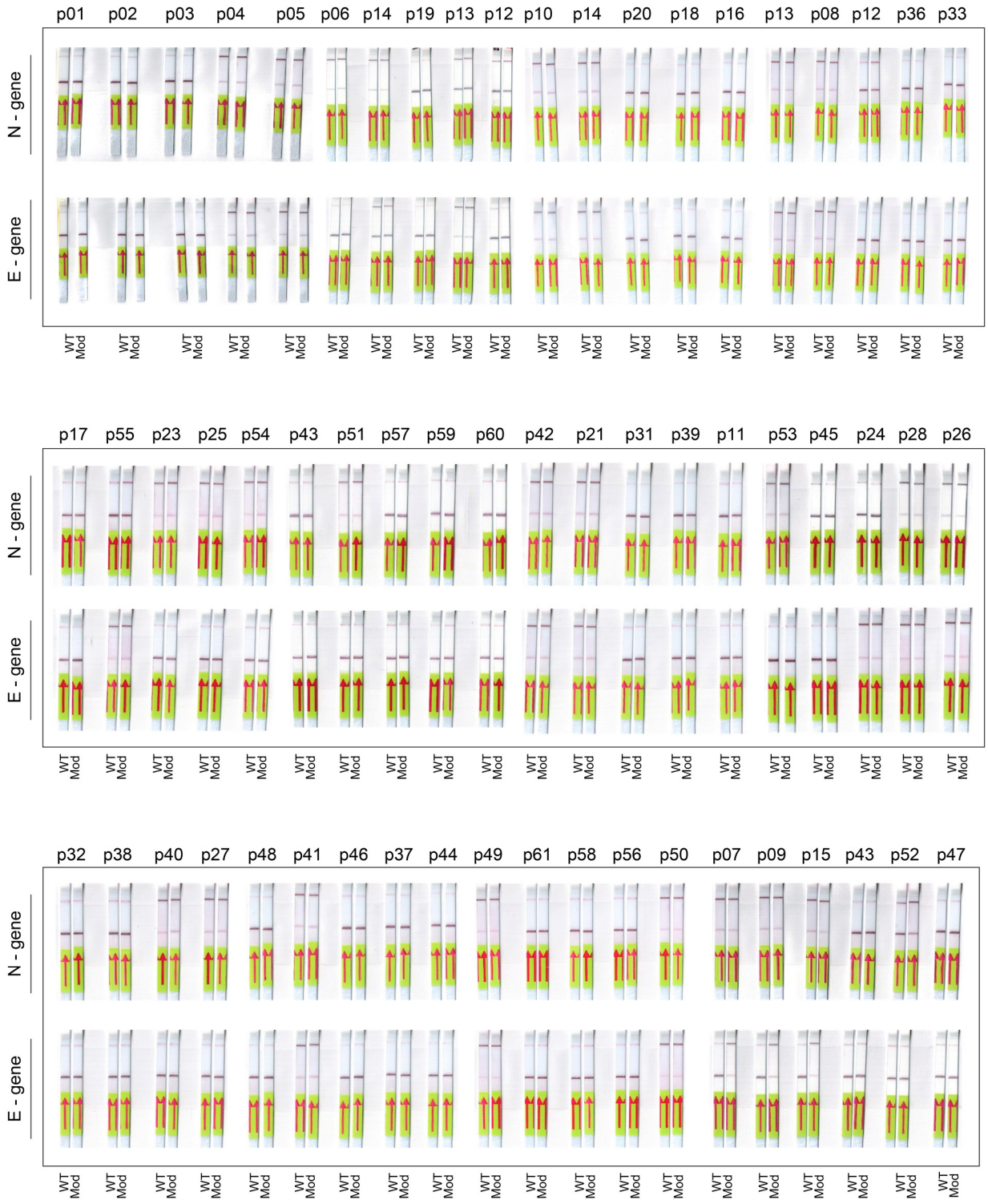

**Supplementary Figure S1. LoD determination of N2 gene when UDG is not incorporated in the pre-amplification step.** (**a**) Estimated LoD experiments were performed as described in figure 2 in the main text. An estimated LoD was determined based on the lowest copies/µL with 2/3 replicates detected positive. (**b**) 20 replicates with the copies/µL at 1X and 2X times the estimated LoD in (a) were repeated to confirm the final LoD. (**c**) Fluorescence image of samples in (b).



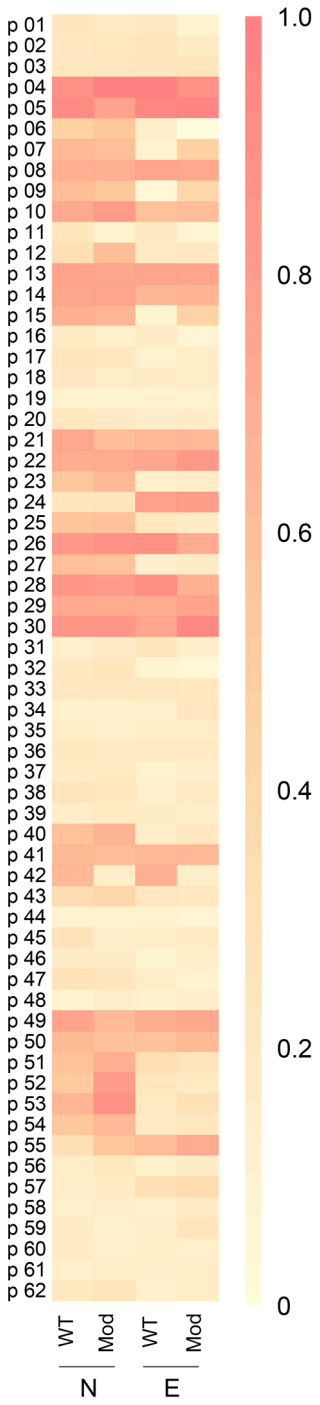

N1 Gene

RNase P Gene

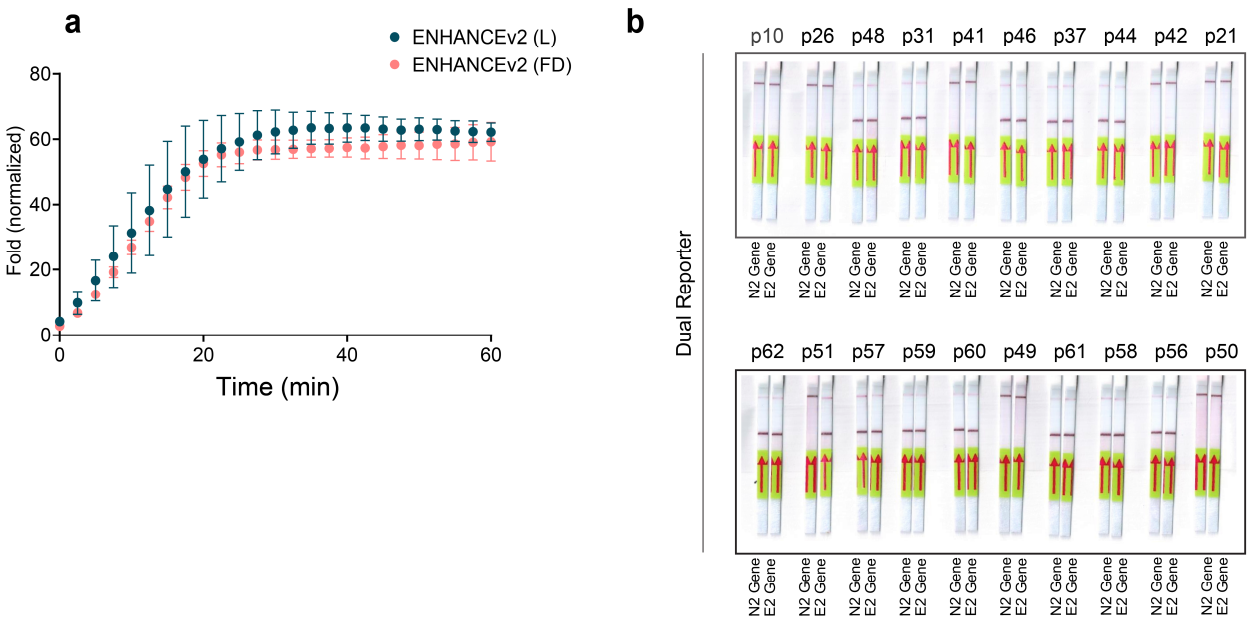
**Supplementary Figure S2. Representation of clinical validation of SARS-CoV-2 detection in 62 patient samples using RT-qPCR.** Obtained patient samples were re-tested with RT-qPCR following CDC-recommended protocol to determine the Ct values.



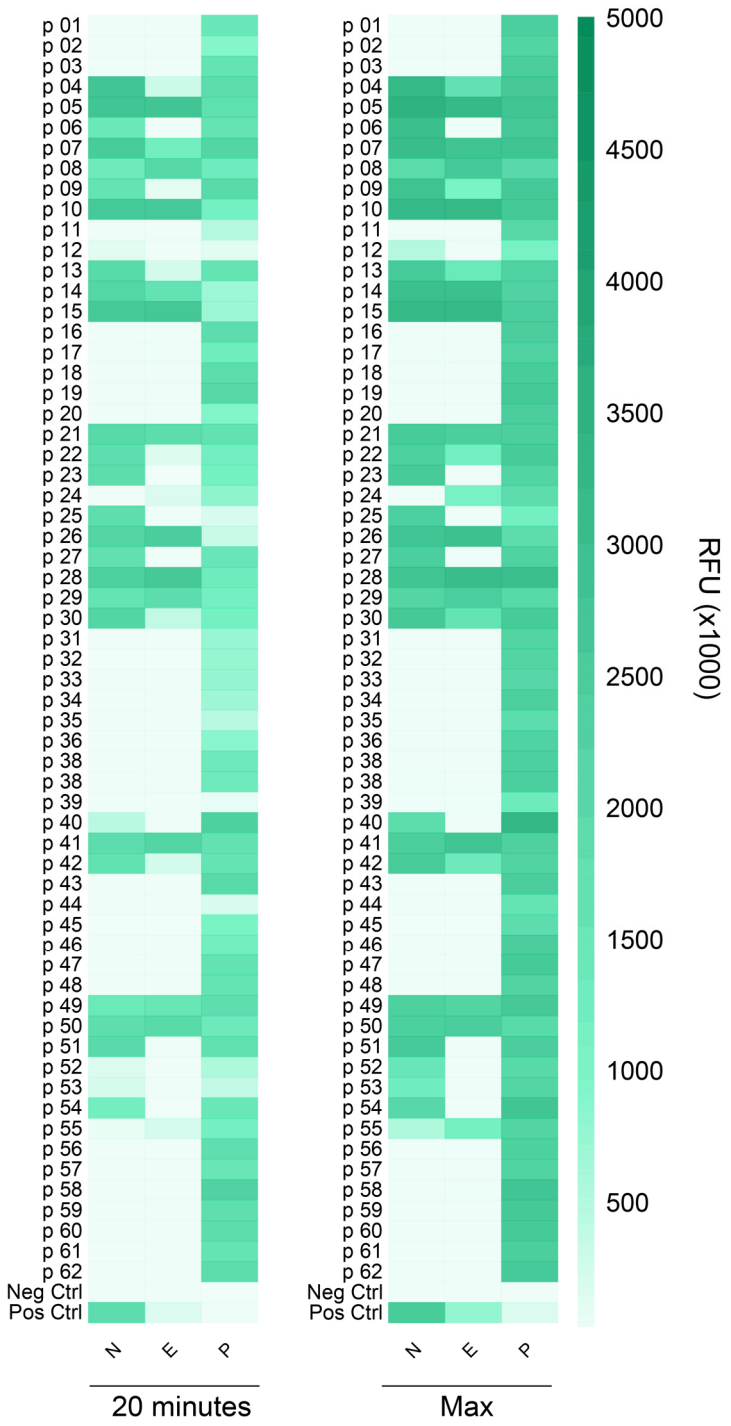

**Supplementary Figure S3. Clinical validation of ENHANCE for the detection of SARS-CoV-2 in 62 patient samples using lateral flow assay.** The patient samples were not in numerical order due to blind testing. The asterisk (*) denotes that patient sample 12 was repeated.



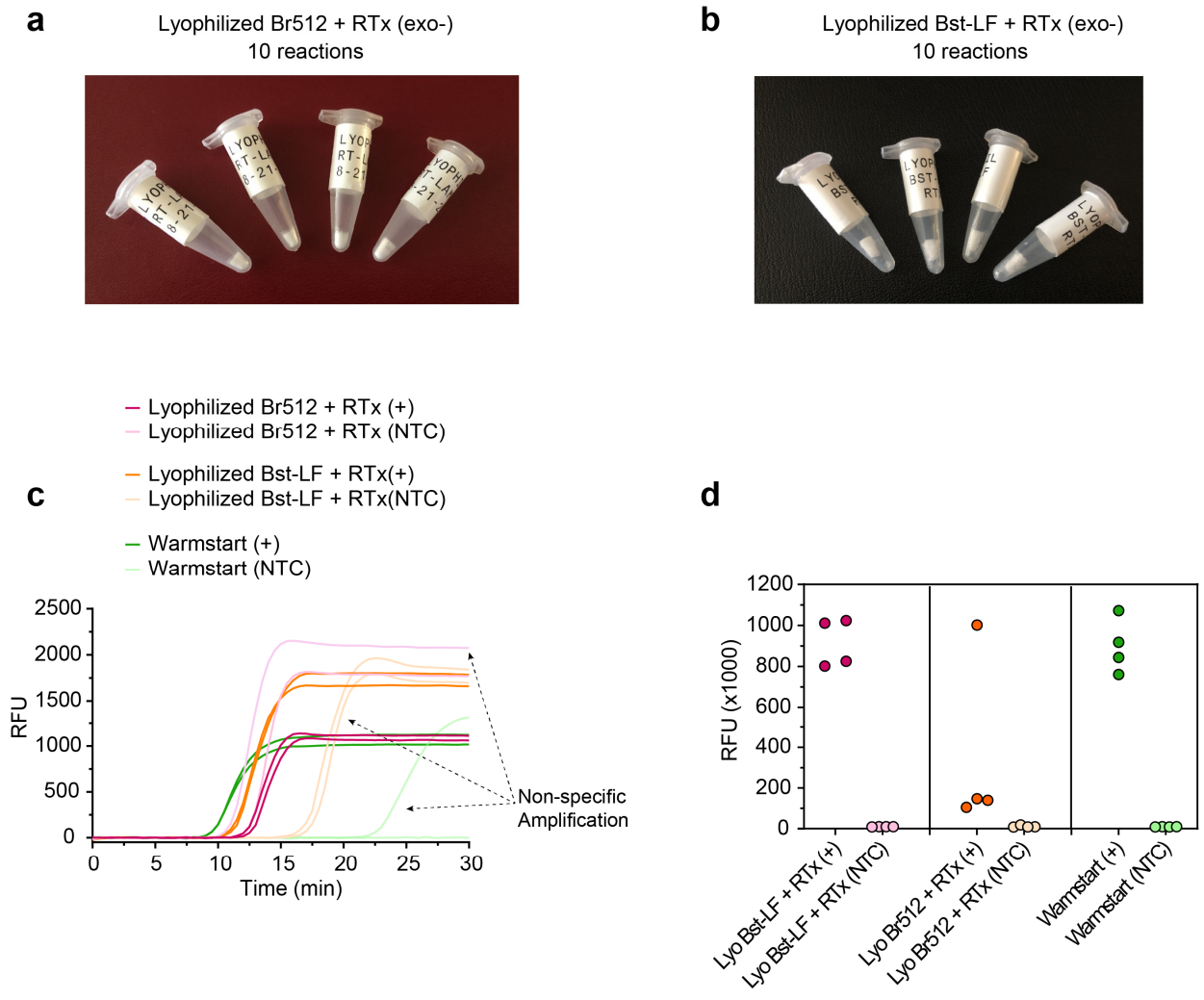

**Supplementary Figure S4. Quantitative analysis of lateral flow assay using ENHANCE on 62 patient samples.** The heat map shows ImageJ quantification of band intensity ratio of positive line (top band) to highest signal obtained in all 31 positive patient samples.




**Supplementary Figure S5. Comparison between liquid version and lyophilized version of ENHANCEv2. (a)** Fold change in fluorescence intensity normalized to corresponding NTC reactions targeting N2gene between liquid ENHANCEv2 (L) and freeze-dried ENHANCEv2 (FD) in one hour. Error bars represent standard deviation. (**b**) Representation of lateral flow paper strips showing compatibility of the dual reporter. Sample reactions from the fluorescence-based reporter assay using dual reporter version 2 in ENHANCEv2 was diluted down to a final concentration of 125 nM reporter followed by the lateral flow assay.




**Supplementary Figure S6. Clinical validation of ENHANCEv2 for the detection of SARS-CoV-2 in 62 patient samples using fluorescence-based reporter assay.** The heat map shows fluorescence intensities taken at t = 20 minutes (left) and maximum fluorescence intensities within an hour. The heat map is supplemental to fig. 4e in the main text whose fluorescence intensities were taken at t = 2.5 minutes.




**Supplementary Figure S7. Lyophilization and functional testing of RT-LAMP reactions. (a)** Representation of lyophilized RT-LAMP reactions using Br512 polymerase and reverse transcriptase RTx(exo). (**b**) Representation of lyophilized RT-LAMP reactions using Bst-LF polymerase and reverse transcriptase RTx(exo). (**c**) RT-LAMP amplification of lyophilized reagents compared to commercial Warmstart® Master mix (New England Biolabs). SYTO9 dye was used to track amplification for each replicate (n = 2 biological replicates). As seen above, Bst-LF + RTx performs as robustly as the Warmstart® Master mix, whereas the Br512 + RTx combination produces non-specific signal the earliest. (+) signifies samples containing positive SARS-CoV-2. NTC denotes non-template control. (**d**) CRISPR detection reaction of amplified target using the lyophilized RT-LAMP reagents from (a) and (b) compared to the commercial Warmstart® Master mix (New England Biolabs). The fluorescence intensities were taken at t = 30 minutes, with n = 4 biological replicates.

**Supplementary Table S1. Comparison of CRISPR-based detection methods**

**Supplementary Table S1.1 Comparing selected CRISPR-based detection methods for COVID-19**

| | DETECTR | SHERLOCK | StopCOVID.v2 | RT-AIOD | ENHANCE |
|---|---|---|---|---|---|
| Target genes | N, E | N, ORF1ab | N | N | N, E |
| Enzyme | Cas12a | Cas13a | Cas12b | Cas12a | Cas12a |
| LoD copies/µL<br><br>(Total copies) | 10-100 copies/µL<br><br>(20 copies input) | 0.9-6.75 copies/µL<br><br>(7-54 copies input) | 0.033 copies/µL<br><br>(100 copies per sample) | 4.6 copies/µL<br><br>(5 copies input) | 15-25 copies/µL<br><br>(1-40 copies input ) |
| Time to detect patient samples<br><br>(RNA to detection) | 30-40 min | 60 min | 45-80 min | 40 min | 33 min |
| Positive Prediction Rate | 95% | 96.30% | 98.40% | 100% | 96.7% |
| Negative Prediction rate | 100% | 100% | 93.40% | 100% | 96.7% |

**Supplementary Table S1.2 Benchmarking ENHANCEv2 against ENHANCEv1 & DETECTR**

| | DETECTR | ENHANCEv1 | ENHANCEv2 |
|---|---|---|---|
| Reference | Chen et al., *Science*, 2018 | Nguyen et al., *Nat. Comms.*, 2020; *Methods*, 2021 | This manuscript [includes ENHANCEv1 clinical validation] |
| Highlights | First study on Cas12a-mediated trans-cleavage activity. Several applications including COVID-19 diagnostics reported. | First study on enhancing collateral activity of Cas12a by engineering crRNAs and detecting RNA as a heteroduplex. | First study on developing and clinically validating a CRISR-based diagnostic assay with dual mode reporters. Lyophilized kit stable for at least 30 days at room temperature. |
| crRNA | Wild-type | Engineered: 7-nt DNA on 3' end of crRNA | Engineered: 7-nt DNA on 3' end of crRNA |
| Cas | LbCas12a | LbCas12a | LbCas12a$^{D156R}$ |
| Kcat/Km (dsDNA) | $1.7 \times 10^7$ $s^{-1}$ $M^{-1}$ | $5.1 \times 10^7$ $s^{-1}$ $M^{-1}$ (3.2-fold higher) | --- |
| Pre-amplification | DNA: RPA | DNA: RPA; RNA: RT-RPA & RT-LAMP | RNA: RT-LAMP |
| Targets | HPV16 & HPV18 | HIV, HCV, PCA3, & SARS-CoV-2 | SARS-CoV-2 |
| Clinical validation | HPV16: 100% accuracy; HPV18: 92% accuracy | None | SARS-CoV-2: 97% accuracy |
| LOD range | -pre-amplification: pM; +pre-amplification: aM | -pre-amplification: fM; +pre-amplification: aM | -UDG: 0.2-7.9 copies/µL; +UDG: 15-25 copies/µL |
| Assay time, temperature | 60-120 min, 37°C (RPA+CRISPR) | 30 min, 65°C (RT-LAMP) + 10-20 min, 37°C (CRISPR) | 30 min, 65°C (RT-LAMP) + 3 min, 37°C (CRISPR) |
| Readout mode | Single-mode: Fluorescence (FL) | Single-mode: FL or lateral flow (LF) | Dual-mode: FL+LF in one reaction |
| Key equipment | FL mode: Heater & plate reader | FL mode: Heater & plate reader; LF mode: Heater | FL mode: Heater & 460 nm LED; LF mode: Heater |
| Sample type | DNA & anal swabs | Simulated urine, DNA, RNA & heteroduplex | RNA & nasal swabs |
| Extraction | Crude DNA extraction | Purified DNA and RNA | Optimization: DNA extraction buffer, 15 min @ 65°C + 2 min @ 98°C; Clinical: Maxwell® RSC |
| Cold chain | Freezer required | Freezer required | All components lyophilized. Stable for at least 30 days at 25°C. |
| Cost/rxn | <$1 (F) | <$1 (F), <$3 (LFA) | <$1 (F), <$3 (LFA) |

**Supplementary Table S2. Assay controls and interpretation of results**

**Supplementary Table S2.1 Fluorescence-based reporter detection assay: Assay controls**

| | Positive Control | Negative Control | Interpretation |
|---|---|---|---|
| SARS-CoV-2 N2 Gene ($N2_{t=20}/N2_{t=0}$) | ≥ 5 | < 5 | Valid for N2 gene. |
| | < 5 | < 5 | Invalid for positive control. Indicates QC failure. |
| | ≥ 5 | ≥ 5 | Invalid for negative control. Indicates N2 gene contamination |
| SARS-CoV-2 E2 Gene ($E2_{t=20}/E2_{t=0}$) | ≥ 5 | < 5 | Valid for E2 gene. |
| | < 5 | < 5 | Invalid for positive control. Indicates QC failure. |
| | ≥ 5 | ≥ 5 | Invalid for negative control. Indicates E2 gene contamination |
| Human RNASE-P Gene (RNASE-$P_{t=20}$ / RNASE-$P_{t=0}$) | N/A | < 5 | Valid for RNASE-P. |
| | | ≥ 5 | Invalid. Indicates RNASE-P gene contamination. |

**Supplementary Table S2.2 Fluorescence-based reporter detection assay: Fold-change interpretation**

| SARS-CoV-2 N2 gene ($N2_{t=20}$ /$NTC_{t=20}$) | SARS-CoV-2 E2 gene ($E2_{t=20}$ /$NTC_{t=20}$) | Human RNase-P gene (RNase-$P_{t=20}$ /$NTC_{t=20}$) | Interpretation |
|---|---|---|---|
| ≥ 5 | ≥ 5 | N/A | Positive for SARS-CoV-2 |
| ≥ 5 | < 5 | N/A | |
| < 5 | ≥ 5 | N/A | |
| < 5 | < 5 | ≥ 5 | Negative for SARS-CoV-2 |

**Supplementary Table S2.3 Lateral flow assay: Assay controls**

| | Positive control | Negative control | Interpretation |
|---|---|---|---|
| SARS-CoV-2 N2 gene visual readout | + | - | Valid for N2 gene. |
| | - | + | Invalid for positive control. Indicates QC failure. |
| | + | + | Invalid for negative control. Indicates N2 gene contamination |
| SARS-CoV-2 E2 gene visual readout | + | - | Valid for E2 gene. |
| | - | + | Invalid for positive control. Indicates QC failure. |
| | + | + | Invalid for negative control. Indicates E2 gene contamination |
| Human RNASE-P gene visual readout | N/A | - | Valid for RNASE-P. |
| | | + | Invalid. Indicates RNASE-P gene contamination. |

**Supplementary Table S2.4 Lateral flow assay: Visual readout interpretation**

| SARS-CoV-2 N2 gene visual readout | SARS-CoV-2 E2 gene visual readout | Human RNase-P gene visual readout | Interpretation |
|---|---|---|---|
| + | + | N/A | Positive for SARS-CoV-2 |
| + | - | N/A | |
| - | + | N/A | |
| - | - | + | Negative for SARS-CoV-2 |

**Supplementary References:**


1. Chen, J. S. *et al.* CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* **360**, 436-+, doi:10.1126/science.aar6245 (2018).

2. Nguyen, L. T., Smith, B. M. & Jain, P. K. Enhancement of trans-cleavage activity of Cas12a with engineered crRNA enables amplified nucleic acid detection. *Nat Commun* **11**, doi:ARTN 4906.
3. Nguyen, L. T. *et al.* CRISPR-ENHANCE: An enhanced nucleic acid detection platform using Cas12a. *Methods*, doi:10.1016/j.ymeth.2021.02.001 (2021)