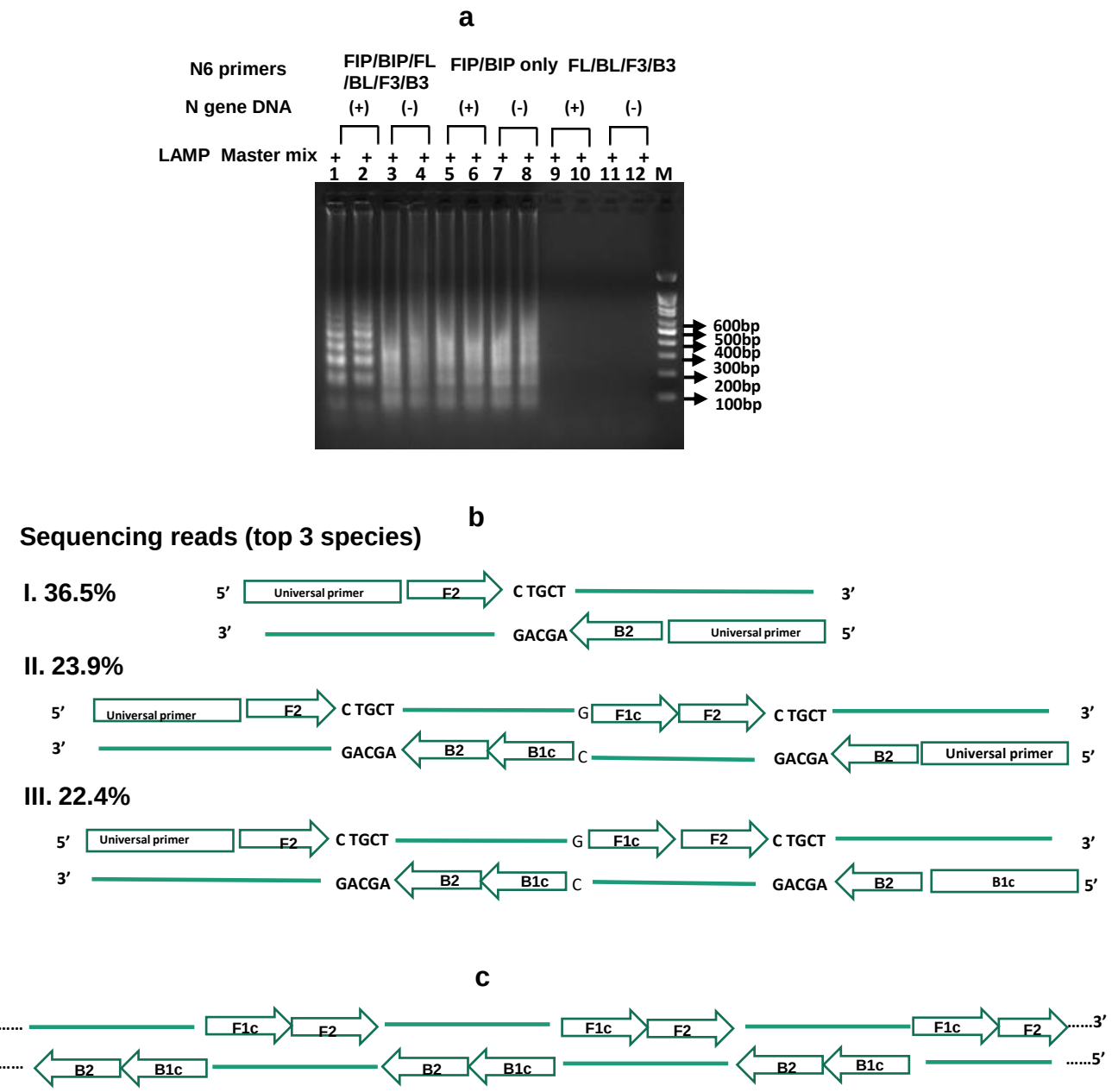
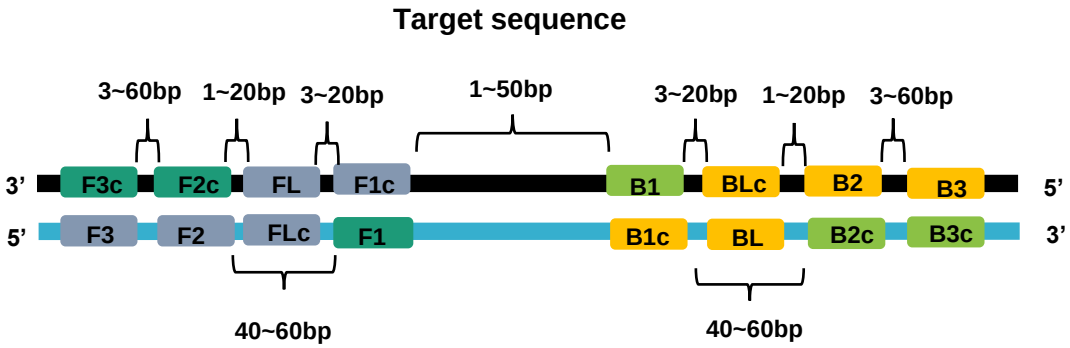



**Figure S1. Analysis of the false-positive products from the N6 assay.** **a.** Gel electrophoresis of the LAMP products from the reactions, in the presence or absence of the template DNA (the plasmid containing the N gene sequence of SARS-CoV-2) (lanes 1-12): in the presence of full set primers (lanes 1-4); in the presence FIP/BIP primers only (lanes 5-8), or in the presence of other primers only (reactions 9-12). **b.** Illumina sequencing analysis of the non-specific products as shown in lanes 3-8. **c.** Hypothesized model for the formation of non-specific LAMP products.

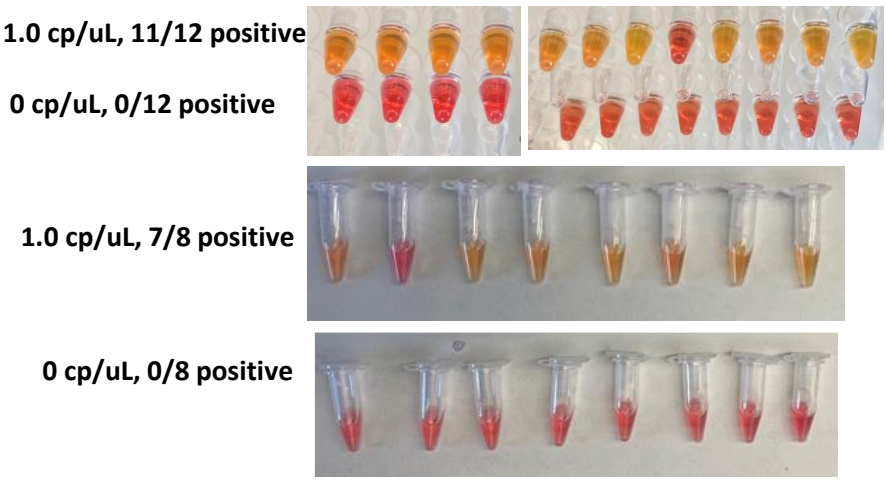



Primer Length

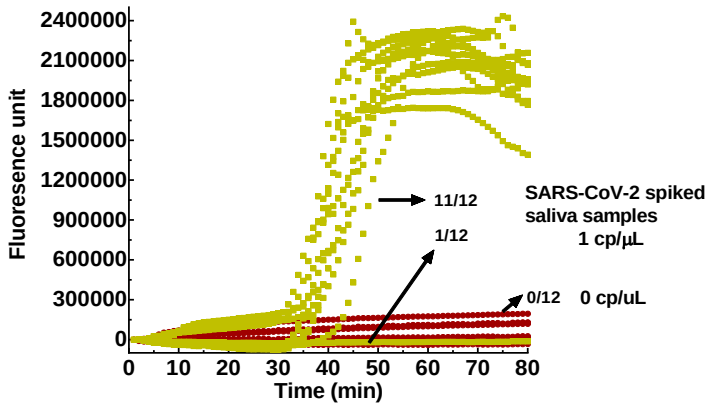
| bp | F3 | F2 | FL | F1 | B1 | BL | B2 | B3 | FIP(F2+F1c) | BIP(B2+B1c) |
|---|---|---|---|---|---|---|---|---|---|---|
| Min | 19 | 19 | 19 | 12 | 12 | 19 | 19 | 19 | | |
| Max | 25 | 25 | 25 | 17 | 17 | 25 | 25 | 25 | 37 | 37 |
| Opti | 22 | 22 | 22 | | | 22 | 22 | 22 | | |

**Figure S2. Restraints on the relative position and length of the LAMP primers.**

**a. SARS-CoV-2 spiked saliva (1.0 cp/µL)**




**b. Real-time monitoring of the reaction on a RT-qPCR instrument**




**c. SARS-CoV-2 spiked saliva (1.5 cp/µL)**

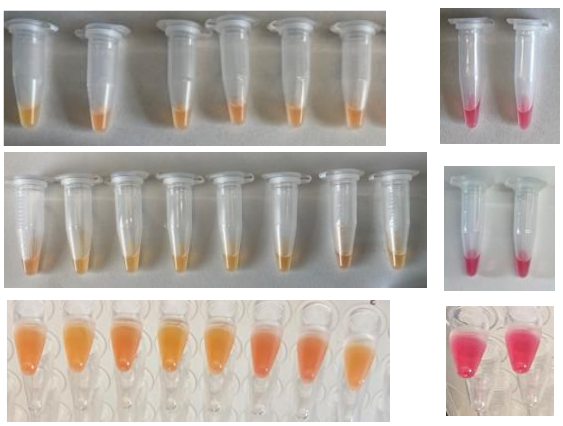
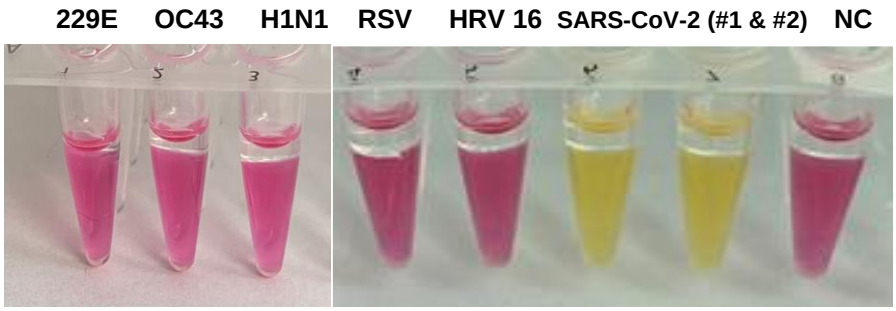



**Figure S3. The sensitivity and specificity of the N27/N5 assay (120 µL reaction volume) assessed by colorimetric/fluorescence readout. a.** Saliva samples spiked with 1.0 cp/µL SARS-CoV-2 particles. N27/M5 assays were performed either in 96-well PCR plate (0.2 mL) in a real-time PCR instrument (first panel of **a**) or in 0.5 mL Eppendorf tubes in a heating block (2nd & 3rd panels of **a**). **b.** Real-time monitoring of the spiked-in reactions as well as negative control reactions as shown in the first panel of **a**. **c.** Colorimetric readout of saliva samples (in 1.5 mL tubes or 96-well PCR plate) spiked with 1.5 cp/µL SARS-CoV-2 particles.

**a. Colorimetric change at the end of reaction**




**b. Real-time monitoring of the reaction on a real-time PCR instrument**

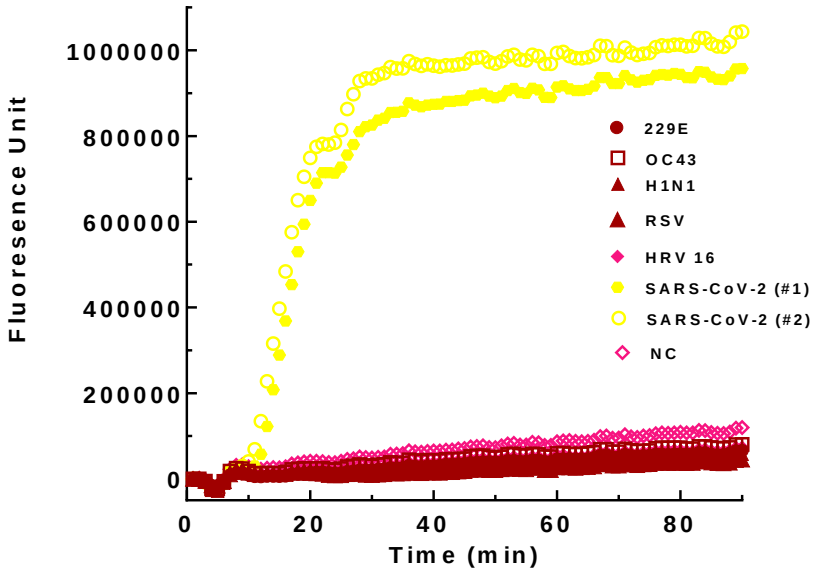



**Figure S4. The cross-reactivity of the N27/M5 assay with other common respiratory viruses.** Genomic RNAs (9 µL) from human coronavirus 229E (Ct ~23), human coronavirus OC43 (Ct ~23), influenza A virus (H1N1, Ct ~16), human respiratory syncytial virus (RSV) (Ct ~14), human rhinovirus 16 (HRV 16, Ct ~14), SARS-CoV-2 (Ct ~24 and ~25 for #1 and #2, respectively), and HeLa cell RNA (1 ng/µL, as negative control) were added to the N27/M5 RT-LAMP assay (120 µL volume). Assay positivity was defined by: (**a**) colorimetric changes at the end of the reaction, or (**b**) fluorescent signals detected by the real-time PCR instrument. The relative concentration of the genomic RNA from each virus was evaluated by standard RT-qPCR with ~0.5 µL RNA input, using primers specific to each viral genome sequence (Supplementary Table S8). SARS-CoV-2 RNA (samples #1 and #2) were isolated from 300 µL saliva of healthy subjects spiked with 6,000 cp/µL and 3,000 cp/µL heat-inactivated SARS-CoV-2, respectively. The results shown here represent three independent measurements with similar findings. NC: negative control.