Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

Supplementary information

SARS-CoV-2 recombinase polymerase amplification assay with lateral flow readout and duplexed full process internal control

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This Supplementary Information document describes:

Table S1: Primer and probe sequences for the MS2 assay

MS2 Forward Primer	TCGAGAGAAAGATCGCGAGGAAGATCAATACATA
MS2 Reverse Primer	CCTCAGCAATCGCAGCAAACTCCGGCATCTACT
MS2 Reverse Primer – Lateral Flow	CCTCAGCAATCGCAGCAAACTCCGGCATCTACT-[DIG]
MS2 Probe – Fluorescence	CTTCTTTGTTGTCTTCGACATGGGTAA-[T(ROX)]-C-dSpacer-[T(BHQ2)]-CATGTTTGAATGGCC-Spacer C3
MS2 Probe – Lateral Flow	[FAM]-CTTCTTTGTTGTCTTCGACATGGGTAATC-dSpacer-TCATGTTTGAATGGCC-Spacer C3

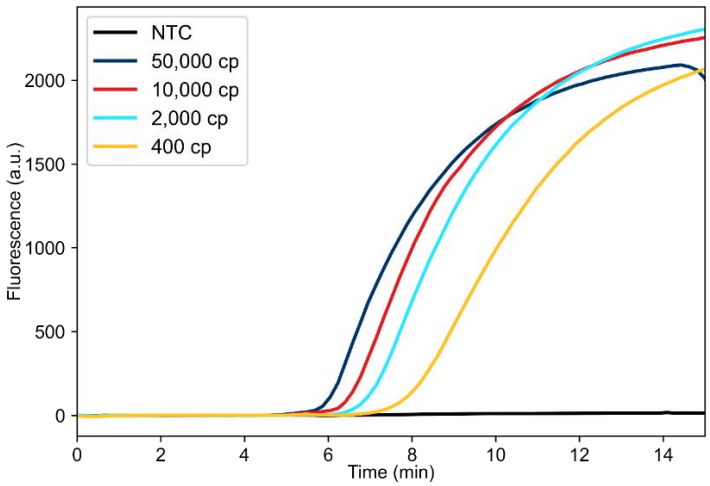

Figure S1: SARS-CoV-2 detection at high copy input. Detection of copies per reaction of RNA by the single target SARS-CoV-2 assay. All tested RNA input from 50,000-25 copies per reaction amplified successfully in one pot RT-RPA.

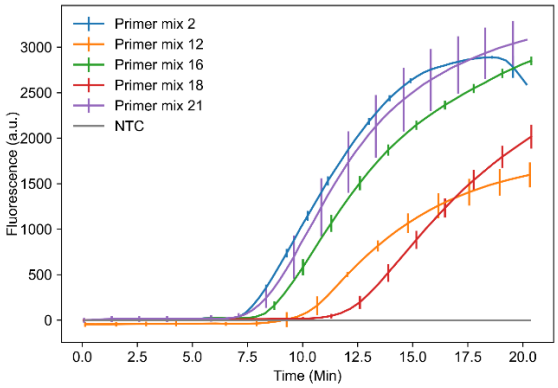

Figure 2: SARS-CoV-2 primer screening. Primers were designed following the Twist RPA design manual. All primer mixes were tested at 10^3 copies per reaction. Lines show an average of three replicates with error bars as the replicate standard deviation. Primer mix 2 was selected as the optimal primer combination and used for all subsequent testing.

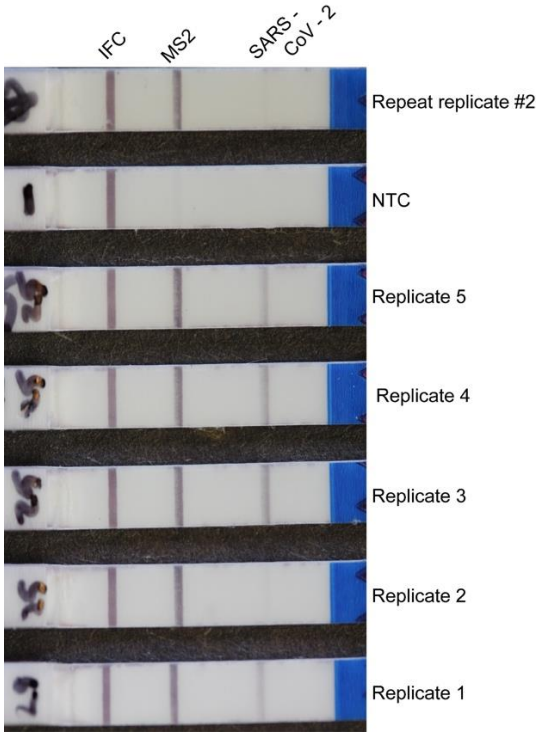

Figure 3: Duplexed LFA at 25 copies per reaction. Scanned LFA of the duplexed 25 copy replicates. Replicates 25.3, 25.4, 25.5 exhibit strong banding. Replicate 25.1 displays weak banding but is determinable by eye and by code analysis (Supplementary figure 3). Replicate 25.2 failed to resolve visible banding, the reaction solution was re-tested as displayed on the top LFA noted by 2 25.2 star. Repeat LFA of replicate 25.2 failed to resolve banding eliminating LFA capture failure as mechanism of failure.

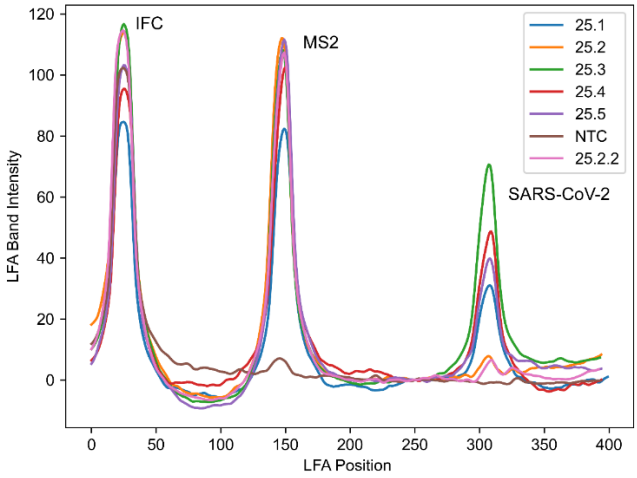

Figure 4: Line intensity plot profiles duplexed LFA at 25 copies per reaction produced by the analysis code. As seen above in S3, replicates 25.3, 25.4, and 25.5 exhibit strong banding. Note replicate 25.3 shows the highest SARS-CoV-2 intensity. Replicate 25.1 displays weak banding but is above the positive threshold (dash-dot magenta line) for SARS-CoV-2 and MS2 while also showing the lowest IFC intensity. Replicate 25.2 and subsequent re-test of (25.2.2) show similar banding intensity which is lower than the positive threshold.

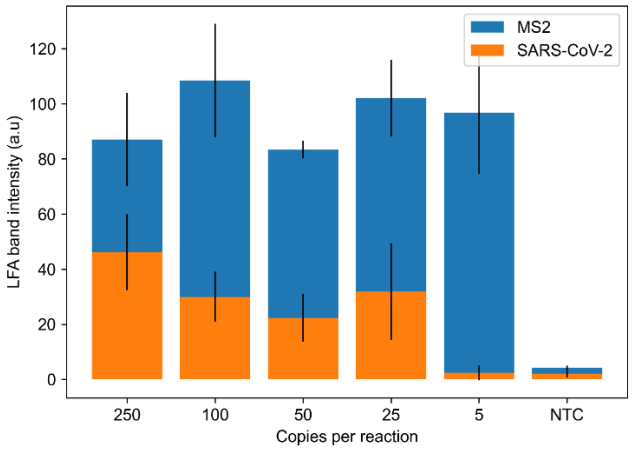

Figure 5: Average Duplexed intensity levels of SARS-CoV-2 and MS2 test bands. The intensity of the MS2 bands was higher than the SARS-CoV-2 test bands and was stable. The intensity of the Sars-CoV-2 test bands decreased with decreasing copy input as expected except for 25 copies per reaction.

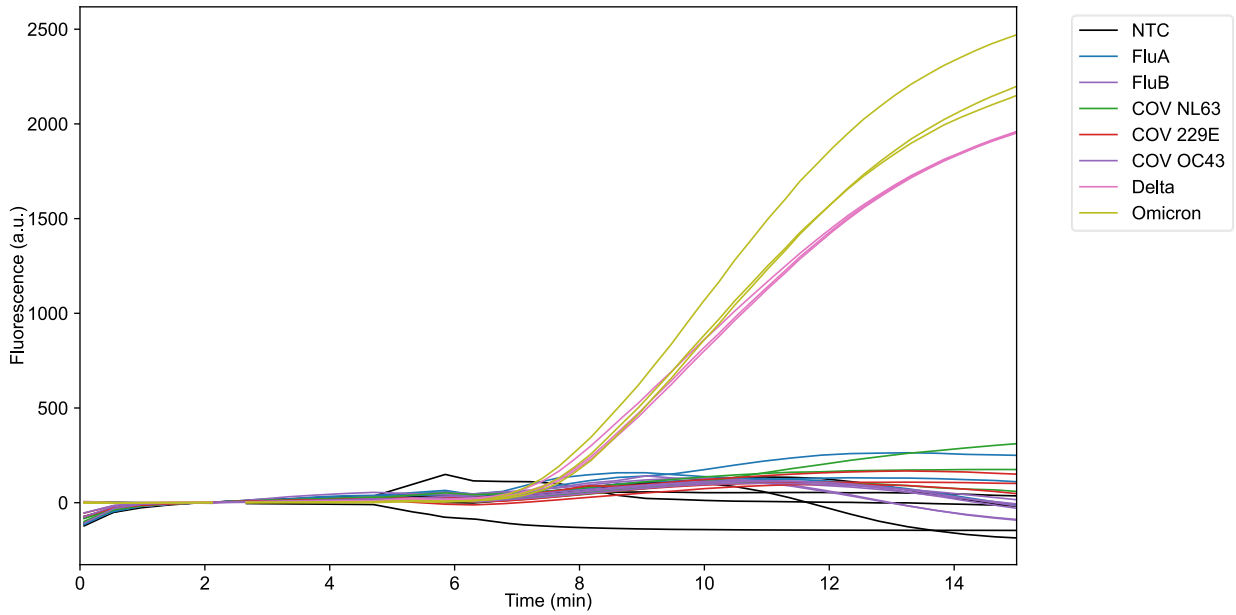

Figure 6: Fluorometer readings of other viral RNA screened with the duplexed RT-RPA LFA assay.

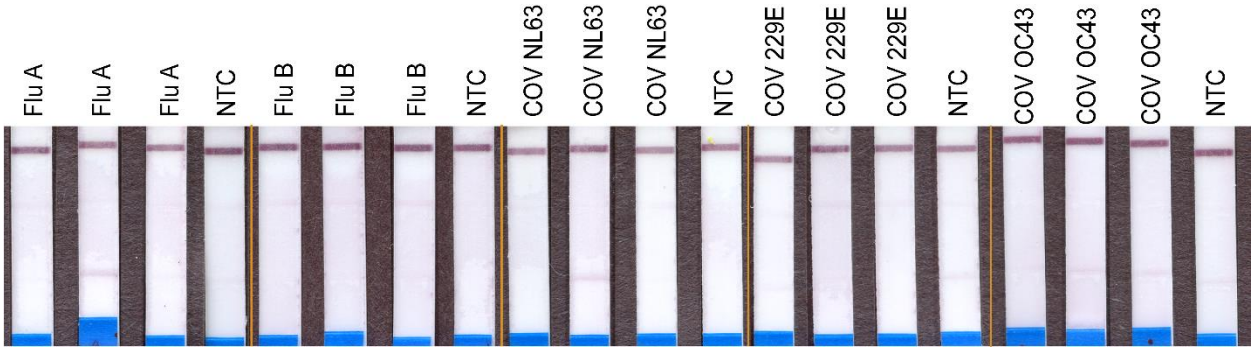

Figure 7: Scanned LFA readings of other viral RNA screened with the duplexed RT-RPA LFA assay. Orange bars denote separates images merged together.