Electronic Supplementary Material (ESI) for Lab on a Chip.

Development and Translation of a Paper-based Top Readout Vertical Flow Assay for SARS-CoV-2 Surveillance

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Supplementary Information

Table S1. List of non-specific pathogens used for the cross-reactivity study

S/N	Biological Agent	Source	Lot #	Test Concentration	Remarks/expire date
1	Human adenovirus 3	ATCC VR-3	70033218	4×10^{6} $TCID_{50}$/mL	
2	Human adenovirus 5	ATCC VR-5	70024114	4×10^{7} $TCID_{50}$/mL	
3	Coronavirus-229E	ATCC VR-740	70035459	4×10^{4} $TCID_{50}$/mL	
4	Coronavirus-OC43	ATCC VR-1558	70036255	4×10^{4} $TCID_{50}$/mL	
5	Human adenovirus 4	ATCC VR-1572	58527797	$1.25 \times 10^{5.5}$ $TCID_{50}$/mL	
6	Coronavirus NL63	ZEC. 0810228CFHI	325222	1.17×10^{3} $TCID_{50}$/mL	HI (15-10-2023)
7	RSV Type A	ZEC. 0810040ACFHI	324924	1.25×10^{4} $TCID_{50}$/mL	HI (25-08-2023
8	RSV Type B	ZEC. 0810040CFHI	323000	1.25×10^{4} $TCID_{50}$/mL	HI (04-09-2022
9	Rhinovirus A16	ZEC. 0810285CFHI	316699	$2.5 \times 10^{4.1}$ $TCID_{50}$/mL	HI (N/A)
10	Influenza A	AMR in house culture		1.13×10^{9} PFU/mL	
11	Influenza B	AMR in house culture		1×10^{5} PFU/mL	
12	Klebsiella pneumoniae	AMR in house culture		3×10^{7} CFU/mL	
13	Streptococcus pneumoniae	AMR in house culture		4×10^{6} CFU/mL	

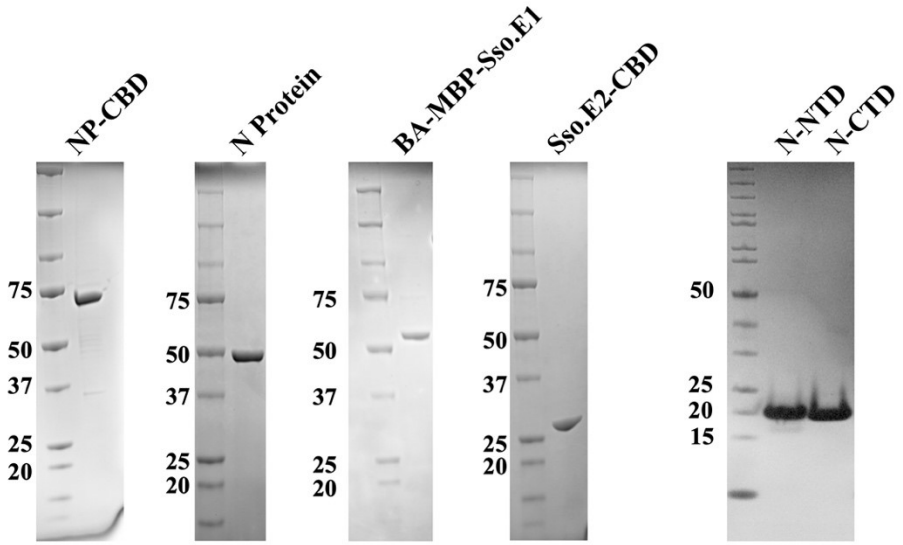

Figure S1. SDS-PAGE gel images of purified proteins.

SDS-PAGE gel images for NP-CBD (SARS-CoV-2 N Protein fused with CBD), NP (SARS-CoV-2 N Protein), BA-MBP-Sso.E1 (Sso.E1 tagged with BA-MBP), Sso.E2-CBD (Sso.E2 fused with CBD), N-NTD (SARS-CoV-2 N Protein N terminal domain) and N-CTD (SARS-CoV-2 N Protein C terminal domain).

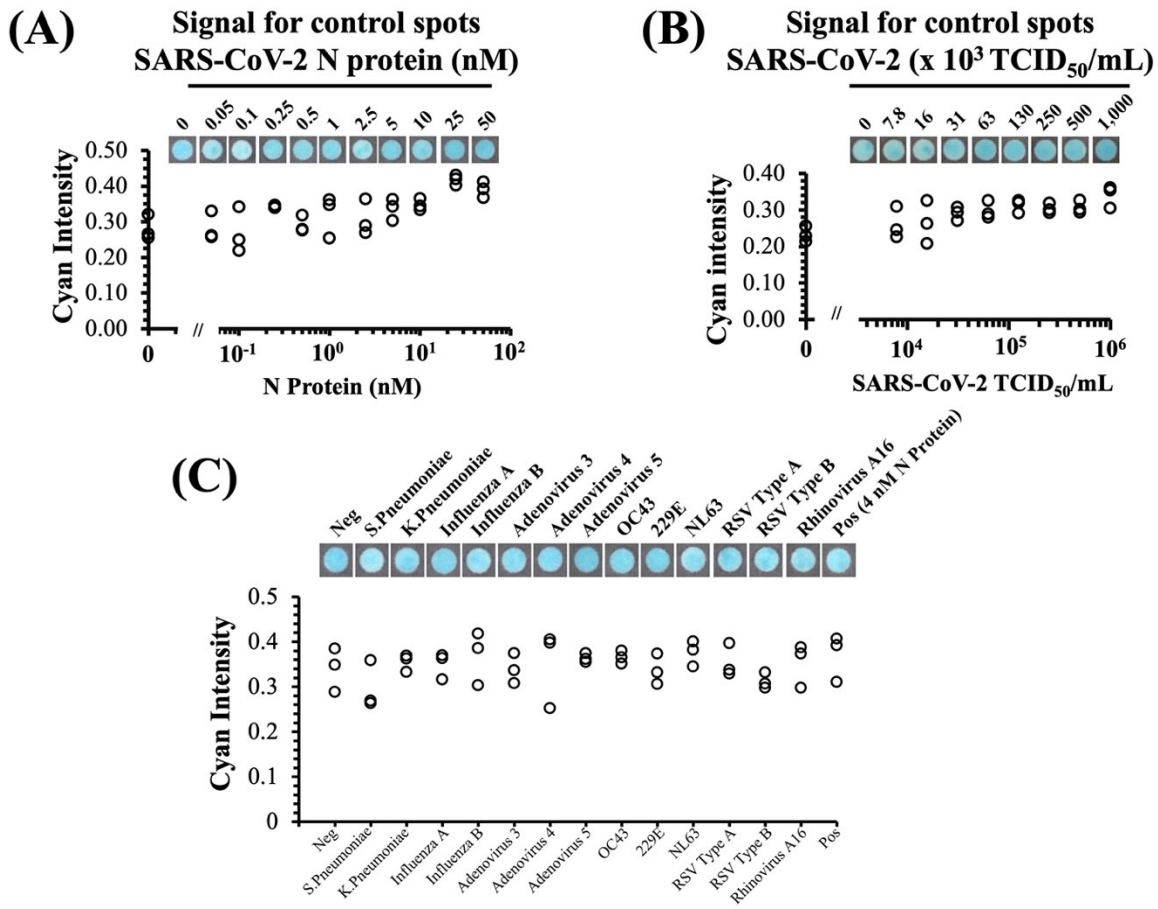

Figure S2. Analysis of colorimetric signals obtained from control spots.

(A). Cyan intensity at control spot with different concentrations (0, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50 nM) of SARS-CoV-2 N Protein spiked in saliva test matrix; **(B)**. Cyan intensity at control spot with different concentrations (0, 7.8, 16, 31, 63, 130, 250, 500, 1,000 x 103 $TCID_{50}$/mL) of SARS-CoV-2 virus spiked in saliva test matrix; **(C)**. Cyan intensity at control spot with 13 different pathogen samples spiked in saliva test matrix.

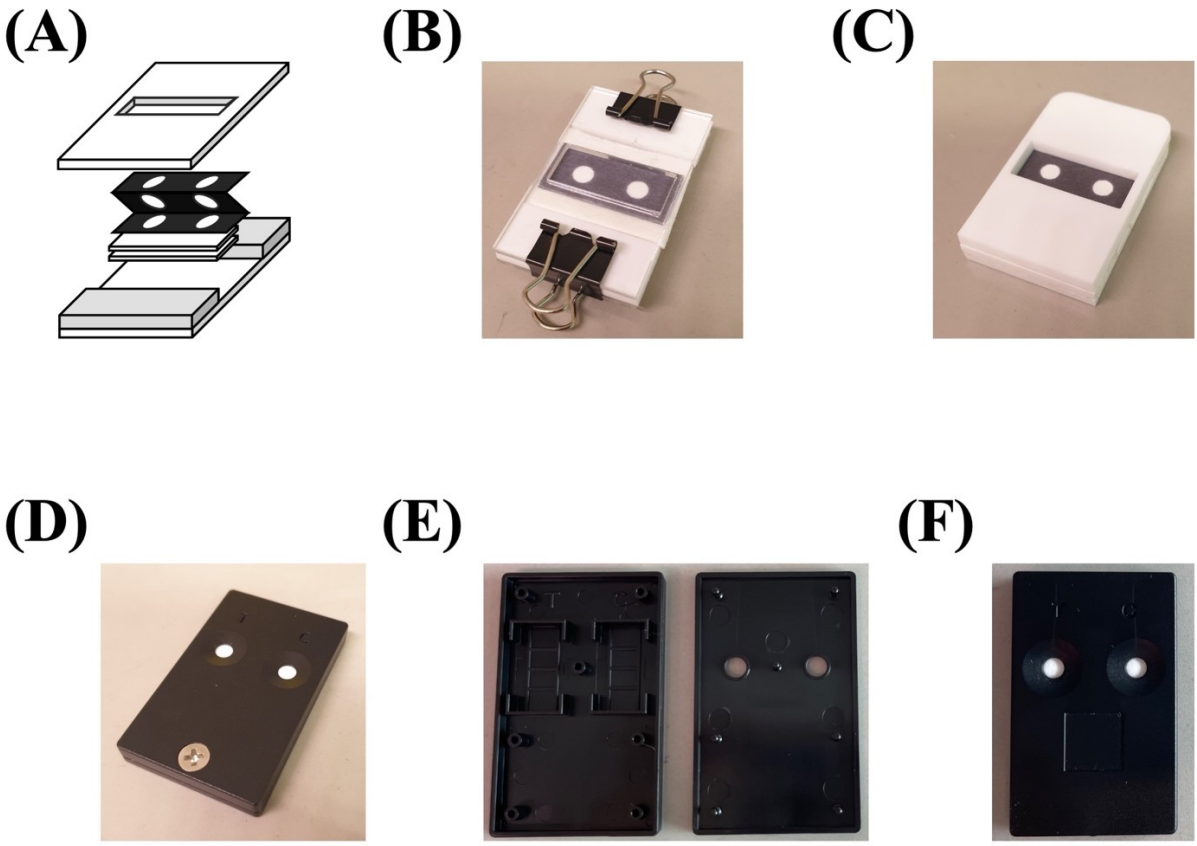

Figure S3. Images of test strip construction.

(A). Schematic representation of cassette assembly; **(B)**. Cassette assembled with plastic manifold and paper clips; **(C)**. Cassette assembled with plastic manifold and double-sided tape; **(D)**. Aluminum cassette assembled with screw; **(E)**. Injection molding produced bottom (left) and top (right) pieces of the test cassette and **(F)**. Injection molded plastic cassette after assembly.

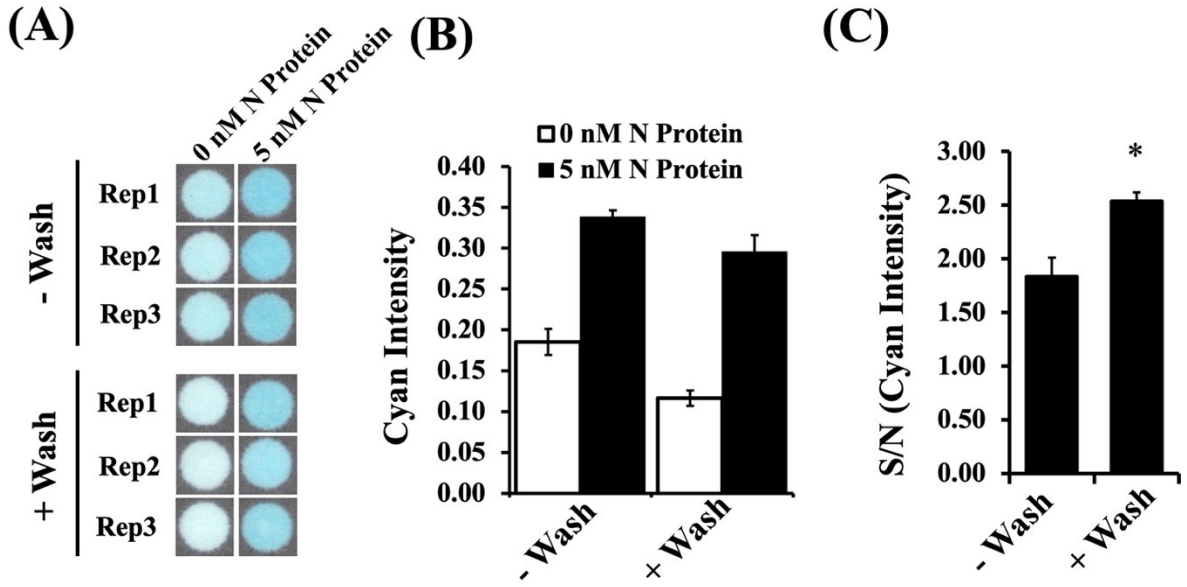

Figure S4. Assessment of washing step.

(A). Test spot images of VFAs carried out with and without washing step and with or without 5 nM SARS-CoV-2 N Protein respectively: **(B)**. Cyan intensity analysis for VFAs carried out with and without washing step; **(C)**. Signal to noise ratio (S/N) analysis for VFAs carried out with and without washing step, the difference is significant.

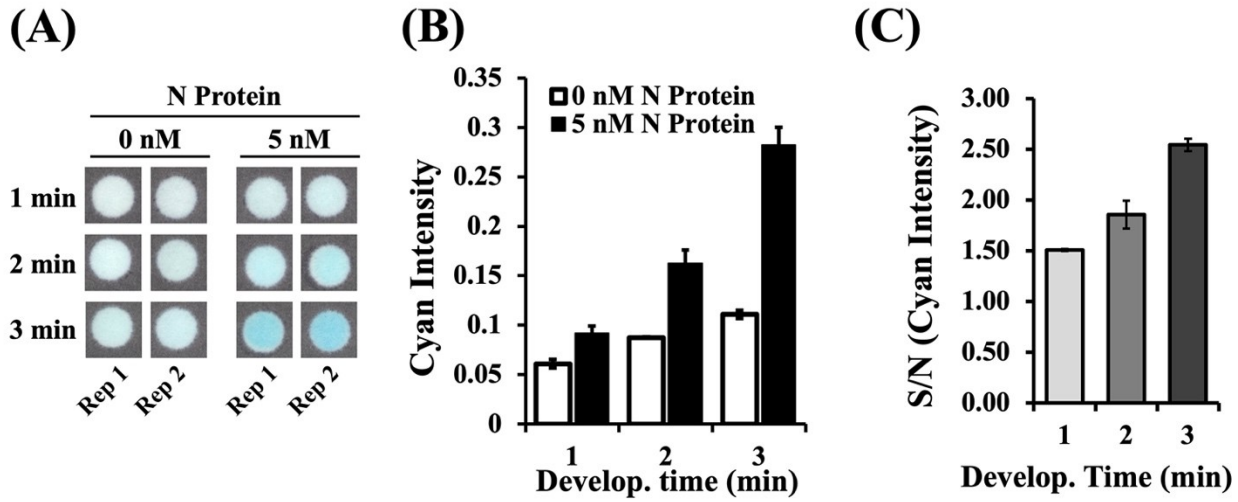

Figure S5. Assessment of different colorimetric developmental times.

(A). Test spot images of VFAs carried out with or without 5 nM SARS-CoV-2 N Protein that are developed for 1 or 2 or 3 minutes; **(B)**. Cyan intensity analysis for VFAs carried out with or without SARS-CoV-2 N Protein for different development times; **(C)**. Signal to noise ratio (S/N) analysis of VFAs that have been developed for signal for 1 or 2 or 3 minutes.

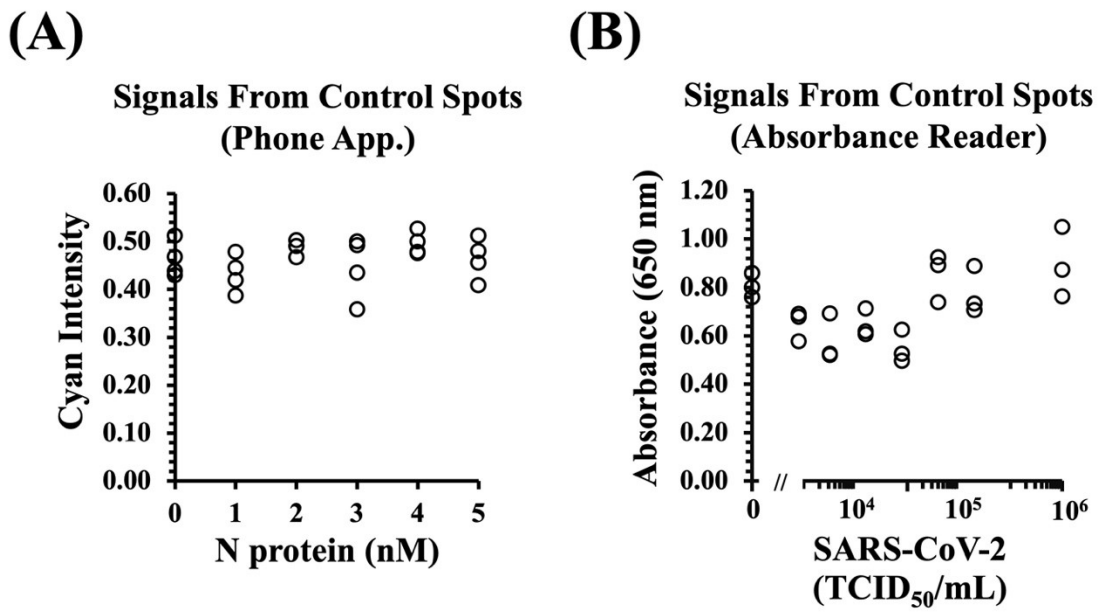

Figure S6. Analysis of colorimetric signals obtained from control spots of the PoC tests. **(A)**. Cyan intensity at control spot with different concentrations (0, 1, 2, 3, 4, 5 nM) of SARS-CoV-2 N Protein spiked in saliva test matrix; **(B)**. Absorbance (650 nm) at control spot using Attonics spectrophotometer absorbance reader system with different concentrations (0, 0.032, 0.16, 0.8, 4, 20, 1,000 x 10^3 $TCID_{50}$/mL) of SARS-CoV-2 virus spiked into saliva test matrix.