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Development and performance of a point-of-care rapid antigen test for detection of SARS-COV-2 variants

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ABSTRACT

Background: SARS-CoV-2 antigen-based tests are well-calibrated to infectiousness and have a critical role to play in the COVID-19 public health response. We report the development and performance of a unique lateral flow immunoassay (LFA).

Methods: Combinations of several monoclonal antibodies targeting multiple antigenic sites on the SARS-CoV-2 nucleocapsid protein (NP) were isolated, evaluated, and chosen for the development of a LFA termed CoV-SCAN (BioMedomics, Inc.). Clinical point-of-care studies in symptomatic and asymptomatic individuals were conducted to evaluate positive predictive agreement (PPA) and negative predictive agreement (NPA) with RT-PCR as comparator.

Results: In laboratory testing, CoV-SCAN detected 14 recombinant N-proteins of SARS-CoV-2 variants with sensitivity in the range of 0.2–3.2 ng/mL, and 10 authentic SARS-CoV-2 variants with sensitivity in the range of 1.6–12.5 TCID50/swab. No cross reactivity was observed with other human coronaviruses or other respiratory pathogens. In clinical point-of-care testing on 148 individuals over age 2 with symptoms of ≤5 days, PPA was 87.2% (CI 95: 78.3–94.8%) and NPA was 100% (CI 95: 94.2–100%). In another 884 asymptomatic individuals, PPA was 85.7% (CI 95: 42.1–99.6%) and 99.7% (99.0–99.9%). Overall, CoV-SCAN detected over 97.2% of specimens with CT values <30 and 93.8% of nasal swab specimens with the Omicron variant, even within the first 2 days after symptom onset.

Conclusions: The unique construction of CoV-SCAN using two pairs of monoclonal antibodies has resulted in a test with high performance that remains durable across multiple variants in both laboratory and clinical evaluations. CoV-SCAN should identify almost all individuals harboring infectious SARS-CoV-2.

Summary: Unique construction of a point-of-care rapid antigen test using two pairs of monoclonal antibodies has led to good performance that remained durable across multiple variants in laboratory and clinical evaluations. Test should identify almost all individuals harboring infectious SARS-CoV-2.

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1. Introduction

Preventing viral transmission of SARS-CoV-2 requires identifying whether an individual is infectious, not whether that person is infected. SARS-CoV-2 viral load increases rapidly after infection, with the peak viral load associated with the highest risk of transmission to others. A high analytic sensitivity test used infrequently, particularly with a delay in result reporting like that often seen with Real-Time Polymerase Chain Reaction (RT-PCR), can miss the earliest stages of infection and remain positive even after the patient is no longer infectious. Modeling studies suggest that a lower analytic sensitivity assay like a rapid antigen assay, when administered frequently, has a better chance of identifying a person during peak viral load than a high analytic sensitivity test administered less frequently [1–3]. Real world studies have borne out these modelled assertions [4–6]. Rapid antigen tests have played a key role in public health responses in many countries since 2020 by protecting vulnerable populations, releasing individuals from isolation, enabling a return to activities, and testing during surges or cluster investigations [7].

The continual emergence of new variants presents challenges to testing strategies. Ensuring that rapid antigen tests can continue to detect new variants at or near an optimal performance level is a priority. We describe our experience with the development and performance of a point-of-care lateral flow immunoassay (LFA) that detects all known SARS-CoV-2 variants of concern, including Omicron, through a partnership between an academic institution and a commercial company.

2. Development of rapid antigen test

2.1. Isolation and characterization of nucleocapsid (N) protein-specific monoclonal antibodies

Monoclonal antibodies against the SARS-CoV-2 N-protein were derived by isolating antibody-producing CD19+CD27+ memory B cells from patients who have had COVID-19 infection that could bind the N-protein, followed by the use of 10x genomics to amplify and sequence each pair of H-chain and L-chain antibody genes (Supplementary Figure S1) [9,10].

Initial analyses yielded several N-protein-specific monoclonal antibodies with high binding affinity (Fig. 1A) and equilibrium dissociation constants (K_D) in the sub-nM range (Fig. 1B).

Epitope mapping studies were performed so that antibodies with different binding sites could be chosen for the assay (Fig. 1C and Supplemental Figure S2). Multiple combinations of monoclonal antibodies were first tested in immunoassays to identify specific sets that would optimally capture and detect SARS-CoV-2 N-protein, including ones containing mutations that exist in variants of concern (VOC). The antibody combination of 9-24+9-11 and 8-05+8-08 had the highest detection signal at the same level of SARS-CoV-2 nucleocapsid proteins (Fig. 1D). Moreover, we also compared these antibody pairs on the lateral flow immunoassay and found the antibody pair, 9-24+9-11 and 8-05+8-08 showed the best detection results (Data not shown). These efforts ultimately led to the identification of 9-24+9-11/8-05+8-08 as the best combination of antibodies to format into a LFA (Fig. 1D) termed with the commercial name of CoV-SCAN.

2.2. Lateral flow immunoassay components

The CoV-SCAN test cassette consists of 6 components – a sample pad, conjugate pad, filter pad, nitrocellulose membrane, and absorption pad all laminated to an adhesive backing card (Fig. 2A). The conjugate pad is first sprayed with antibodies 9-24 and 9-11, each tightly conjugated to gold nanoparticles. Additionally, antibodies 8-05 and 8-08 and the control mouse anti-human antibody are sprayed onto the Test Zone and Control Zone, respectively. The gold nanoparticles allow the assay to be read visually without the assistance of additional equipment by imparting a red color to the T-line, if the N-protein is present, and C-line. Test results are interpreted 15 minutes after application of the sample: a colored line at the C-line indicates that the fluid containing the sample material has moved laterally appropriately; two colored lines at the C-line and T-line locations indicates the sample is COVID-19 positive; only one colored C-line indicates the sample is COVID-19 negative; and the absence of a colored C-line indicates an invalid test (Fig. 2B).

2.3. Limits of detection for SARS-CoV-2 mutant N proteins and variants of concern

A series of studies were performed to determine the sensitivity and specificity of CoV-SCAN using N-proteins with different amino acid mutations at different concentrations. Fourteen recombinant SARS-CoV-2 variant N-proteins (Supplemental Table S1) were detected by CoV-SCAN, typically with sensitivity in the range of 0.2–0.8 ng/mL while mutant proteins such as D401Y resulted in slightly lower sensitivity (3.2 ng/mL) (Fig. 3A and Supplemental Fig. S3).

D401Y is not found in currently circulating SARS-CoV-2 variants of concern (VOCs) and variants of interest (VOIs); however, R203K is found in approximately 30% of all circulating viruses and presented in several VOCs and VOIs, including Omicron. CoV-SCAN showed excellent specificity. It did not detect N-proteins of human coronaviruses MERS, 229E, HKU1, NL63 and OC43, although the N-protein of SARS-CoV could be detected at concentrations >2 ng/mL (Fig. 3A, Supplemental Fig. S4a and S4b).

Moreover, CoV-SCAN detected a number of chemically-inactivated SARS-CoV-2 authentic viruses, including Alpha/B.1.1.7, Beta/B.1.351, Gamma/P.1, Delta/B.1.617.2, and Omicron/B.1.1.529 variants with sensitivity in the range of 1.6–12.5 TCID50/swab (Fig. 3B).

2.4. Cross-reactivity studies on other respiratory pathogens

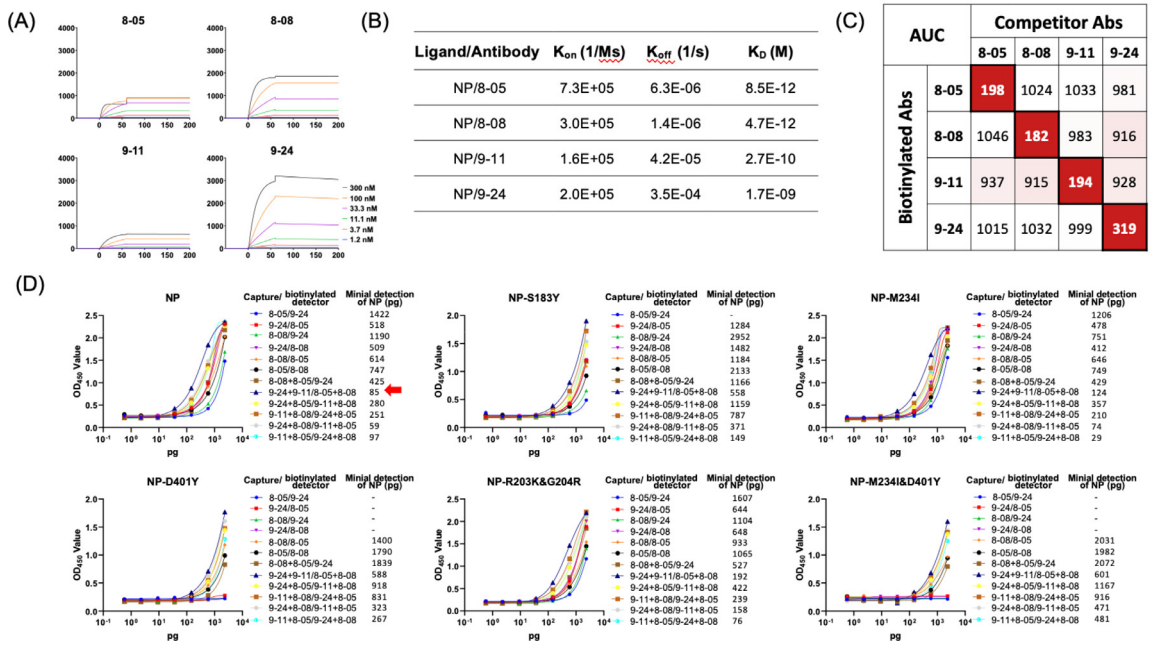
The specificity of CoV-SCAN was also confirmed using a panel of inactivated respiratory pathogens recommended by the FDA, including 19 viruses (2.2×10^5 to 2.8×10^8 PFU), 9 bacteria (1.9×10^6 to 7.8×10^9 CFU), and one yeast (8.4×10^8 CFU), along with nasal washes pooled from 10 healthy individuals (Fig. 3C).

3. Point-of-care clinical evaluation studies

Two prospective clinical evaluation studies were performed in adults and children at two separate medical sites in New York City for point-of-care use in non-laboratory settings. **Study 1:** Symptomatic participants were recruited prospectively from Columbia University Irving Medical Center (CUIMC) through the COVID-19 testing site for employees and patients, the Emergency Department, inpatient units, and from household contacts of patients with COVID-19. Inclusion criteria included (1) age over 2; and (2) self-reported presence of any symptoms consistent with COVID-19 beginning ≤5 days prior to enrollment. Participants were enrolled sequentially and tested blindly in separate periods to evaluate impact of different variants of concern on the performance of the LFA: 1/7/21 to 2/4/21 (wildtype), 3/5/21 to 9/10/21 (Delta), and 12/13/21 to 12/21/21 (Omicron). **Study 2**: Symptomatic and asymptomatic participants were tested prospectively in all-comer fashion from a Health and Hospitals COVID-19 testing site in New York City from 10/14/21 to 11/30/21.

3.1. Study procedure

Two anterior (mid-turbinate) nasal swabs were collected from each participant by rolling the swab in each nostril 5 times and utilized for CoV-SCAN and RT-PCR testing. For the CoV-SCAN test, a swab was



Ligand/Antibody	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)
NP/8-05	7.3E+05	6.3E-06	8.5E-12
NP/8-08	3.0E+05	1.4E-06	4.7E-12
NP/9-11	1.6E+05	4.2E-05	2.7E-10
NP/9-24	2.0E+05	3.5E-04	1.7E-09

AUC		Competitor Abs			
		8-05	8-08	9-11	9-24
Biotinylated Abs	8-05	198	1024	1033	981
	8-08	1046	182	983	916
	9-11	937	915	194	928
	9-24	1015	1032	999	319

Fig. 1. Isolation and characterization of N-protein monoclonal antibodies.
(A) Binding of antibodies to SARS-CoV-2 nucleocapsid protein was determined by surface plasmon resonance (SPR). The nucleocapsid protein-bound sensors were incubated with six different concentrations of antibodies. Kinetic data from one representative experiment were fit to a 1:1 binding model. (B) Summary of SPR kinetic and affinity measurements. (C) Epitope mapping by competition ELISA of N-protein monoclonal antibodies. (D) Evaluation of antibody pairs for lateral flow rapid test. The red arrow represents the antibody pair used for Lateral flow assay (LFA) antigen tests. A representative result of three experimental replicates is shown.

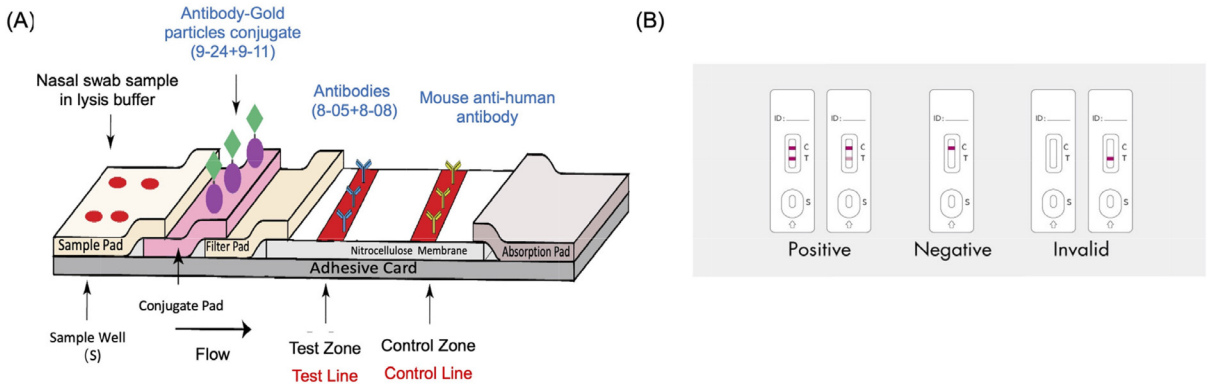


Fig. 2. CoV-SCAN for rapid SARS-CoV-2 nucleocapsid protein test.
Schematic of laminated CoV-SCAN test strip. (B) CoV-SCAN result display. C: control line, T: test line.

placed directly into the mixing tube, mixed with 10 drops of lysis buffer, and 3 drops of the sample were placed on the S-well of the test cassette. After 15 min, the test results were determined by visual inspection. Results were recorded as positive for presence of a T-line, and negative if the T-line was not present by visualization. All valid tests required the presence of a C-line; tests without C-line were not recorded, and the test was repeated using a new test cassette. For the RT-PCR test, the nasal swab was kept in viral transport media (VTM) at 2–8 °C until the RT-PCR was performed. For Study 1, RT-PCR was performed in the Center for Advanced Laboratory Medicine (CALM) at CUIMC with Xpert Xpress SARS-CoV-2/Flu/RSV (Version 4.0) on a Cepheid GeneXpert® Xpress System (Version 4.8). For Study 2, RT-PCR was performed at the Pandemic Response Laboratory. [11] All RT-PCR tests were performed by technicians blinded to the CoV-SCAN test results.

3.1.1. *Genotyping*

We determined the presence of variants of concern/interest from RT-PCR-positive specimens obtained in Study 1 using PCR-based single-nucleotide polymorphism (SNP) assays and whole genome nanopore sequencing [12]. The SNP genotyping assay was performed at the positions of del69-70, L452R, S477N, T478K, E484K, G496S/Q498R, and N501Y in the S gene. The presence of L452R in combination with T478K is highly suggestive of the Delta variant (B.1.617.2 or AY lineages) and G496S/Q498R is highly suggestive of the Omicron variant (B.1.1.529 or BA lineages). For whole genome sequencing, reverse transcription was performed using LunaScript RT SuperMix (NEB), tiling PCR was performed on the cDNA targeting 1200 bp regions, and amplicons barcoded using the Oxford Nanopore Rapid Barcoding 96 kit. Pooled barcoded libraries were then sequenced on an Oxford Nanopore GridION

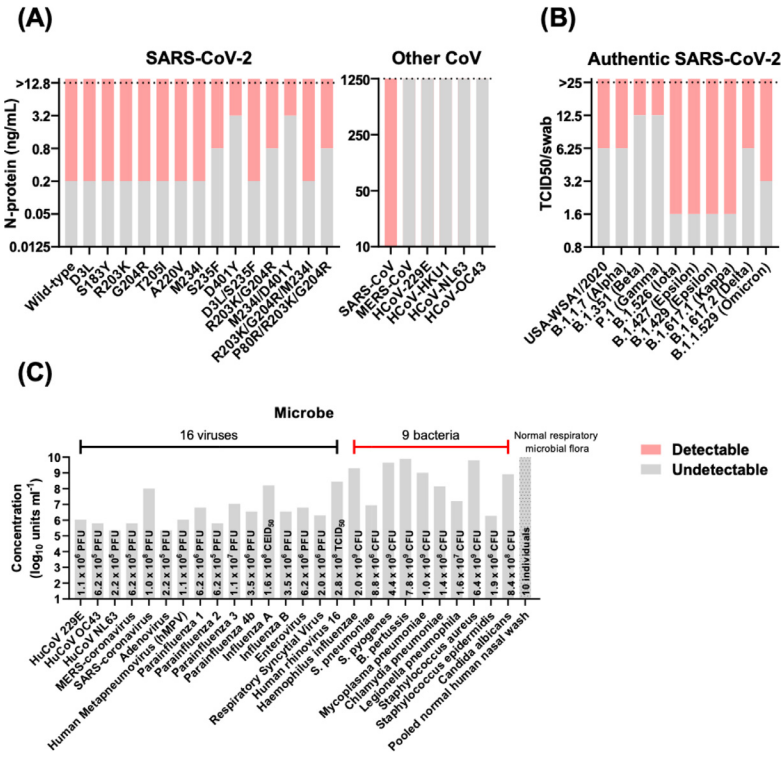


Fig. 3. The analytical sensitivity and specificity of CoV-SCAN. Detection of nucleocapsid proteins of SARS-CoV-2 mutant and related coronaviruses. (B) Limits of detection for SARS-CoV-2 variants. (C) Microbes tested for cross-reactivity. 3 reps were run for each experiment. The tests were repeated with 4 independent experiments (12 reps for each concentration),a representative result of four experimental replicates is shown.

sequencer using R9.4.1 flow cells. Basecalling was performed in the MinKNOW software v21.02.1. Genomes were manually curated by visually inspecting sequencing alignment files for verification of key residues in Geneious v10.2.6. Nextclade and Pangolin were used to identify lineages and genomic mutations.

3.1.2. Data analyses

Positive Percent Agreement (PPA), the proportion correctly identified as positive by CoV-SCAN if detected by RT-PCR, and Negative Percent Agreement (NPA), the proportion correctly identified as negative by CoV-SCAN not detected by RT-PCR, were calculated with 95% exact binomial confidence intervals.

3.1.3. Ethics Statement

Columbia University Irving Medical Center Institutional Review Board approved Study 1. BRANY IRB approved Study 2. All participants provided informed consent.

4. Results

For **Study 1**, 148 participants were enrolled with a mean age of 46.7±19.9 (range 2–89) with 82 (55%) females and 40 (27%) Black and 55 (37%) Hispanic/Latinx. Mean duration from symptom onset to enrollment and specimen collection was 2.7±1.6 days. Among the 86 clinical specimens that were RT-PCR-positive, 75 paired specimens were CoV-SCAN-positive, resulting in a PPA of 87.2% (95% CI 78.3%-94.8%) (Table 1a).

Among the 62 clinical specimens that were RT-PCR-negative, all 62 paired specimens were CoV-SCAN-negative, resulting in a NPA of 100% (95% CI 94.2–100%).

Among the 86 RT-PCR-positive specimens, 43 of the more recent specimens were assessed for SARS-CoV-2 VOCs/VOIs using PCR-based SNP typing and/or whole-genome sequencing (Table 2). The following VOCs/VOIs were identified: Alpha (B.1.1.7), Delta (B.1.617.2 and AY lineages), Omicron (B.1.1.529 and BA.1), Iota (B.1.526, including B.1.526-E484K and B.1.526-S477N), and Mu (B.1.621). CoV-SCAN was positive for 41/43 (95.3%) of specimens with VOCs/VOIs. Importantly,

Table 1
Positive percent agreement (PPA) and negative percent agreement (NPA) of CoV-SCAN and RT-PCR results for symptomatic and asymptomatic participants performed at different sites.

a. Symptoms ≤ 5 Days (Columbia)			
01/07/2021 to 12/21/2021		**PCR-Comparator**	
CoV-SCAN		**Detected**	**Not Detected**
	Positive	**75**	**0**
	Negative	**11**	**62**
	Total	**86**	**62**
PPA (sensitivity)	**87.2**	(CI 95: 78.3– 94.8%)	
NPA (specificity)	**100.0**	(CI 95: 94.2–100.0%)	
b. Symptomatic (NYC H&H)			
10/12/2021 to 12/01/2021		**PCR-Comparator**	
CoV-SCAN		**Detected**	**Not Detected**
	Positive	**8**	**0**
	Negative	**1**	**103**
	Total	**9**	**103**
PPA (sensitivity)	**88.9**	(CI 95: 51.8–99.7%)	
NPA (specificity)	**100.0**	(CI 95: 96.5–100.0%)	
c. Asymptomatic (NYC H&H)			
10/12/2021 to 12/01/2021		**PCR-Comparator**	
CoV-SCAN		**Detected**	**Not Detected**
	Positive	**6**	**3**
	Negative	**1**	**874**
	Total	**7**	**877**
PPA (sensitivity)	**85.7**	(CI 95: 42.1– 99.6%)	
NPA (specificity)	**99.7**	(CI 95: 99.0–99.9%)	

CoV-SCAN was positive for 15/16 (93.8%) of specimens with the Omicron variant, including 7/7 (100%) from specimens obtained within the first 2 days after symptom onset. Among RT-PCR specimens, 20 occurred in participants who developed COVID-19 infection after completing a full series of EUA approved COVID-19 vaccinations with and without booster (Table 2). CoV-SCAN was positive for 19/20 (95%) specimens representing breakthrough infections.

Subsequent to the completion of Study 1, B.A.2 became the predominant variant in New York City during April, 2022. Although we did not

Table 2
Data for all RT-PCR+ specimens including duration of symptoms prior to testing, and Variants of Concern/Interest (VOC/I).

Days Symptoms	CoV-SCAN Result	RT-PCR CT Value	VOC/I	Single Nucleotide Assay	Sequencing Results
3	Positive	20.8	Non-VOC/I	N/A	N/A
4	Positive	32.0	Non-VOC/I	N/A	N/A
3	Negative	33.5	Non-VOC/I	N/A	N/A
3	Negative	33.7	Non-VOC/I	N/A	N/A
2	Positive	21.5	Non-VOC/I	N/A	N/A
4	Negative	35.2	Non-VOC/I	N/A	N/A
3	Positive	25.0	Non-VOC/I	N/A	N/A
0	Positive	23.5	Non-VOC/I	N/A	N/A
0	Positive	22.5	Non-VOC/I	N/A	N/A
1	Positive	15.8	Non-VOC/I	N/A	N/A
3	Positive	24.1	Non-VOC/I	484-/501-	N/A
1	Positive	17.4	Non-VOC/I	N/A	N/A
2	Positive	20.3	Non-VOC/I	N/A	N/A
1	Positive	23.2	Non-VOC/I	N/A	N/A
2	Positive	30.0	Non-VOC/I	N/A	N/A
2	Positive	24.4	Non-VOC/I	N/A	N/A
1	Negative	39.5	Non-VOC/I	N/A	N/A
1	Positive	17.0	Iota	N/A	B.1.526-E484K
2	Negative	29.2	Non-VOC/I	N/A	N/A
1	Positive	18.2	Iota	484-/501-	B.1.526
1	Positive	19.7	Non-VOC/I	484-/501-	N/A
5	Positive	20.5	Non-VOC/I	484-/501-	B.1.409
3	Positive	18.0	Non-VOC/I	484-/501-	B.1.1.434
1	Positive	19.7	Iota	484-/501-	B.1.526
5	Positive	21.2	Iota	484-/501-	B.1.526
1	Positive	23.2	Non-VOC/I	484-/501-	B.1.1.434
3	Positive	20.7	Non-VOC/I	484-/501-	B.1.2
2	Positive	25.4	Non-VOC/I	501-	N/A
5	Positive	18.0	Non-VOC/I	484-/501-	B.1.243
3	Positive	27.9	Non-VOC/I	484-	B.1.1.25
2	Positive	29.5	Non-VOC/I	484-/501-	N/A
3	Positive	28.7	Alpha	N/A	B.1.1.7
1	Positive	28.9	Iota +	484+/501-	N/A
1	Positive	29.2	Non-VOC/I	N/A	B.1.637
2	Positive	17.2	Iota	484-/501-	B.1.526-S477N
2	Positive	20.1	Iota	484-/501-	B.1.526-S477N
4	Positive	20.5	Non-VOC/I	484-/501-	B.1.1.222
4	Positive	21.1	Iota	484+/501-	B.1.526-E484K
3	Negative	38.5	Iota +	484-/501-/477+	N/A
5	Positive	33.6	Non-VOC/I	N/A	N/A
0	Positive	24.5	Non-VOC/I	484-/501-	B.1.2
5	Positive	24.8	Iota +	484+/501-/477-/452-	NA
3	Positive	22.5	Iota +	484+/501-/477-/452-	N/A
2	Positive	23.6	Iota +	484+/501-/477-/452-	N/A
0	Positive	24.1	Non-VOC/I	N/A	B.1.637
5	Positive	23.5	Iota	484-/501-/477+/452-	B.1.526-S477N
2	Positive	17.3	Non-VOC/I	N/A	N/A
5	Positive	29.8	Non-VOC/I	N/A	N/A
3	Positive	23.5	Alpha	N/A	B.1.1.7
4	Positive	28.0	Alpha	N/A	B.1.1.7
2	Positive*	27.8	Non-VOC/I	452+	B.1.637
1	Positive	25.3	Iota	484+/501-/477-/452-	B.1.526-E484K
3	Positive*	19.3	Iota +	484+/501-/477-/452-	N/A
5	Positive	23.4	Iota +	484-/501-/477+	N/A
5	Positive*	17.8	Iota +	484-/501-/477+/452+	N/A
0	Positive*	28.6	Non-VOC/I	484-/501-/477-/452+	B.1.637
5	Positive	23.5	Iota +	484-/501-/477+/452+	N/A
5	Positive	25.5	Alpha +	484-/501+/478-/452-	N/A
2	Positive	19.8	Mu	484+/501+/478-/452-	B.1.629
5	Positive	20.0	Alpha +	484-/501+/478-/452-	N/A
2	Positive*	26.6	Delta	484-/501-/478+/452+	B.1.617.2
5	Negative	34.5	Non-VOC/I	N/A	N/A
5	Negative	38.5	Non-VOC/I	N/A	N/A
1	Positive	16.8	Delta	N/A	AY.2
3	Positive	21.4	Delta	484-/501-/478+/452+	AY.47
5	Positive	23.1	Non-VOC/I	N/A	N/A
4	Positive*	21.6	Non-VOC/I	N/A	N/A
1	Negative	31.3	Non-VOC/I	N/A	N/A
5	Negative	36.1	Non-VOC/I	N/A	N/A
3	Positive*	19.2	Non-VOC/I	N/A	N/A
4	Positive*	24.1	Omicron	501+/452-/496&498+	BA.1
0	Positive	22.3	Omicron	501+/452-/496&498+	BA.1
1	Positive*	23.0	Omicron	501+/452-/496&498+	BA.1

(*continued on next page*)

Table 2 (*continued*)

Days Symptoms	CoV-SCAN Result	RT-PCR CT Value	VOC/I	Single Nucleotide Assay	Sequencing Results
2	Positive*	21.2	Omicron	501+/452-/496&498+	BA.1
1	Positive*	27.9	Omicron +	501+/452-/496&498+	N/A
2	Positive	24.6	Omicron +	501+/452-/496&498+	N/A
5	Positive*	24.9	Omicron	501+/452-/496&498+	BA.1
5	Positive*	27.6	Omicron +	501+/452-/496&498+	N/A
4	Positive*	20.4	Omicron	501+/452-/496&498+	BA.1
3	Positive*	22.7	Omicron	501+/452-/496&498+	BA.1
0	Positive	24.1	Omicron	501+/452-/496&498+	BA.1
3	Negative*	25.1	Omicron	501+/452-/496&498+	BA.1
5	Positive*	27.2	Omicron	501+/452-/496&498+	BA.1
5	Positive*	26.0	Omicron	501+/452-/496&498+	BA.1
4	Positive*	29.8	Omicron +	501+/452-/496&498+	N/A
1	Positive*	21.8	Omicron	501+/452-/496&498+	BA.1

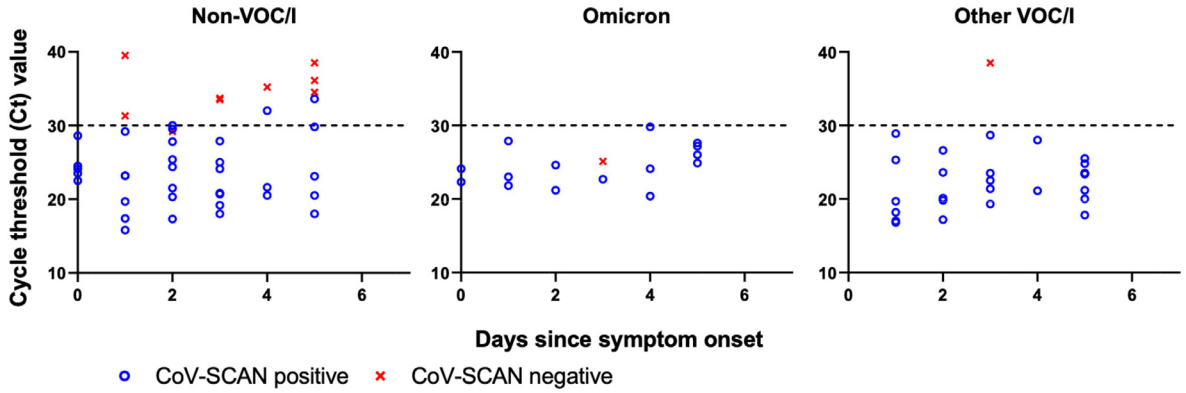


Fig. 4. CoV-SCAN and RT-PCR results on paired samples by CT value and days after symptom onset.
True Positive (blue): paired samples in which the RT-PCR is positive and CoV-SCAN is positive.

perform a formal study, CoVSCAN was positive for 3/3 tested specimens from symptomatic participants, obtained between 1-3 days after symptom onset. These specimens had RT-PCR CT values between 21.1 and 27.4 and were subsequently confirmed to be B.A.2 by whole genome sequencing

In Fig. 4, CoV-SCAN and RT-PCR results on paired samples are plotted by CT value and days between symptom onset and sample collection for Non-VOCs/VOIs, Omicron, and all other VOCs/VOIs. False Negative paired samples, with RT-PCR-positive and CoV-SCAN-negative results, are represented in red. Among the 86 RT-PCR-positive specimens, 12 (14.0%) had a RT-PCR CT value ≥30.0. CoV-SCAN was positive for 3/12 (25.0%) of specimens with a CT value ≥30.0 and positive for 72/74 (97.2%) of specimens with CT values <30.0.

False Negative (red): paired samples in which the RT-PCR is positive and the CoV-SCAN is negative.

For **Study 2,** 1000 participants were enrolled with a mean age of 43±15 (range 12 to 78) and 539 (53.9%) females. Overall, 996 paired samples were included in the analyses, excluding 4 with inconclusive or unavailable RT-PCR results or invalid CoV-SCAN results. In total, there were 112 participants with symptoms and 884 asymptomatic participants. Among participants with symptoms, the PPA was 88.9% (95% CI 51.8–99.7%) and NPA was 100% (96.5–100%) (Table 1b). Among asymptomatic participants, the PPA was 85.7% (95% CI 42.1%-99.6%) and NPA was 99.7% (99.0–99.9%) (Table 1c). Among the 16 specimens that were RT-PCR-positive, 5/16 (31.3%) had a RT-PCR CT value ≥30.0. CoV-SCAN was positive for 3/5 (60%) specimens with a RT-PCR CT value ≥30.0 and positive for 11/11 (100%) of specimens with CT values < 30.0.

5. Discussion

We developed a unique SARS-CoV-2 rapid antigen LFA that utilizes two pairs of high-affinity monoclonal antibodies to capture and detect N-protein. Formulated before the emergence of variants, the test demonstrates levels of detection for all known variants of concern that are similar to wild type. In clinical point-of-care testing on 1144 symptomatic and asymptomatic participants, the PPA is over 85.7% and NPA is over 99.7%, with detection of over 97.2% of specimens with CT values <30. In addition, our test has performed well on the detection of the Omicron variant (B.1.1.529, BA.1 and BA.2) in the clinical setting even within the first 2 days after symptom onset.

An independent comparative assessment of 122 rapid antigen tests self-certified by manufacturers to conform to European health, safety, and environmental protection standards and thereby granted access to the European Union Common Market, identified 26 (21%) that did not reach 75% sensitivity for high viral load samples (defined as CT ≤25) [13]. Among the 96 tests with reasonable sensitivity in the high viral load samples, overall sensitivity across all CT values ranged from 28% to 86%. CoV-SCAN outperforms 94 out of 96 tests in overall sensitivity and has near equivalent sensitivity to the two best performing tests (85.7% versus 86.0%). A separate comparison of the 6 rapid antigen tests across a panel of 100 patient-derived samples, places CoV-SCAN's PPA on par with the most sensitive test, Innova, (PPA 89%, 95% CI 81.4–93.8) and superior to the remaining 5 tests assessed. CoV-SCAN's specificity was also among the highest [14].

Virus isolated from samples with CT values >30 are extremely difficult to culture in laboratory settings, suggesting that such samples may not be infectious [15–17]. For this reason, some experts have suggested that antigen tests act as "tests of infectivity" as they are, by design, highly sensitive only when high levels of virus are present [6,18]. In contrast, highly sensitive PCR tests can detect viral fragments with no real-world consequence for weeks after infection [19,20]. CoV-SCAN's high sensitivity (>97%) for samples with CT <30 suggest that it is well calibrated for the detection of infectious cases. The lower sensitivity of antigen tests relative to PCR should be treated as a unique and beneficial attribute of the test. If deployed frequently and strate-

gically, widespread use of low-cost antigen tests can provide great societal utility and has the potential to minimize the disruption of SARS-CoV-2 to workplaces, educational settings, and congregant living settings.

The US FDA recommends that antigen tests makers routinely assess the impact of mutations or viral variants on test performance [21]. Most recently, there has been concern that approved antigen tests were less sensitive in the detection of Omicron [8]. Analytic testing with cultured virus as a proxy for clinical sensitivity that compared the sensitivity of seven antigen tests to detect Omicron versus earlier variants found slightly reduced sensitivity in all but one test [22]. However a clinical evaluation comparing the sensitivity of Abbott's BinaxNow to RT-PCR during the Omicron surge in San Francisco reported >95% sensitivity in samples with CT<30 [23].

The Omicron surge has elevated the profile of rapid antigen tests in the US and the government has made home tests available and accessible to all Americans through an influx of one billion free mailed tests and guaranteed reimbursement of eight tests per month by insurance [24,25]. How such tests can be optimally used to interrupt transmission chains remains unclear. In addition, guidance on timing of testing post-exposure or post symptom onset might vary by variants and/or vaccine/past infection status due to differential time course of viral proliferation and clearance. Clear guidance on how to use approved rapid antigen tests to maximize public health and individual benefit is urgently needed.

6. Conclusion

The unique construction of CoV-SCAN, with two paired antibodies, has led to durable performance of the test across multiple variants in both laboratory and clinical evaluations, and is pending review at multiple agencies. Pending authorization, testing with CoV-SCAN can be deployed with confidence as a component of any rapid antigen testing strategy to identify individuals harboring infectious virus.

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Conflicts of Interest

Potential conflicts of interest: MNVH, WS, ZC, BJ, FW are employed by BioMedomics, Inc, the manufacturer of the CoV-SCAN rapid antigen test.

Potential conflicts of interest: DDH and LL are inventors on a patent application on monoclonal antibodies used in the construction of CoV-SCAN, and the rights to this invention has been licensed to Biomedomics by Columbia University.

No other authors declare any conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2022.100080.

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