



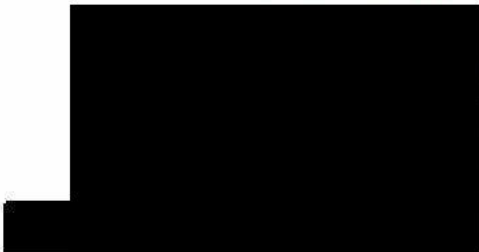
Sona Nanotech COVID-19 Lateral Flow Assay

EUA Testing Preliminary Report

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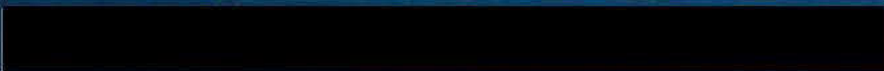
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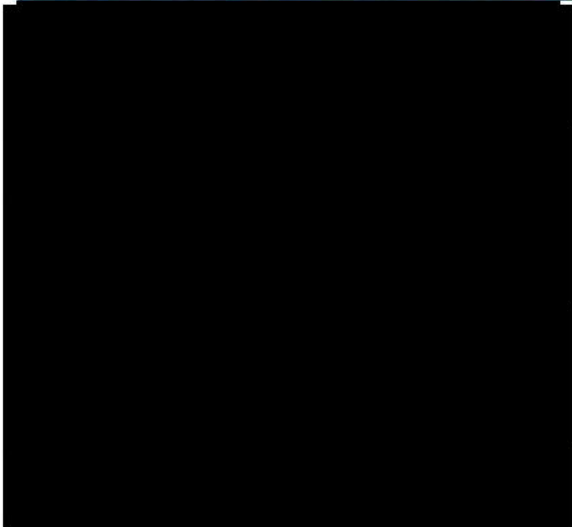
 Project No. 311640.01 .004

July 24, 2020





Approvals
Sona Nanotech COVID-19 Lateral Flow Assay
EUA Testing Preliminary Report



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Section 1. Executive Summary

- FDA Emergency Use Authorization (EUA) studies consisted of the following:
 - Limit of Detection (LoD)
 - Exclusivity and Microbial Interference
 - Interfering Substances
 - Inclusivity and Hook Effect
- The confirmed LoD using live SARS-CoV-2 virus diluted in simulated nasal matrix (SNM) was 4.5×10^3 TCID₅₀/mL. The LoD of sample diluted in Reagent Solution is 2.14×10^2 TCID₅₀/mL.
- A total of 28 viruses, bacteria, and fungi, and pooled nasal wash were tested for exclusivity and microbial interference. Only SARS-CoV resulted in a positive test result. None of the organisms tested resulted in a false-negative result in SARS-CoV-2 spiked samples.
- At the initial concentration tested (5% v/v), false-positive results were observed with Zicam and NeilMed Naso GEL. False positive results were not observed at 2.5% v/v.
- False-negative results were observed at the initial concentrations tested with Oxymetazoline nasal spray (15% v/v) and nasal gel (10% v/v). False negative results were not observed at 7.5% v/v nasal spray and 3.75% v/v nasal gel.
- No hook effect was seen with SARS-CoV-2 isolate USA_WA1/2020 or Hong Kong/VM20001061/2020.

Section 2.

Coronavirus Isolates and Determination of TCID₅₀

The SARS-CoV-2 isolate used for these studies, which is known as USA_WA1/2020, was isolated from the first documented US case of a traveler from Wuhan, China.¹ SARS-CoV-2 was sourced from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA). SARS-CoV and MERS coronaviruses were sourced from BEI Resources. All coronaviruses were cultured in Vero E6 cells per established procedures. Briefly, 3×10^6 Vero E6 cells were plated into a T75 flask with 15 mL infection media (Dulbecco's Modified Eagle's medium supplemented with 5% fetal bovine serum and nonessential amino acids) and incubated in a humidified incubator with 5% CO₂. The following day the Vero cells were re-fed with infection media and inoculated with 0.5 ml of virus stock. Cells were incubated for 4 days at which point widespread cytopathic effect (CPE) was apparent. At this point, supernatant was collected and 1 mL aliquots of virus stock frozen at $\leq -70^\circ\text{C}$.

For determination of TCID₅₀, an aliquot of virus stock was thawed and TCID₅₀ determined following established procedures. In brief, 10-fold serial dilutions of virus stock were prepared and plated (8 wells per dilution) in a 96 well plate containing 5,000 Vero E6 cells/well. After 5 days of incubation, each well was scored as positive or negative for CPE and TCID₅₀/mL, as determined by the Reed and Muench method. The coronavirus source information and TCID₅₀/mL concentration of the neat virus stocks prepared by [REDACTED] is summarized in Table 1.

Table 1. Summary of coronaviruses used in the studies

Virus	Isolate	Source/#	Lot	Culture Date	TCID ₅₀ /mL	GC/mL
SARS-CoV-2	USA_WA1/2020	WRCEVA	TVP23156	3/24/20	6.81×10^4	5.7×10^5
SARS	Urbani	BEI NR-18925	200300592	3/6/20	3.15×10^5	N/A
MERS	EMC/2012	BEI NR-44280	62043787	3/19/20	1.36×10^5	N/A

GC/mL = Genomic Copies/mL

2.1 Quantitative RT-PCR of SARS-CoV-2 Stock Using N1 Primers and Probes (CDC Method)

Viral genomic copies per mL (GC/mL) was determined by quantitative RT-PCR using a Bio-Rad CFX96 Real-Time Detection System. The standard curve was prepared from a custom gBlock (Integrated DNA Technologies) containing the entire SARS-CoV-2 N1 target amplicon sequence plus 30 bp of flanking sequence on the 5' and 3' ends. The gBlock sequence was derived from NCBI accession number MN908947 (Severe acute respiratory syndrome coronavirus 2 isolate

¹ Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A, Diaz G, Cohn A, Fox L, Patel A, Gerber SI, Kim L, Tong S, Lu X, Lindstrom S, Pallansch MA, Weldon WC, Biggs HM, Uyeki TM, Pillai SK; Washington State 2019-nCoV Case Investigation Team. 2020. First Case of 2019 Novel Coronavirus in the United States. N Engl J Med. Mar 5;382(10):929-936. doi: 10.1056/NEJMoa2001191. Epub January 31, 2020.

Wuhan-Hu-1, complete genome). The copy number concentration of the gBlock was determined based on the total amount of oligonucleotide (ng) and the length (bp).

The qPCR procedure used N1 primer and probe sequences published by the CDC.² The primer and probe sequences used are summarized in Table 2

NOTE: That N1 probe fluorophore labels are different from what is published by the CDC.

Table 2. CDC assay primer and probe sequences

Description	Sequence
2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'
2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'
2019-nCoV_N1 Probe	5'-6-FAM/ZEN-ACC CCG CAT TAC GTT TGG TGG ACC- IBFQ-3'

Primers and probes were purchased as custom items from Integrated DNA Technologies. TaqPath™ 1-step RT-qPCR Master Mix, CG was sourced from ThermoFisher. Thermal cycling conditions followed those published in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Instructions for Use and are summarized in Table 3.³

² Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. 2020. Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primer and Probe Information. Retrieved from:

<https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html>.

³ Centers for Disease Control and Prevention, Respiratory Viruses Branch, Division of Viral Diseases. 2020. Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus: Instructions for Use. Retrieved from:

<https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-for-detection-instructions.pdf>.

Table 3. CDC assay thermal cycling parameters

Stage	Temperature	Time	Cycles
1	25°C	2 min	1
2	50°C	15 min	1
3	95°C	2 min	1
4	95°C	3 sec	45
	55°C	30 sec	

The gBlock standard curve consisted of the following concentrations: 1×10^1 , 1×10^2 , 1×10^3 , and 1×10^4 GC/ μ L. SARS-CoV-2 culture supernatant was diluted in nuclease-free water for testing at the following dilutions: 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} . Master mix was prepared as noted in the Table 4.

Table 4. CDC assay master mix preparation

Reagent	Volume per reaction
Nuclease-free water	8.5 μ L
Primer/Probe mix	1.5 μ L
TaqPath™ 1-step RT-qPCR Master Mix	5.0 μ L
TOTAL	15 μL

For the RT-PCR reaction, 15 μ L of prepared master mix was added to each well followed by 5 μ L of standard or sample, for a final total volume of 20 μ L per reaction well. Both gBlock standards and SARS-CoV-2 sample dilutions were run in duplicate wells.

The GC/mL of the SARS-CoV-2 dilutions was determined using the slope and y-intercept of the gBlock standard curve, as determined by linear regression analysis. The GC/mL of the virus stock was determined based on the average of the duplicate well results for all dilutions tested. For the SARS-CoV-2 stock used for these studies, the concentration was calculated to be 5.7×10^9 GC/mL.

Section 3.

Limit of Detection

A preliminary LoD was determined by first testing serial ten-fold dilutions (1/10, 1/100, and 1/1000) of SARS-CoV-2 stock diluted in simulated nasal matrix (SNM – phosphate buffered saline with 15% glycerol, 2.5% porcine mucin, and 1% human blood). A 50 µL sample of SARS-CoV-2 diluted in SNM was added to a tube, then a dry nasopharyngeal swab added to the tube followed by 1000 µL of Reagent Solution (1:20 dilution). The swab was used to mix the sample, then 100 µL of diluted sample loaded onto triplicate Test Cartridges. The 1/10 dilution tested positive on all three replicates, and the 1/100 and 1/1000 dilutions tested negative on all three replicates. To further define the preliminary LoD, the 1/10 dilution was serially diluted two-fold in SNM to prepare 1/20, 1/40, and 1/80 dilutions of SARS-CoV-2. The 1/20 dilution tested positive on all three replicates, and the 1/40 and 1/80 dilutions tested negative. The 1/20 dilution was selected as the preliminary LoD of 3.41×10^3 TCID₅₀/mL. The results of the preliminary LoD testing are summarized in Table 5, and component information is listed in Table 6.

Table 5. Preliminary LoD Determination

Sample	TCID ₅₀ /mL (in SNM)	Test Results					
		Replicate 1		Replicate 2		Replicate 3	
		T	C	T	C	T	C
Positive Control	-	+	+	+	+	TNP*	TNP*
Negative Control	-	-	+	-	+	-	+
SNM (unspiked)	-	-	+	-	+	-	+
1/10 SARS-CoV-2	6.81×10^3	+	+	+	+	+	+
1/100 SARS-CoV-2	6.81×10^2	-	+	-	+	-	+
1/1000 SARS-CoV-2	6.81×10^1	-	+	-	+	-	+
1/20 SARS-CoV-2	3.41×10^3	+	+	+	+	+	+
1/40 SARS-CoV-2	1.70×10^3	-	+	-	+	-	+
1/80 SARS-CoV-2	8.51×10^2	-	+	-	+	-	+

T = Test Line.

C = Control Line.

* Not enough positive control reagent to perform test.

Table 6. Components used for LoD Determination

Component	Vendor/ Manufacturer	Part No.	Lot No.	Expiration
Test Cassettes	Sona Nanotech	TC-CV2	FG20176-001	N/A
Reagent Solution	Sona Nanotech	RS-CV2	N/A	N/A
1.5 mL Microfuge Tubes	Costar	3213	04920000	18 Mar 2023
External Positive Control	Sona Nanotech	EPC-CV2	N/A	N/A
External Negative Control	Sona Nanotech	ENC-CV2	N/A	N/A
Nasopharyngeal (NP) Swabs	Puritan	25-3316-H	7209	01 Apr 2025
Simulated Nasal Matrix (SNM)		N/A	NH052820	28 May 2021

N/A = Not Available.

[illegible]

C = Control Line.

The LoD for the Sona Nanotech COVID-19 Lateral Flow Assay was determined to be 4.50×10^3 TCID₅₀/mL using live SARS-CoV-2 virus diluted in SNM. When the 1:20 sample dilution factor is considered (50 µL of SARS-CoV-2 spiked SNM added to 1000 µL of Reagent Solution), the LoD for the for the Sona Nanotech COVID-19 Lateral Flow Assay as loaded onto the Test Cassette is 2.14×10^2 TCID₅₀/mL.

Section 4.

Exclusivity and Microbial Interference

To determine if other respiratory pathogens that could be present in a nasopharyngeal (NP) swab sample could cause a false-positive test result, a panel of sixteen (16) viruses, eight (8) bacteria, three (3) fungi, and pooled human nasal wash were tested by spiking 1:20 SNM:Reagent Solution with high concentrations of organism stocks. Target organism concentrations were $\geq 10^5$ TCID₅₀/mL, PFU/mL, or CEID₅₀/mL for viruses, and $\geq 10^6$ cfu/mL for bacteria and fungi. When the target concentration was not achievable due to the titer of the stock culture, a 1/10 dilution was tested. A 1/10 dilution in SNM:Reagent Solution was used for testing pooled nasal wash.

The organisms utilized for exclusivity and microbial interference testing are listed in Table 8. Exclusivity test results are summarized in Table 9. False-positive results were seen only with SARS-CoV. The cross-reactivity with SARS-CoV was not surprising due to similarities in amino acid sequence between SARS-CoV-2 and SARS-CoV (76% homology with multiple regions of identical amino acid sequence).

Table 8. Exclusivity and Microbial Interference Organisms

ID	Organism	Source/Strain/ID No.	Lot#/Harvest Date	Stock Conc.	Test Conc.	Units
229E	Human coronavirus 229E	ATCC VR-740	70033323	1.6×10^7	1.6×10^5	TCID ₅₀ /mL
OC43	Human coronavirus OC43	Zeptomatrix 0810024CF	323852	1.51×10^6	1.5×10^5	TCID ₅₀ /mL
NL63	Human coronavirus NL63	MRI	N/A	4.4×10^5	4.4×10^4	TCID ₅₀ /mL
SARS	SARS-coronavirus	MRI Urbani	WS#1 3/24/20	1.58×10^4	1.58×10^3	TCID ₅₀ /mL
MERS	MERS-coronavirus	MRI EMC/2012	WS#1 3/25/20	5.0×10^4	5.0×10^3	TCID ₅₀ /mL
AV1	Adenovirus 1	ATCC VR-1	70007874	2.2×10^7	2.2×10^5	TCID ₅₀ /mL
hMPV	hMPV	BEI NR-22227	355	2.8×10^6	2.8×10^5	TCID ₅₀ /mL
P1	Parainfluenza virus 1	ATCC VR-94	70016021	1.6×10^7	1.6×10^5	TCID ₅₀ /mL
P2	Parainfluenza virus 2	ATCC VR-92	70004593	5.9×10^6	3.0×10^5	TCID ₅₀ /mL
P3	Parainfluenza virus 3	ATCC VR-93	70027437	1.6×10^8	1.6×10^6	TCID ₅₀ /mL
P4	Parainfluenza virus 4a	ATCC VR-1377	64398052	1.6×10^5	1.6×10^4	TCID ₅₀ /mL
FluA	Influenza A	ATCC VR-1894	70014833	5.2×10^7	5.2×10^5	CIED ₅₀ /mL
FluB	Influenza B	ATCC VR-1931	70020870	7.8×10^6	3.9×10^5	TCID ₅₀ /mL

Table 8. Exclusivity and Microbial Interference Organisms (Continued)

ID	Organism	Source/Strain/ID#	Lot#/Harvest Date	Stock Conc.	Test Conc.	Units
EV68	Enterovirus 68	ATCC VR-1826	70019851	1.6×10^7	1.6×10^5	TCID ₅₀ /mL
RSV	Respiratory syncytial virus	ATCC VR-26	70024483	8.0×10^6	8.0×10^5	PFU/mL
RV	Rhinovirus	ATCC VR-1601	57866034	8.89×10^5	8.89×10^4	TCID ₅₀ /mL
HI	<i>Haemophilus influenzae</i>	ATCC 49247	70023140	5.4×10^8	5.4×10^6	cfu/mL
SPN	<i>Streptococcus pneumoniae</i>	ATCC 49619	70027361	8.95×10^5	8.95×10^4	cfu/mL
SPY	<i>Streptococcus pyogenes</i>	ATCC 19615	70016309	1.39×10^7	1.39×10^6	cfu/mL
CA	<i>Candida albicans</i>	ATCC 14503	08/24/18	1.7×10^9	1.7×10^7	cfu/mL
BP	<i>Bordetella pertussis</i>	ATCC 9797	09/04/18	2.4×10^9	2.4×10^7	cfu/mL
MP	<i>Mycoplasma pneumoniae</i>	ATCC 15531-TTR	70022921	1.0×10^9	1.0×10^7	cfu/mL
CP	<i>Chlamydia pneumoniae</i>	ATCC VR-1356	70019109	4.0×10^7	4.0×10^6	IFU/mL
LP	<i>Legionella pneumophila</i>	Zeptomatrix 801645	323903	1.88×10^{10}	1.88×10^8	cfu/mL
MT	<i>Mycobacterium tuberculosis</i>	Zeptomatrix 801660	323674	6.86×10^7	6.86×10^6	cfu/mL
PC	<i>Pneumocystis carinii</i>	ATCC PRA-159	62297170	1.0×10^8	1.0×10^6	nuclei/mL
PJ	<i>P. jiroveci</i> - <i>S. cerevisiae</i> recombinant	Zeptomatrix 801698	322301	1.56×10^8	1.56×10^6	cfu/mL
PNW	Pooled Human Nasal Wash	Lee Biosolutions 991-26	20-03-511 T6557	NA	NA	NA

Table 9. Exclusivity Test Results

Sample ID	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
	Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
External Positive Control	+	+	+	+	+	+	Pass
External Negative Control	-	+	-	+	-	+	Pass
229E	-	+	-	+	-	+	Pass
OC43	-	+	-	+	-	+	Pass
NL63	-	+	-	+	-	+	Pass
SARS	+	+	+	+	+	+	Fail
MERS	-	+	-	+	-	+	Pass
AV1	-	+	-	+	-	+	Pass
hMPV	-	+	-	+	-	+	Pass
P1	-	+	-	+	-	+	Pass

Table 9. Exclusivity Test Results (Continued)

Sample ID	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
	Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
P2	-	+	-	+	-	+	Pass
P3	-	+	-	+	-	+	Pass
P4	-	+	-	+	-	+	Pass
FluA	-	+	-	+	-	+	Pass
FluB	-	+	-	+	-	+	Pass
EV68	-	+	-	+	-	+	Pass
RSV	-	+	-	+	-	+	Pass
RV	-	+	-	+	-	+	Pass
HI	-	+	-	+	-	+	Pass
SPN	-	+	-	+	-	+	Pass
SPY	-	+	-	+	-	+	Pass
CA	-	+	-	+	-	+	Pass
BP	-	+	-	+	-	+	Pass
MP	-	+	-	+	-	+	Pass
CP	-	+	-	+	-	+	Pass
LP	-	+	-	+	-	+	Pass
MT	-	+	-	+	-	+	Pass
PC	-	+	-	+	-	+	Pass
PJ	-	+	-	+	-	+	Pass
PNW	-	+	-	+	-	+	Pass

Components listed in Table 6.

To determine if any of the respiratory pathogens in the test panel could interfere with the detection of a true positive test result, 1:20 SNM:Reagent Solution was spiked with both high concentration of potential interfering organism stock and a low level of SARS-CoV-2 (3X LoD). Results from Microbial Interference testing are summarized in Table 10. None of the organisms tested caused a false-negative test result in diluted SNM spiked with both organism and 3X LoD SARS-CoV-2.

Table 10. Microbial Interference Test Results

Sample ID	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
	Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
External Positive Control	+	+	+	+	+	+	Pass
External Negative Control	-	+	-	+	-	+	Pass
229E	+	+	+	+	+	+	Pass
OC43	+	+	+	+	+	+	Pass
NL63	+	+	+	+	+	+	Pass
SARS	TNP	TNP	TNP	TNP	TNP	TNP	TNP
MERS	+	+	+	+	+	+	Pass
AV1	+	+	+	+	+	+	Pass
hMPV	+	+	+	+	+	+	Pass
P1	+	+	+	+	+	+	Pass
P2	+	+	+	+	+	+	Pass
P3	+	+	+	+	+	+	Pass

Table 10. Microbial Interference Test Results (Continued)

Sample ID	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
	Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
P4	+	+	+	+	+	+	Pass
FluA	+	+	+	+	+	+	Pass
FluB	+	+	+	+	+	+	Pass
EV68	+	+	+	+	+	+	Pass
RSV	+	+	+	+	+	+	Pass
RV	+	+	+	+	+	+	Pass
HI*	+	+	+	+	+	+	Pass
SPN	+	+	+	+	+	+	Pass
SPY	+	+	+	+	+	+	Pass
CA*	+	+	+	+	+	+	Pass
BP*	+	+	+	+	+	+	Pass
MP*	+	+	+	+	+	+	Pass
CP	+	+	+	+	+	+	Pass
LP	+	+	+	+	+	+	Pass
MT	+	+	+	+	+	+	Pass
PC	+	+	+	+	+	+	Pass
PJ	+	+	+	+	+	+	Pass
PNW*	+	+	+	+	+	+	Pass

TNP = Test Not Performed.

Components listed in Table 6 except as noted below.

* Test Cassette Lot FG20177-001.

Pneumocystis jirovecii, was included in the exclusivity and microbial interference test panel as it is the causative agent of *P. jirovecii* pneumonia (PJP). However, *P. jirovecii* is difficult to culture. As a surrogate for *P. jirovecii*, a *P. jirovecii*-*S. cerevisiae* recombinant that was utilized for exclusivity and microbial interference testing in a predicate FDA EUA cleared COVID-19 lateral flow immunoassay was tested. Additionally, *Pneumocystis carinii* strain M167-6 was included in the test panel. *Pneumocystis jirovecii* was previously known as *Pneumocystis carinii*. In 2002 the name for the causative agent of *Pneumocystis carinii* pneumonia (PCP) in humans was changed from *Pneumocystis carinii* to *Pneumocystis jirovecii*.⁴

Human coronavirus HKU1 was not included in the wet testing panel, as it is difficult to culture and a source for this virus could not be found. Attempts to source clinical samples positive for HKU1 were also unsuccessful. Due to the inability to wet test HKU1 for exclusivity and interference, an *in silico* analysis was performed in which the spike protein amino acid sequences of HKU1 and SARS-CoV-2 were compared. The results of the *in silico* analysis are attached as Appendix A. In summary, one potential cross-reacting linear epitope was identified, and no potential cross-reacting conformational epitopes were identified. If one of the monoclonal antibodies used in the Sona Nanotech COVID-19 Lateral Flow Assay targets this common linear epitope, then cross-reactivity or interference could occur in a patient sample containing HKU1 coronavirus.

⁴ Stringer JR, Beard CB, Miller RF, Wakefield AE. 2002. A new name (*Pneumocystis jirovecii*) for *Pneumocystis* from humans. *Emerg Infect Dis.* Sep;8(9):891-6.

Section 5. Interfering Substances

To determine if endogenous or exogenous substances that might be present in a nasopharyngeal (NP) swab sample could cause a false-positive test result, a panel of eighteen (18) substances were tested by spiking 1:20 SNM:Reagent Solution at the concentrations recommended by the FDA.⁵ The FDA recommended interfering substances information was obtained from the Quidel Sofia Analyzer and Influenza A+B FIA Influenza A+B immunological test system 510(k) decision summary (K112177). The substances and concentrations tested are listed in Table 11, and test results summarized in Table 12. At the initial recommended test concentration, false-positive results were seen with Naso GEL (NeilMed) and Zicam at 5% v/v. No false-positive results were seen when the substances were tested more dilute at 2.5% v/v.

Table 11. Interfering Substances

Substance	Source/Item#	Lot No.	Expiration Date
Human Whole Blood (EDTA tube)	BIOIVT	HMN421040	7/29/2020
Mucin (bovine submaxillary type I-S)	Sigma M1778	SLCD6129	6/9/2021
Ricola (Menthol)	Ricola	N280101	10/31/2022
Sucrets (Dyclonin/Menthol)	Sucrets	026320T11	3/1/2022
Chloraseptic (Menthol/Benzocaine)	Chloraseptic Max	00989M11	8/1/2021
Naso GEL (NeilMed)	NeilMed	NG-214	2/1/2023
Nasal Drops (Phenylephrine)	CVS Health	OAK0862	10/1/2022
Nasal Spray (Oxymetazoline)	CVS Health	72008	1/1/2022
Nasal Spray (Cromolyn)	Nasal Crom	294521	6/1/2021
Nasal Gel (Oxymetazoline)	Afrin	TN000AM	9/1/2020
Zicam	Zicam	B73765	11/1/2021
Homeopathic (Alkalol)	Alkalol	P8A004	1/1/2020
Fisherman's Friend	Lofthouse of Fleetwood	BL00136	9/3/2022
Sore Throat Phenol Spray	Chloraseptic	8161	10/1/2022
Tobramycin	Sigma TL1014	SLCB7407	6/9/2021
Mupirocin	TNP	TNP	TNP
Tamiflu (Oseltamivir Phosphate)	Sigma SML-1606	0000097332	N/A
Fluticasone Propionate	CVS Health	RL7205	9/1/2021

N/A = Not Available.

Table 12. Interfering Substances Test Results

Sample ID	Test Concentration	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
		T	C	T	C	T	C	
External Positive Control	NA	+	+	+	+	+	+	Pass
External Negative Control	NA	-	+	-	+	-	+	Pass
Human Whole Blood (EDTA tube)	4% v/v	-	+	-	+	-	+	Pass

⁵ U.S. Food & Drug Administration. Antigen Template for Manufacturers. Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) - Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff. Version May 11, 2020. Retrieved from: <https://www.fda.gov/media/137907/download>.

Table 12. Interfering Substances Test Results (Continued)

Sample ID	Test Concentration	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
		T	C	T	C	T	C	
Mucin (bovine submaxillary type I-S)	0.5%	-	+	-	+	-	+	Pass
Ricola (Menthol)	1.5 mg/mL	-	+	-	+	-	+	Pass
Sucrets (Dyclonin/Menthol)	1.5 mg/mL	-	+	-	+	-	+	Pass
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	-	+	-	+	-	+	Pass
Naso GEL (NeilMed)	5% v/v	+	+	+	+	+	+	Fail
Naso GEL (NeilMed)	2.5% v/v	-	+	-	+	-	+	Pass
Nasal Drops (Phenylephrine)	15% v/v	-	+	-	+	-	+	Pass
Nasal Spray (Oxymetazoline)	15% v/v	-	+	-	+	-	+	Pass
Nasal Spray (Oxymetazoline)	7.5% v/v	-	+	-	+	-	+	Pass
Nasal Spray (Cromolyn)	15% v/v	-	+	-	+	-	+	Pass
Nasal Gel (Oxymetazoline)	10% v/v	-	+	-	+	-	+	Pass
Nasal Gel (Oxymetazoline)	5% v/v	-	+	-	+	-	+	Pass
Nasal Gel (Oxymetazoline)	3.75% v/v	-	+	-	+	-	+	Pass
Zicam	5% v/v	+	+	+	+	+	+	Fail
Zicam	2.5% v/v	-	+	-	+	-	+	Pass
Homeopathic (Alkalol)	10% v/v	-	+	-	+	-	+	Pass
Fisherman's Friend	1.5 mg/mL	-	+	-	+	-	+	Pass
Sore Throat Phenol Spray	15% v/v	-	+	-	+	-	+	Pass
Tobramycin	4 µg/mL	-	+	-	+	-	+	Pass
Mupirocin	10 mg/mL	TNP	TNP	TNP	TNP	TNP	TNP	TNP
Tamiflu (Oseltamivir Phosphate)	5 mg/mL	-	+	-	+	-	+	Pass
Fluticasone Propionate	5% v/v	-	+	-	+	-	+	Pass

TNP = Test Not Performed.

NA = Not Applicable.

Components listed in Table 5 except for Test Cassette Lot = FG20177-001.

T = Test Line.

C = Control Line.

To determine if any of the potential interfering substances in the test panel could interfere with the detection of a true positive test result, 1:20 SNM:Reagent Solution was spiked with both potential interfering substance and a low level of SARS-CoV-2 (3X LoD). The test results are summarized in Table 13. At the initial recommended test concentration, false-negative results were observed with Nasal Spray (Oxymetazoline) at 15% v/v and Nasal Gel (Oxymetazoline) at 10% v/v. The false-negative results dissipated when lower concentrations of substance were tested (Nasal Spray (Oxymetazoline) at 7.5% v/v and Nasal Gel (Oxymetazoline) at 3.75% v/v).

Table 13. Interfering Substances Spiked with 3X LoD SARS-CoV-2 Test Results

Sample ID	Test Concentration	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
		T	C	T	C	T	C	
External Positive Control	NA	+	+	+	+	+	+	Pass
External Negative Control	NA	-	+	-	+	-	+	Pass
Human Whole Blood (EDTA tube)	4% v/v	+	+	+	+	+	+	Pass
Mucin (bovine submaxillary type I-S)	0.5%	+	+	+	+	+	+	Pass
Ricola (Menthol)	1.5 mg/mL	+	+	+	+	+	+	Pass

Table 13. Interfering Substances Spiked with 3X LoD SARS-CoV-2 Test Results (Continued)

Sample ID	Test Concentration	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
		T	C	T	C	T	C	
Sucrets (Dyclonin/Menthol)	1.5 mg/mL	+	+	+	+	+	+	Pass
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	+	+	+	+	+	+	Pass
Naso GEL (NeilMed)	5% v/v	TNP	TNP	TNP	TNP	TNP	TNP	TNP
Naso GEL (NeilMed)	2.5% v/v	+	+	+	+	+	+	Pass
Nasal Drops (Phenylephrine)	15% v/v	+	+	+	+	+	+	Pass
Nasal Spray (Oxymetazoline)	15% v/v	-	+	+	+	+	+	Fail
Nasal Spray (Oxymetazoline)	7.5% v/v	+	+	+	+	+	+	Pass
Nasal Spray (Cromolyn)	15% v/v	+	+	+	+	+	+	Pass
Nasal Gel (Oxymetazoline)	10% v/v	-	+	-	+	-	+	Fail
Nasal Gel (Oxymetazoline)	5% v/v	-	+	-	+	-	+	Fail
Nasal Gel (Oxymetazoline)	3.75% v/v	+	+	+	+	+	+	Pass
Zicam	5% v/v	TNP	TNP	TNP	TNP	TNP	TNP	TNP
Zicam	2.5% v/v	+	+	+	+	+	+	Pass
Homeopathic (Alkalol)	10% v/v	+	+	+	+	+	+	Pass
Fisherman's Friend	1.5 mg/mL	+	+	+	+	+	+	Pass
Sore Throat Phenol Spray	15% v/v	+	+	+	+	+	+	Pass
Tobramycin	4 µg/mL	+	+	+	+	+	+	Pass
Mupirocin	10 mg/mL	TNP	TNP	TNP	TNP	TNP	TNP	TNP
Tamiflu (Oseltamivir Phosphate)	5 mg/mL	+	+	+	+	+	+	Pass
Fluticasone Propionate	5% v/v	+	+	+	+	+	+	Pass

TNP = Test Not Performed.

NA = Not Applicable.

Components listed in Table 5 except for Test Cassette Lot = FG20177-001.

T = Test Line.

C = Control Line.

Section 6.

Inclusivity and Hook Effect

The ability of the Sona Nanotech COVID-19 Lateral Flow Assay to detect an additional SARS-CoV-2 isolate (Hong Kong/VM20001061/2020) was determined by testing serial 1/5 dilutions of virus stock in SNM. The Hong Kong isolate was detectable at a concentration of 3.90×10^4 TCID₅₀/mL in SNM (1.90×10^3 TCID₅₀/mL after dilution in Reagent Solution).

Potential hook effect was assessed by testing neat virus stock diluted 1/20 in Reagent Solution. No hook effect was seen with either the USA-WA1/2020 or Hong Kong SARS-CoV-2 isolates. Isolate information is listed in Table 14 and results of inclusivity and hook effect testing are summarized in Table 15.

Table 14. Inclusivity and Hook Effect Strain Information

Sample ID	Source/Strain/ID No.	Lot No./Harvest Date	Stock Conc. (TCID ₅₀ /mL)	Test Conc. (TCID ₅₀ /mL)
1/5 Hong Kong	Hong Kong/VM20001061/2020	6/12/2020	1.95×10^5	3.90×10^4
1/25 Hong Kong	Hong Kong/VM20001061/2020	6/12/2020	1.95×10^5	7.8×10^3
1/125 Hong Kong	Hong Kong/VM20001061/2020	6/12/2020	1.95×10^5	1.56×10^3
1/625 Hong Kong	Hong Kong/VM20001061/2020	6/12/2020	1.95×10^5	3.12×10^2
USA - Hook	USA-WA1/2020	WS1/24Mar20	6.81×10^4	3.24×10^3
Hong Kong - Hook	Hong Kong/VM20001061/2020	6/12/2020	1.95×10^5	9.29×10^3

Table 15. Inclusivity and Hook Effect Test Results

Sample ID	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
	Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
External Positive Control	+	+	+	+	+	+	Pass
External Negative Control	-	+	-	+	-	+	Pass
1/5 Hong Kong	+	+	+	+	+	+	Pass
1/25 Hong Kong	-	+	-	+	-	+	Fail
1/125 Hong Kong	-	+	-	+	-	+	Fail
1/625 Hong Kong	-	+	-	+	-	+	Fail
USA - Hook	+	+	+	+	+	+	Pass
Hong Kong - Hook	+	+	+	+	+	+	Pass

Components listed in Table 6.



Section 7. Conclusions

The Limit of Detection for the Sona Nanotech COVID-19 Lateral Flow Assay was determined to be 4.5×10^3 TCID₅₀/mL in a simulated nasal matrix sample using SARS-CoV-2 isolate USA-WA1/2020. The LoD of 50 µL sample diluted 1/20 in Reagent Solution is 2.14×10^2 TCID₅₀/mL. SARS-CoV cross-reacts in the Sona Nanotech COVID-19 Lateral Flow Assay, resulting in a false-positive test result. No cross-reactivity was seen with any of the other organisms tested, and none of the organisms tested interfere with the detection of a true positive test result. High concentrations (5% v/v) of Naso GEL (NeilMed) and Zicam caused a false-positive test result that was eliminated by reducing the concentration to 2.5% v/v. Oxymetazoline Nasal Gel (15% v/v) and Nasal Spray (10% v/v) both interfered with the detection of a true positive test result at the initial concentrations tested, but did not interfere at lower test concentrations of 7.5% v/v and 3.75% v/v, respectively. The Sona Nanotech COVID-19 Lateral Flow Assay can detect the Hong Kong/VM20001061/2020 SARS-CoV-2 isolate as well as the USA-WA1/2020 isolate. No hook effect was seen with either isolate.

Appendix A.

***In silico* analysis of HKU1 Coronavirus Spike Protein for
Potential Cross Reactivity in the Sona Nanotech COVID-19
Lateral Flow Assay**

SARS-CoV-2 B-cell Epitope Evaluation

Results

Computational estimation of B-cell linear and discontinuous epitopes was performed using Discotope 2.0 and Ellipro through the Immune Epitope Database (IEDB) web interface. The following Protein Data Bank (PDB) entries were used as input for comparisons between SARS-CoV-2 and HKU1.

Table 1. The PDB entry for SARS-CoV-2 was selected because it represents the virus in the "open" configuration with the receptor-binding domain exposed. The only available structure for HKU1 spike is in the "closed" conformation

Organism	PDB ID
Human Coronavirus HKU1	5I08
SARS-CoV-2	6VSB

Linear epitopes estimated by Ellipro were analyzed for overlaps of 5-mer peptide sequences, as 5 amino acids has been identified as the low limit for typical B cell epitopes.^{1,2} This yielded an overlap of 7 consecutive amino acids between a single pair of estimated linear epitopes for HKU1 and SARS-CoV-2.

Table 2. Estimated linear epitopes by Ellipro that contain overlapping peptides. Exactly matching sequence is highlighted red

Org	Start	End	Epitope Sequence	Ellipro Score
HKU1	948	1017	PILSETQISGYTTAATVAAMFPPWSAAAGVPFSLNVQYRINGLGVTMDVLN KNQKLIANAFNKALLSIQN	0.812
SARS2	879	925	AGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIAN	0.839

This AA overlap was identified to be located at the N-terminal end of the Heptad Repeat 1 (HR1) domain of the S2 region of Spike protein in both viruses. An investigation into the antigenic capability of this region revealed that both small peptide therapeutics and antibodies targeting this region in SARS have been demonstrated to have a neutralizing effect as this protein region's interaction with the Heptad Repeat 2 (HR2) domain is required for membrane fusion.^{3,4} While the SARS amino acid conformation of this region is slightly different, in a study

¹ Kringelum, J. V., Nielsen, M., Padkjær, S. B., & Lund, O. (2013). Structural analysis of B-cell epitopes in antibody:protein complexes. *Molecular Immunology*, 53(1-2), 24-34. doi:10.1016/j.molimm.2012.06.001.

² Paull, M. L., Johnston, T., Ibsen, K. N., Bozakowski, J. D., & Daugherty, P. S. (2019). A general approach for predicting protein epitopes targeted by antibody repertoires using whole proteomes. *Plos One*, 14(9). doi:10.1371/journal.pone.0217668.

³ Elshabrawy, H. A., Coughlin, M. M., Baker, S. C., & Prabhakar, B. S. (2012). Human Monoclonal Antibodies against Highly Conserved HR1 and HR2 Domains of the SARS-CoV Spike Protein Are More Broadly Neutralizing. *PLoS ONE*, 7(11). doi:10.1371/journal.pone.0050366.

⁴ Yuan, Y., Cao, D., Zhang, Y., Ma, J., Qi, J., Wang, Q., Gao, G. F. (2017). Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. *Nature Communications*, 8(1). doi:10.1038/ncomms15092.

of sera from 42 original SARS patients, 40% had antibodies that positively reacted with a linear peptide covering this equivalent region in HR1.⁵

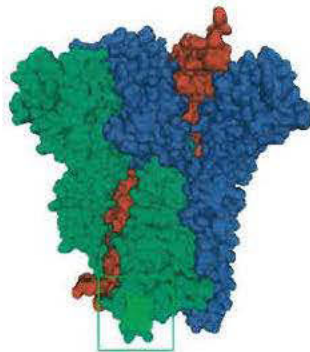


Figure 1. Location of overlapping amino sequence epitope in HKU1 Spike

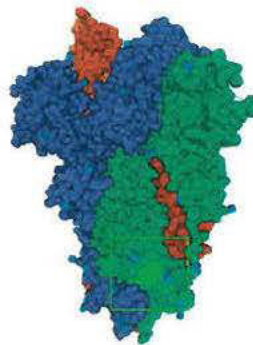


Figure 2. Location of overlapping amino acid sequence epitope in SARS-CoV-2. Resolved glycans are indicated by blue cubes

The surrounding N-linked glycans identified in SARS-CoV-2 were considered to not provide adequate shielding of this epitope since 22 of the 23 original SARS glycosylation sites are

⁵ Hu, Y., Zhou, Y., Wu, H., Luo, B., Chen, J., Li, W., & Jiang, S. (2004). Identification of Immunodominant Sites on the Spike Protein of Severe Acute Respiratory Syndrome (SARS) Coronavirus: Implication for Developing SARS Diagnostics and Vaccines. *The Journal of Immunology*, 173(6), 4050-4057. doi:10.4049/jimmunol.173.6.4050.

conserved in SARS-CoV-2, including those that appear in the proximal space surrounding this candidate epitope.

Table 3. Corresponding N-linked glycan sites in SARS and SARS-CoV-2

Org	Position	Amino
SARS	699	NFSI
SARS	783	NFSQ
SARS_CoV_2	717	NFTI
SARS_CoV_2	801	NFSQ

From this we concluded that neutralizing antibodies that target the S2 subunit for SARS-CoV-2 could potentially produce a cross reaction with HKU1 if the paratope of the antibody recognized the HR1 region. However, it should be noted that this homology only exists in one genotype for HKU1. The S2 subunit for HKU1 appears to have two distinct lineages as demonstrated by BLAST results below in Table 4.

Table 4. BLAST results for NQKLIAN peptide demonstrate two distinct HKU1 S2 genotypes (drop in Query cover highlighted in red)

Description	Max Score	Total Score	Query cover	E Value	Per. Ident	Accession
spike glycoprotein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	AGT17758.1
spike glycoprotein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	AB07438.1
spike protein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	BBA20985.1
RecName: Full=Spike glycoprotein; Short=S glycoprotein	25.2	25.2	100%	0.004	100.00	Q14EB0.1
spike glycoprotein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	AB075545.1
spike glycoprotein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	AB075513.1
spike glycoprotein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	AB075625.1
spike glycoprotein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	AB075505.1
RecName: Full=Spike glycoprotein; Short=S glycoprotein	25.2	25.2	100%	0.004	100.00	Q02ME7.1
spike glycoprotein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	AZ552618.1
spike glycoprotein [Human coronavirus HKU1]	25.2	25.2	100%	0.004	100.00	AB075617.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AMN88694.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AB075497.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AB075601.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	YP_173238.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AB075553.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AB075593.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AGT17777.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AGW27872.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AB075609.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	ADN03339.1
spike protein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	BBA20983.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AGW27863.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AMN88686.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AB075528.1
spike protein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	ARU07577.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AGW27898.1
spike glycoprotein [Human coronavirus HKU1]	21.8	33.1	85%	0.082	100.00	AGT17769.1

Additionally, a method to do comparisons between conformational epitopes estimated by Discotope and Ellipro was developed. This approach iterated through each residue in the PDB structural data for each virus spike protein and created a dictionary of proximal residues within 6Å of the target amino acid. This parameter corresponds with the default radius parameter for discontinuous epitope estimation in Ellipro as well as a literature review that identified the recognition site of most antibody Complementarity-determining regions (CDRs) as being at least

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Figure 4

SARS-CoV-2 spike amino/position	Cluster amino/count	HKU1 spike amino/position
C301	K1L1S1T1	N29
A688	I2Q1S2	G404
T941	A2L1S1	N1023
E990	D1I1Q1R1V1	Q1071
V991	D1I1L1Q1R1	V1072
Q992	D1I2L1R1	Q1073
R1000	L2Q2S1	N1041
A1022	A2L1N1T1	L1082

[illegible]

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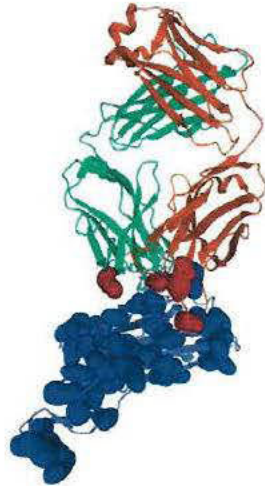


Figure 5. Residues highlighted in blue are conserved between SARS-CoV-2 and HKU1. Residues highlighted in red are CDR residues in the light and heavy chain of the antibody that are identified as having hydrogen bonding interactions with epitope residues.

Table 6. Most contact residues between SARS-CoV-2 antibody CR3022 and the spike RDB are not conserved between SARS-CoV-2 and HKU1.

Chain	Residue	Conserved between SARS-CoV-2 and HKU1
Light	Y55	Yes
Light	S33	No
Light	Y38	No
Heavy	Y52	Yes
Heavy	D55	No
Heavy	D57	No
Heavy	I102	Yes
Heavy	S103	No
Heavy	D107	Yes

This analysis suggests that CR3022 would not cross react with HKU1 antibodies that target the RBD, and that the significant divergence in sequence identity between the two viruses suggest that this is generally true for antibodies that target the S1 domain of these coronaviruses.

Supplemental Material

Discotope Results SARS-CoV-2 with -3.7 Score Cutoff

chain_id	residue_id	residue_name	contact_number	propensity_score	discotope_score
A	281	GLU	0	-3.366	-2.979
A	282	ASN	7	-2.664	-3.162
A	415	THR	0	-3.819	-3.38
A	420	ASP	4	-3.618	-3.662
A	449	TYR	4	-0.567	-0.962
A	450	ASN	11	-1.78	-2.841
A	454	ARG	14	-1.224	-2.894
A	491	PRO	7	-0.72	-1.442
A	492	LEU	15	-0.95	-2.565
A	493	GLN	9	-0.572	-1.541
A	494	SER	7	-0.846	-1.553
A	496	GLY	3	0.041	-0.309
A	498	GLN	4	0.68	0.142
A	499	PRO	5	0.178	-0.417
A	500	THR	0	1.907	1.688
A	503	VAL	5	-1.856	-2.218
A	505	TYR	8	-1.528	-2.272
A	556	ASN	2	-3.79	-3.584
A	558	LYS	2	-1.479	-1.539
A	560	LEU	2	-1.137	-1.236
A	561	PRO	0	-0.961	-0.851
A	562	PHE	0	-2.061	-1.824
A	703	ASN	4	-2.02	-2.248
A	704	SER	3	-1.469	-1.645
A	705	VAL	10	-2.821	-3.646
A	793	PRO	1	-2.278	-2.131
A	794	ILE	1	-2.5	-2.327
A	809	PRO	4	-2.691	-2.841
A	810	SER	9	-0.669	-1.627
A	914	ASN	7	-1.117	-1.794
A	917	TYR	9	-2.702	-3.426
A	918	GLU	13	-2.285	-3.517
A	1071	GLN	9	-2.775	-3.491
A	1099	GLY	1	-3.789	-3.468
A	1100	THR	0	-3.877	-3.431
A	1101	HIS	8	-2.903	-3.489
A	1111	GLU	19	-1.693	-3.684
A	1118	ASP	4	-3.018	-3.129
A	1140	PRO	7	-0.961	-1.656
A	1141	LEU	5	-0.257	-0.802
A	1142	GLN	7	0.318	-0.523
A	1143	PRO	6	1.067	0.255
A	1144	GLU	6	0.718	-0.056
A	1145	LEU	5	0.162	-0.431
A	1146	ASP	5	0.731	0.072

Discotope Results HKU1 with -3.7 Score Cutoff

chain_id	residue_id	residue_name	contact_number	propensity_score	discotope_score
A	27	ASP	0	-3.728	-3.3
A	29	ASN	2	-3.797	-3.591
A	30	LYS	0	-2.368	-2.096
A	114	ASN	3	-2.775	-2.801
A	115	ASN	0	-1.918	-1.699
A	406	SER	0	-3.719	-3.291
A	441	PRO	12	-2.204	-3.33
A	442	SER	17	-1.252	-3.063
A	443	SER	10	-2.347	-3.227
A	444	TRP	8	-1.574	-2.313
A	445	ASN	8	-0.908	-1.723
A	447	ARG	1	-1.12	-1.106
A	463	SER	12	-2.52	-3.61
A	467	PHE	2	-3.911	-3.692
A	488	SER	0	-3.25	-2.876
A	573	LEU	2	-3.178	-3.043
A	639	ALA	2	-3.785	-3.58
A	642	ASN	2	0.121	-0.123
A	643	ASN	3	-2.252	-2.338
A	644	TRP	0	-2.333	-2.064
A	651	SER	0	-2.045	-1.809
A	652	ASN	0	-2.099	-1.858
A	771	ASN	0	-3.499	-3.096
A	993	THR	0	-3.314	-2.933
A	994	MET	0	-3.418	-3.025
A	995	ASP	5	-3.383	-3.569
A	999	LYS	6	-2.826	-3.19

Ellipro Results

SARS-CoV-2 Linear Epitopes

No.	Chain	Start	End	Peptide Number of residues	Score
1	8	1070	1146		
				AQEKNFITTAICHGKAHFPREGVFSNGTHWFTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELD	77 0.88
2	8	433	526		
				VIAWNSNNLDSYNYLYRNLPFERDISTEIYNCYFPLQSYGFQPTVGYPYRVVLSFELLHAPATVCG	
3	8	92	192		
				FASTKSNIIIRGWIFGTTLDSTQSLLIYNHATNVIKVECFQFCNDPFLSEFRVYSSANNCTFEYVSQPFKNLREF	78 0.798
4	8	328	364		
				RFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVAD	37 0.758
5	8	553	564		
				TESNKKFLPFQQ	12 0.733
6	8	392	428		
				FTNVYADSFVIRGDEVQRQIAPGQTGKIADYNKLPDD	37 0.713
7	8	62	85		
				VTWFHNPVLP	10 0.702
8	8	236	269		
				TRFQTLAAYVVG	13 0.688
9	8	575	585		
				AVRDPQTLEIL	11 0.674
10	8	701	718		
				AENSVAYSNNSIAIPTNF	18 0.67
11	8	879	925		
				AGTITSGWTFGAGAAIQIPFAYQMAYRFNGIGYTNVLYENQKLIAN	47 0.639
12	8	703	815		
				AQVKQIYKTFPIKDFGGFNFSQILPDPSKR	30 0.63
13	8	203	219		
				IYSKHTPPQG	10 0.613
14	8	528	537		
				KKSTNLVKNK	10 0.501
15	8	371	376		
				SASFST	6 0.551

HKU1 Linear Epitopes

No.	Chain	Start	End	Peptide Number of residues	Score
1	A	427	589	VS LPLVNTINNFPSSWNRVYGGFNLSSYDVVSDHCFVSNSDFPCFLGWSFDSCISNRRNCRIF	
	68	0.869			
2	A	237	255	VMPLTCNAISSNTDNETLE	19 0.822
3	A	948	1017	PILSETQISGYTTAATAVAHFPPWSAAAGVPFS LNWQYRINGLGVTDVLMKNQELIANAFNKALLSIQN	
	70	0.812			
4	A	106	123	VKNTKLYVWNTLYSEFST	18 0.805
5	A	634	648	KEVSAAYYNNQNL	15 0.784
6	A	15	34	IGDFNCTNSFINDYNTIPR	20 0.766
7	A	851	905	DTQLQVANALMQGVTLSSNLTLLED	27 0.742
8	A	140	183	QPHNGILEITACQTYKEYPHTVCKSKGSIRNESWHDSSSEPLC	44 0.731
9	A	057	068	GFKDFLTNKTYYT	12 0.713
10	A	76	94	DLALKGSIYLSLTIWYKPPF	19 0.687
11	A	1104	1135	SKAIEKVNECVKSSQSPRINFCGNGVHILSLVQ	32 0.687
12	A	319	353	RRIPNLPCDDIDNLNIVSVSPUNWERRIFSNCN	35 0.679
13	A	766	799	TFEPFNFTIAGH	13 0.669
14	A	186	197	KKNFTYVNSADW	12 0.666
15	A	403	418	LSSSGFLQSSNYKIDI	16 0.664
16	A	304	401	NSITVDKFAIPNRRDDL	10 0.639
17	A	723	731	AVNLTSYSV	9 0.602
18	A	925	929	CTGGS	5 0.552
19	A	609	617	YSNTEISTG	9 0.519

SARS-CoV-2 Discontinuous Epitopes

No.	Residues	Number of residues	Score
1	"B:A701, B:E702, B:N703, B:S704, B:V705, B:A706, B:Y707, B:S708, B:N709, B:N710, B:S711, B:I712, B:A713, B:I714, B:P715, B:T716, B:N717, B:F718, B:A783, B:Q784, B:V785, B:K786, B:Q787, B:I788, B:Y789, B:K790, B:T791, B:P792, B:P793, B:I794, B:K795, B:D796, B:F797, B:G798, B:G799, B:F800, B:N801, B:F802, B:S803, B:Q804, B:I805, B:L806, B:P807, B:D808, B:P809, B:S810, B:K811, B:R815, B:Q872, B:V873, B:S875, B:A876, B:A879, B:G880, B:I882, B:T883, B:S884, B:G885, B:W886, B:T887, B:F888, B:G889, B:A890, B:G891, B:A892, B:A893, B:L894, B:Q895, B:I896, B:P897, B:F898, B:A899, B:M900, B:Q901, B:M902, B:A903, B:Y904, B:F906, B:N907, B:G908, B:G910, B:V911, B:T912, B:Q913, B:N914, B:V915, B:L916, B:Y917, B:E918, B:N919, B:Q920, B:K921, B:L922, B:I923, B:A924, B:N925, B:L1034, B:A1070, B:Q1071, B:E1072, B:K1073, B:N1074, B:F1075, B:T1076, B:T1077, B:A1078, B:P1079, B:A1080, B:I1081, B:C1082, B:M1083, B:D1084, B:G1085, B:K1086, B:A1087, B:M1088, B:F1089, B:P1090, B:R1091, B:E1092, B:G1093, B:V1094, B:F1095, B:V1096, B:S1097, B:M1098, B:G1099, B:T1100, B:M1101, B:W1102, B:F1103, B:V1104, B:T1105, B:Q1106, B:A1107, B:M1108, B:F1109, B:V1110, B:E1111, B:P1112, B:Q1113, B:I1114, B:I1115, B:T1116, B:T1117, B:D1118, B:M1119, B:T1120, B:F1121, B:V1122, B:S1123, B:G1124, B:M1125, B:C1126, B:D1127, B:V1128, B:V1129, B:I1130, B:G1131, B:I1132, B:V1133, B:N1134, B:N1135, B:T1136, B:V1137, B:V1138, B:D1139, B:P1140, B:L1141, B:Q1142, B:P1143, B:E1144, B:L1145, B:D1146"	174	0.747
2	"B:R320, B:F329, B:P330, B:N331, B:I332, B:T333, B:N334, B:L335, B:C336, B:P337, B:F338, B:G339, B:E340, B:V341, B:F342, B:N343, B:A344, B:T345, B:R346, B:F347, B:A348, B:S349, B:V350, B:V351, B:A352, B:W353, B:N354, B:R355, B:K356, B:R357, B:I358, B:S359, B:N360, B:C361, B:V362, B:A363, B:D364, B:V367, B:S371, B:A372, B:S373, B:F374, B:S375, B:T376, B:C391, B:F392, B:T393, B:N394, B:V395, B:Y396, B:A397, B:D398, B:S399, B:F400, B:V401, B:I402, B:R403, B:G404, B:D405, B:E406, B:V407, B:R408, B:Q409, B:I410, B:A411, B:P412, B:G413, B:Q414, B:T415, B:G416, B:K417, B:I418, B:A419, B:D420, B:Y421, B:N422, B:Y423, B:K424, B:L425, B:P426, B:D427, B:D428, B:T430, B:V433, B:I434, B:A435, B:W436, B:N437, B:S438, B:N439, B:N440, B:L441, B:D442, B:S443, B:Y449, B:N450, B:Y451, B:L452, B:Y453, B:R454, B:N460, B:L461, B:K462, B:P463, B:F464, B:E465, B:R466, B:D467, B:I468, B:S469, B:T470, B:E471, B:I472, B:Y473, B:N487, B:C488, B:Y489, B:F490, B:P491, B:L492, B:Q493, B:S494, B:Y495, B:G496, B:F497, B:Q498, B:P499, B:T500, B:V503, B:G504, B:V505, B:Q506, B:P507, B:Y508, B:R509, B:V510, B:V511, B:V512, B:L513, B:S514, B:E516, B:L517, B:L518, B:M519, B:A520, B:P521, B:A522, B:T523, B:V524, B:C525, B:G526, B:P527, B:K528, B:K529, B:S530, B:T531, B:N532, B:L533, B:V534, B:K535, B:N536, B:K537, B:N544, B:T553, B:E554, B:S555, B:N556, B:K557, B:F558, B:L560, B:P561, B:F562, B:Q563, B:Q564, B:A575, B:V576, B:R577, B:D578, B:P579, B:Q580, B:T581, B:L582, B:E583, B:I584, B:L585"	185	0.735
3	"B:A27, B:Y28, B:T29, B:N30, B:F32, B:R34, B:Y38, B:F59, B:V62, B:T63, B:W64, B:F65, B:M66, B:N81, B:P82, B:V83, B:L84, B:P85, B:F86, B:F92, B:A93, B:S94, B:T95, B:E96, B:K97, B:S98, B:N99, B:I100, B:I101, B:R102, B:G103, B:W104, B:I105, B:F106, B:G107, B:T108, B:T109, B:L110, B:D111, B:S112, B:K113, B:T114, B:Q115, B:S116, B:L117, B:L118, B:I119, B:V120, B:N121, B:N122, B:A123, B:T124, B:N125, B:V126, B:V127, B:I128, B:K129, B:V130, B:C131, B:E132, B:F133, B:Q134, B:F135, B:C136, B:N137, B:D138, B:P139, B:F140, B:L141, B:S155, B:E156, B:F157, B:V159, B:Y160, B:S161, B:S162, B:A163, B:N164, B:N165, B:C166, B:T167, B:F168, B:E169, B:Y170, B:V171, B:S172, B:Q173, B:P174, B:F175, B:L176, B:K187, B:N188, B:L189, B:R190, B:E191, B:F192, B:S205, B:K206, B:H207, B:T208, B:P209, B:P217, B:Q218, B:G219, B:A222, B:L223, B:E224,		

B:N234, B:I235, B:T236, B:R237, B:F138, B:Q239, B:T240, B:L241, B:L242, B:A263, B:A264, B:Y265, B:Y266,
 B:V267, B:G268" 122 0.731
 4 "B:T747, B:E748, B:S750, B:N751" 4 0.578
 5 "B:I203, B:L226, B:V227, B:L229, B:P230, B:I231" 6 0.523
 6 "B:V656, B:N657, B:N658" 3 0.509

HKU1 Discontinuous Epitopes

No.	Residues	Number of residues	Score
1	"A:Y28, A:N29, A:K30"	3	0.828
2	"A:N793, A:F794, A:T795, A:I796, A:A797, A:G798, A:H799, A:D848, A:D851, A:I852, A:T853, A:Q854, A:L855, A:Q856, A:V857, A:A858, A:N859, A:A860, A:L861, A:M862, A:Q863, A:G864, A:V865, A:T866, A:L867, A:S868, A:S869, A:N870, A:L871, A:N872, A:T873, A:L902, A:L903, A:E904, A:D905, A:P948, A:I949, A:L950, A:S951, A:E952, A:T953, A:Q954, A:I955, A:S956, A:G957, A:Y958, A:T959, A:T960, A:A961, A:A962, A:T963, A:V964, A:A965, A:A966, A:M967, A:F968, A:P969, A:P970, A:M971, A:S972, A:A973, A:A974, A:A975, A:G976, A:V977, A:P978, A:F979, A:S980, A:L981, A:N982, A:V983, A:Q984, A:Y985, A:R986, A:I987, A:N988, A:G989, A:L990, A:G991, A:V992, A:T993, A:M994, A:D995, A:V996, A:L997, A:N998, A:K999, A:N1000, A:Q1001, A:K1002, A:L1003, A:T1004, A:A1005, A:N1006, A:A1007, A:F1008, A:N1009, A:K1010, A:A1011, A:L1012, A:L1013, A:S1014, A:I1015, A:Q1016, A:N1017, A:S1104, A:Q1117, A:S1118, A:P1119, A:R1120, A:I1121, A:N1122, A:E1112, A:C1113, A:V1114, A:K1115, A:S1116, A:Q1117, A:S1118, A:P1119, A:R1120, A:I1121, A:N1122, A:F1123, A:C1124, A:G1125, A:N1126, A:G1127, A:N1128, A:M1129, A:I1130, A:L1131, A:S1132, A:L1133, A:V1134, A:Q1135, A:F1143, A:I1144, A:H1145, A:F1146, A:S1147"	141	0.761
3	"A:I321, A:P322, A:N323, A:L324, A:P325, A:D326, A:C327, A:D328, A:I329, A:D330, A:N331, A:W332, A:L333, A:N334, A:N335, A:V336, A:S337, A:V338, A:P339, A:S340, A:P341, A:L342, A:N343, A:W344, A:E345, A:R346, A:R347, A:I348, A:F349, A:S350, A:N351, A:C352, A:N353, A:V354, A:H354, A:T357, A:V358, A:D359, A:K359, A:F359, A:A359, A:I359, A:P359, A:N359, A:R359, A:R359, A:D359, A:D400, A:L401, A:L403, A:Q404, A:S405, A:S406, A:Q407, A:F408, A:L409, A:Q410, A:S411, A:S412, A:N413, A:Y414, A:K415, A:I416, A:D417, A:I418, A:S419, A:Y427, A:S428, A:L429, A:P430, A:L431, A:V432, A:N433, A:V434, A:T435, A:I436, A:N437, A:N438, A:F439, A:N440, A:P441, A:S442, A:S443, A:W444, A:N445, A:R446, A:R447, A:Y448, A:G449, A:F450, A:G451, A:S452, A:F453, A:N454, A:L455, A:S456, A:S457, A:Y458, A:D459, A:V460, A:V461, A:Y462, A:S463, A:D464, A:H465, A:C466, A:F467, A:S468, A:V469, A:N470, A:S471, A:D472, A:F473, A:C474, A:P475, A:C476, A:L477, A:G478, A:M479, A:S476, A:F477, A:D478, A:S479, A:C480, A:I481, A:S482, A:N483, A:N484, A:R485, A:C486, A:N487, A:I488, A:F489, A:G490, A:T491, A:T492, A:S493, A:F494, A:I495, A:N496, A:D497, A:T498, A:L499, A:M499, A:P499, A:N499, A:T499, A:K500, A:L501, A:L502, A:L503, A:L504, A:L505, A:L506, A:L507, A:L508, A:L509, A:L510, A:L511, A:L512, A:L513, A:L514, A:L515, A:L516, A:L517, A:L518, A:L519, A:L520, A:L521, A:L522, A:L523, A:L524, A:L525, A:L526, A:L527, A:L528, A:L529, A:L530, A:L531, A:L532, A:L533, A:L534, A:L535, A:L536, A:L537, A:L538, A:L539, A:L540, A:L541, A:L542, A:L543, A:L544, A:L545, A:L546, A:L547, A:L548, A:L549, A:L550, A:L551, A:L552, A:L553, A:L554, A:L555, A:L556, A:L557, A:L558, A:L559, A:L560, A:L561, A:L562, A:L563, A:L564, A:L565, A:L566, A:L567, A:L568, A:L569, A:L570, A:L571, A:L572, A:L573, A:L574, A:L575, A:L576, A:L577, A:L578, A:L579, A:L580, A:P181, A:L182, A:C183, A:K187, A:N188, A:F189, A:T190, A:V191, A:N192, A:V193, A:S194, A:A195, A:D196, A:W197, A:D215, A:V216, A:V237, A:R238, A:P239, A:L240, A:T241, A:C242, A:N243, A:A244, A:I245, A:S246, A:S247, A:N248, A:T249, A:D250, A:N251, A:E252, A:T253, A:L254, A:E255"	132	0.735
5	"A:R319, A:R320, A:L608, A:S610, A:N611, A:T612, A:E613, A:I614, A:S615, A:T616, A:G617, A:Y625, A:K634, A:E635, A:V636, A:S637, A:A638, A:A639, A:Y640, A:Y641, A:N642, A:N643, A:W644, A:Q645, A:N646, A:L647, A:L648, A:G657, A:F658, A:K659, A:D660, A:F661, A:L662, A:T663, A:N664, A:K665, A:T666, A:Y667, A:T668"	39	0.681
6	"A:L695, A:K696, A:S698, A:F713, A:D714, A:S715, A:Y716, A:L717, A:A723, A:V724, A:N725, A:L726, A:T727, A:S728, A:Y729, A:R763"	16	0.555
7	"A:S730, A:V731, A:S732, A:G741, A:T766, A:F767, A:E768, A:P769, A:F770, A:N771"	10	0.549
8	"A:C925, A:T926, A:G927, A:G928, A:S929, A:E930"	6	0.534
9	"A:N821, A:A823, A:D827, A:S830, A:E831"	5	0.523